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Poster

116. Embryonic Neurogenesis

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 116.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Title: P27kip1 activation by reelin induces preplate splitting during corticogenesis

Authors: J. KIM[1,2,3], H. SUH-KIM[1,2], *Y.-D. LEE[1,2,3];
[1]Sch. of Medicine, Ajou Univ., Suwon, Korea, Republic of; [2]Dept. of Biomed. Sci., Suwon, Korea, Republic of; [3]Ctr. for Cell Death Regulating Bio-drug, Suwon, Korea, Republic of

Abstract: A glycoprotein Reelin secreted into the extracellular matrix by Cajal-Retzius cells has been implicated in the control of neuronal migration and positioning during bran corticogenesis. In the present study, we observed in E12-16 brain of reelin-deficient reeler mouse that production of Tbr2-positive intermediate progenitor cells (IPCs) was suppressed in progenitor compartments (SVZ and IZ). In addition, expression of Ngn2 which directly activates Tbr2 expression was also delayed. Conversion from IPCs to Tbr1-positive postmitotic neurons in preplate is markedly decreased in reeler mice. We found that depression of p27kip1 protein, a CIP/Kip family, was observed in the in the preplate and cortical plate in reeler mice. In particular, cell culture study showed that depressed level of p27kip1 in reelin deficient condition was recovered by reelin treatment. Thus we suggest that reelin-induced p27kip1 pathway may be involved in neuronal differentiation of early-born neurons

Disclosures: J. Kim: None. **H. Suh-Kim:** None. **Y. Lee:** None.

Poster

116. Embryonic Neurogenesis

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: R21AG042585

Title: Compound screen on the proliferation and differentiation of neural progenitor cells

Authors: X. XIA, *S. T. WONG, D. GAO, X. XU, T. ZHOU;
Systems Med. and Bioengineering, Houston Methodist Res. Institute, Weill Cornell Med. Col., Houston, TX

Abstract: Neural progenitor (NP) cells are the multipotent cells that produce neurons and glial cells in the central nervous system, small molecule compounds promoting their proliferation and neuronal differentiation are of pivotal importance to regenerative medicine. We carried out a high-content screen to systematically characterize known bioactive compounds, on their effects on the self-renewing division, neuronal differentiation and dopaminergic neuronal differentiation of NP cells. The screen successfully identified major effective pharmacological classes, and allowed us to compare the pharmacological responsive profiles of several types of widely used NP cells. This study revealed the potential of several small molecule compounds for use in regenerative medicine or transplantation therapy. The screening result also provided insight into the signaling network regulating the neuronal differentiation of NP cells.

Disclosures: **X. Xia:** None. **S.T. Wong:** None. **D. Gao:** None. **X. Xu:** None. **T. Zhou:** None.

Poster

116. Embryonic Neurogenesis

Location: Hall A

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Program#/Poster#: 116.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Support: EY022030-03

Title: Signaling through gp130 and Jak/Stat regulates the proliferation and neurogenic capacity of Müller glia-derived progenitor cells in the avian retina

Authors: *L. J. TODD, A. J. FISCHER;
The Ohio State Univ., Columbus, OH

Abstract: Müller glia can be stimulated to de-differentiate, proliferate and form Müller glia-derived progenitor cells (MGPCs) that are capable of regenerating retinal neurons. The capacity of MGPCs to regenerate retinal neurons varies considerably across vertebrates. The MGPCs in zebrafish retina are able to restore fully functional neurons after injury. By contrast, proliferating MGPCs can be formed in the retinas of birds and mammals, but these cells appear to be biased to form new glia or remain undifferentiated. Thus, one of the key hurdles to harnessing the

regenerative potential of MGPCs is to identify means to stimulate neurogenesis from these cells. In the zebrafish retina, several key cell-signaling pathways have been identified to drive the de-differentiation of mature Müller glia and formation of proliferating of MGPCs. One of these key cell-signaling pathways is the Jak-Stat-pathway. In the retinas of warm-blooded vertebrates, the Jak-Stat pathway is known to stimulate glial differentiation during development and induce gliotic phenotypes in mature glia. We investigated whether Jak-Stat signaling influences the reprogramming of Müller glia and neuronal differentiation of the progeny produced by MGPCs in the avian retina *in vivo*. We observed that pStat3 accumulates selectively in Muller glia in response to intraocular injections of CNTF, FGF2 and retinal damage. We found that inhibition of gp130 receptor, JAK2 kinase or Stat3 suppresses the formation of MGPCs in NMDA-damaged or FGF2-treated retinas. By comparison, activation of Jak-Stat signaling via intraocular injections of CNTF with FGF2 significantly increased numbers of proliferating MGPCs in the absence of retinal damage. Evidence is provided that inhibition of Jak-Stat signaling may impact the formation of MGPCs in the FGF2-treated retina through modulating activation of microglia. Importantly, when signaling through the gp130 co-receptor is inhibited after the proliferation of MGPCs, we found a significant increase in neuronal differentiation at the expense of glial differentiation. Inhibition of gp130 resulted in decreased mRNA levels of notch1, hes1 and hes5, components of the Notch-pathway which are known to inhibit neuronal differentiation. We conclude that Jak-Stat signaling plays important roles during the formation of proliferating MGPCs and differentiation of their progeny. We propose that that targeting gp130 co-receptor and the Jak-Stat pathway may be a means to increase the neurogenic capacity of MGPCs in higher vertebrates.

Disclosures: **L.J. Todd:** None. **A.J. Fischer:** None.

Poster

116. Embryonic Neurogenesis

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: TSRI CIRM Training Grant #01165

NINDS (NS076006)

Title: Transcriptome analysis of neural progenitor cells and immature neurons in optic tectum of Xenopus laevis

Authors: *L.-C. HUANG[1], J. CORNELIUS[2], A. YERI[3], K. KUSUMI[2], K. VAN KEUREN-JENSEN[3], H. T. CLINE[1];
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Abstract: In the developing optic tectum of Xenopus laevis, neural progenitor cells undergo symmetric proliferative division, asymmetric neurogenic division and direct neuronal differentiation (Bestman and Cline, 2012). To provide insights into mechanisms that govern progenitor cell self-renewal and neuronal differentiation, an unbiased analysis on the transcript profile of neural progenitor cells and immature neurons was conducted. The midbrain of stage 46 animals was electroporated with a plasmid from which turboGFP reporter expression was driven by sox2/oct4 enhancer and minimal FGF promoter. TurboGFP+ve neural progenitor cells were enriched from animals reared in dark for 24 hours after electroporation, while turboGFP+ve immature neurons were enriched from animals reared in enhanced visual stimulation for 24 hours (Sharma and Cline, 2010; Bestman et al., 2015). These GFP+ve cells were isolated, using Fluorescence-activated cell sorting (FACS), and their mRNA was amplified in order for their transcripts to be analyzed by RNA-seq. Transcriptome analyses revealed distinct gene expression profiles in neural progenitor cells and immature neurons. ~70% of the reads were aligned to the X. laevis genome scaffolds (Xenbase: genome scaffold assembly v7.1 with gff3 v1.6), which confirms the specificity of the RNA amplification protocol. Out of the aligned reads, ~31% were intergenic, and ~61% were mRNA, in average. Over 54,000 annotated genes in X. laevis genome, 724 and 783 of transcripts were differentially expressed between neural progenitor cells and immature neurons, using Tophat2 and STAR as the aligner, respectively. Of these differentially expressed transcripts, ~ 60% were up-regulated, and ~ 40% were down-regulated. Genes with differential expression were clustered into the following categories, based on their molecular function, using DAVID, a functional annotation tool: voltage-gated ion activity, calcium binding, protein serine/threonine kinase activity, extracellular matrix structure constituent, transcriptome co-factor activity, and endoribonuclease activity. Transcriptome analyses revealed the complexity of transcript profiles between neural progenitor cells and immature neurons. These studies enhance our understanding the molecular mechanisms that regulate the fate of neural progenitor cells differentiated into neurons.

Disclosures: L. Huang: None. **J. Cornelius:** None. **A. Yeri:** None. **K. Kusumi:** None. **K. Van Keuren-Jensen:** None. **H.T. Cline:** None.

Poster

116. Embryonic Neurogenesis

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 116.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSERC

Title: DLX transcriptional regulation of neural progenitor cell fate in the developing forebrain

Authors: ***S. JAPONI**[1], M. CASEY[1], J. ZAGOZEWSKI[1], D. D. EISENSTAT[2];
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Abstract: Introduction Differentiation of inhibitory interneurons from neural progenitors and their migration to appropriate regions in the forebrain contributes to maintaining the balance between excitation and inhibition crucial for proper neural function. Dlx homeobox genes encode transcription factors that specifically bind to homeodomain binding sites in regulatory regions and regulate target gene expression. In the Dlx1/2 double knockout (DKO) mouse, tangential migration of inhibitory interneurons from the ganglionic eminences to the neocortex is impaired. Disruption of signaling by the CXCR4 chemokine receptor results in improper migration of interneurons in the forebrain. We hypothesize that Dlx2 promotes interneuron differentiation and migration by activating Cxcr4 expression. Methods We have used chromatin immunoprecipitation (ChIP) of embryonic mouse forebrain (E13.5) using a specific antibody to DLX2 followed by PCR using oligonucleotide primers flanking candidate homeodomain binding TAAT/ATTA motifs. Targets are characterized using gel shift and reporter gene assays *in vitro* and validated by gene expression studies *in vivo* comparing wild-type and DKO forebrain tissues. Results ChIP-based PCR demonstrated that DLX2 binds to regions containing putative DLX2 binding sites upstream of the transcriptional start site of Cxcr4. Dlx2 significantly affected luciferase reporter gene expression *in vitro* when co-expressed with the regulatory regions of Cxcr4 occupied by DLX2 *in vivo*. Furthermore, gel shift assays revealed the critical sites on Cxcr4 necessary for DLX2 binding. Quantitative RT-PCR showed a decrease in transcript level of Cxcr4 in the Dlx1/2 DKO tissues compared to the WT supporting an activating role for DLX2 on Cxcr4 expression *in vivo*. Conclusions Our results support the hypothesis that DLX2 directly activates expression of Cxcr4, thereby contributing to the transcriptional regulation of differentiating and migrating interneurons in the developing forebrain. This proposed research study will contribute to the emerging evidence supporting a role for DLX transcription factors in maintaining the balance of excitation to inhibition during brain development.

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Poster

116. Embryonic Neurogenesis

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Topic: A.01. Neurogenesis and Gliogenesis

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NIH Grant P30HD03352

UW SCRMC postdoctoral fellowship

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IRSF Research Grant

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Title: Positive feedback between RNA binding protein HuD and transcription factor SATB1 promotes neurogenesis

Authors: F. WANG[1], J. J. TIDEI[1], E. D. POLICH[1], Y. GAO[1], N. PERRONE-BIZZOZERO[2], W. GUO[3], *X. ZHAO[1];
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Abstract: The mammalian ELAV-like protein HuD is a neuronal RNA-binding protein implicated in neuronal development, plasticity, and diseases. Although HuD has long been associated with neuronal development, the function of HuD in neural stem cell differentiation and the underlying mechanisms have gone largely unexplored. Here we show that HuD promotes neuronal differentiation of neural stem/progenitor cells (NSCs) in the adult subventricular zone by stabilizing the mRNA of special AT-rich DNA-binding protein 1 (SATB1), a critical transcriptional regulator in neurodevelopment. We find that SATB1 deficiency impairs the neuronal differentiation of NSCs, whereas SATB1 overexpression rescues the neuronal differentiation phenotypes resulting from HuD deficiency. Interestingly, we also discover that SATB1 is a transcriptional activator of HuD during NSC neuronal differentiation. In addition, we demonstrate that NeuroD1, a neuronal master regulator, is a direct downstream target of SATB1. Therefore, HuD and SATB1 form a positive regulatory loop that enhances NeuroD1 transcription and subsequent neuronal differentiation. Our results here reveal a novel positive

feedback network between an RNA-binding protein and a transcription factor, which plays critical regulatory roles in neurogenesis.

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Poster

116. Embryonic Neurogenesis

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Program#/Poster#: 116.07/A7

Topic: A.01. Neurogenesis and Gliogenesis

Title: Maternal high-fat diet alters neurogenesis in the embryonic mouse forebrain

Authors: ***M. NAMIHIRA**[1], M. YAMANO[2], K. HIRANO[1], M. I. OTSUKA[3], K. IGARASHI[3]; [1]Biomed. Res. Institute, AIST, Ibaraki, Japan; [2]Dept. of Comprehensive Rehabil., Osaka Prefecture Univ., Osaka, Japan; [3]Life Sci. Tokyo Advanced Res. center (L-StaR), Hoshi Univ. Sch. of Pharm. and Pharmaceut. Sci., Tokyo, Japan

Abstract: Epidemiological and animal studies have reported that maternal overconsumption of saturated fats during pregnancy influence on offspring health, ranging from metabolic to behavioral disorders. However, little is known about the influence of the maternal unbalanced diet on the development of embryonic brain including neurogenesis of neural stem cells. Here, we show that maternal high-fat diet (HFD) impact on epigenetic regulation of neural precursors and alter the differentiation of these cells in fetal cortex of mice. In late-gestational stage, embryonic cortex exposed maternal HFD exhibited the enhancement of neuronal production and hyper-acetylation of histone H3 lysine in neural precursors. The analysis of genome-wide gene expression by RNA-seq revealed that the expression of several genes involved in neuronal and glial differentiation of neural precursors were significantly altered in these embryonic cortex. We also found that Hippo pathway effector YAP was inactivated in the cortex. Furthermore, postnatal young male mice exposed maternal HFD display hyperactivity only at night. Taken together, these results suggest that an unbalanced diet during pregnancy alters the neurogenesis in embryonic forebrain through the epigenetic changes and the inactivation of Hippo pathway in neural precursors, and that it may be as a potent risk factor of neurodevelopmental disorder. Further analysis of the influence of maternal HFD on fetal brain development is currently underway.

Disclosures: **M. Namihira:** None. **M. Yamano:** None. **K. Hirano:** None. **M.I. Otsuka:** None. **K. Igarashi:** None.

Poster

116. Embryonic Neurogenesis

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grants R21 EY023714-02

the Fight for Sight

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Title: Perturbations of the albino mouse retinal pigment epithelium during retinal ganglion cell genesis

Authors: *L. IWAI, A. RAMOS, A. SCHALER, S. WEINREB, K. ROBINSON, R. BLAZESKI, C. A. MASON;
Pathology and Cell Biol, Columbia Univ., New York, NY

Abstract: During early eye development, the retinal pigment epithelium (RPE) is apposed to the neural retina. As the RPE acquires pigment, retinal ganglion cells (RGCs) are born and specified into the ipsilaterally- and contralaterally-projecting subpopulations. This divergent RGC projection is the basis for binocular vision. In mice, the ipsilateral projection arises from the ventrotemporal (VT) retina from embryonic ages (E) 13.5 and E15.5. Our previous studies identified genes that specify the two RGC subpopulations, e.g., Zic2 and EphB1 that regulate cell fate and projection of ipsilateral RGCs. In albino mammals, disruption of pigmentation in the RPE results in a reduced ipsilateral RGC projection. Our studies showed that in the albino mouse retina, the peak of RGC birth in the VT retina is delayed by about a day, resulting in fewer Zic2-positive RGCs that are born between E13 and E14, mirroring the reduction of ipsilateral RGCs. The RGCs that are born at E17.5, instead, express the contralateral RGC marker Islet2 (Bhansali et al., 2014). These data suggest that the timing of neurogenesis is linked to RGC subtype specification. To further probe how the RPE and the pigment pathway regulate RGC

neurogenesis and cell fate specification, we compared features of wild type RPE with albino RPE during embryonic development. First, using IHC, ISH and EM, we analyzed the morphological and cell biological features of albino and pigmented RPE at E13.5, 15.5 and 17.5. Cell shape, melanosome localization, and gap junction protein (Cx43) expression, localization and phosphorylation state are disrupted in the albino RPE. Second, we are identifying gene expression differences in albino and pigmented RPE at E13.5. Our microarray data analyzed by GSEA indicates that more genes are upregulated than those that are downregulated in albino RPE compared with pigmented RPE. Those genes that are enriched in albino retina include signaling, cytoskeleton, and cell junction/adhesion genes. Signaling genes more highly expressed in albino RPE compared with pigmented RPE are related to Wnts, BMPs, Hedgehog, and Notch. Our results suggest that perturbation of the cell biology of the albino RPE during RGC genesis may affect the integrity of the RPE and in turn, the transmission of factors affecting RGC specification. These studies should reveal how the RPE normally affects neural retinal development, and provide guidelines for directing stem cells into RGCs for replacement in injured or degenerating visual pathways.

Disclosures: L. Iwai: None. **A. Ramos:** None. **A. Schaler:** None. **S. Weinreb:** None. **K. Robinson:** None. **R. Blazeski:** None. **C.A. Mason:** None.

Poster

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Topic: A.01. Neurogenesis and Gliogenesis

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UBACyT Grant

CONICET

Title: Role of GDNF/GFRα1 on neural stem cells development

Authors: *A. BONAFINA, P. FONTANET, D. IRALA, G. PARATCHA, M. LEDDA;
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Abstract: The cerebral cortex is among the most complex of all biological structures and the major site of higher cognitive functions specific to our species. During its development, progenitors proliferate in the ventricular zone and then migrate into the cortical layers where they differentiate into specific cell types. This timed sequence of the developmental process is controlled by an intrinsic cellular program, as well as extrinsic environmental cues. Because impaired regulation of progenitors causes many developmental disorders, it is crucial to understand the mechanisms underlying the behavior of progenitors, including migration, proliferation, and differentiation. Glial cell line-derived neurotrophic factor (GDNF) was originally discovered because of its ability to promote the survival of ventral midbrain dopaminergic neurons. GDNF signals by binding to the glycosylphosphatidylinositol-anchored receptor GFRα1 in complex with the canonic receptor tyrosine kinase Ret or the neural cell adhesion molecule (NCAM). GDNF and its receptor GFRα1 have been reported to have an essential role in cell migration and differentiation in the peripheral and central nervous system. In particular, GDNF can stimulate migration of precursors of inhibitory interneurons in the rostral migratory stream, and the cerebral cortex. However the role of GDNF/ GFRα1 in the forebrain development is still unknown. In the present work, we set out to investigate new roles of GDNF in the developing nervous system. In particular, we analyzed the role of GDNF and its receptor GFRα1 in the development of cortical precursors. Our results indicate that GDNF and GFRα1 control the transition of neuronal glutamatergic progenitors from a proliferative state towards neuronal differentiation, in a Ret-independent manner. We show that GDNF is able to arrest the proliferation of cultured neuronal precursors induced by bFGF promoting neuronal morphological differentiation. The physiological relevance of this system in cell cycle arrest and differentiation of neuronal precursors is being elucidated in GFRα1-conditional mice.

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Poster

116. Embryonic Neurogenesis

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 0-020-202-B233-5

Title: CLASP2 regulates symmetric divisions of neural progenitor cells in early brain development

Authors: *G. M. DILLON[1], W. TYLER[2], K. OMURO[1], T. F. HAYDAR[2], U. BEFFERT[1], A. HO[1];
[1]Biol., [2]Anat. and Neurobio., Boston Univ., Boston, MA

Abstract: Brain development requires the precise timing and control of progenitor cell divisions to produce neurons which migrate into the six major cell layers of the neocortex. Excitatory projection neurons arise from the proliferative epithelium lining the cerebral ventricles. Within this proliferative zone, post-mitotic neurons can be generated from both radial glial cells (apical progenitors) present at the ventricular zone and by intermediate progenitor cells (basal progenitors) found predominantly in the subventricular zone. During cell division, the mitotic spindle is a polarized, microtubule based structure that acts as a cellular scaffold to facilitate the proper segregation of genetic material. Previous evidence demonstrates that microtubule plus-end binding proteins (+TIPs) are essential factors in regulating orientation of the mitotic spindle through connections between the centrosome and kinetochore. In non-neuronal cells, CLIP-associating proteins (CLASPs) are +TIPs that have been shown to be enriched at both the centrosome and kinetochore during mitosis and play an important role in regulating spindle positioning, pole integrity, and chromosome alignment. However, little is known about the role of CLASPs during neural progenitor divisions of the developing forebrain. To determine whether brain-enriched CLASP2 is directly involved in the timing and control of neurogenesis, we performed *in utero* electroporation loss-of-function studies. We electroporated shRNA constructs of CLASP2 or scrambled control into the lateral ventricle of wild type mice at embryonic day 14.5 and analyzed brain sections 48 hours later. During this time, apical progenitors at the ventricle are transitioning from symmetric, self-renewing divisions to asymmetric divisions producing post-mitotic neurons and/or intermediate progenitor cells. We found that CLASP2 knockdown caused a specific increase in the Sox2-positive, apical progenitor population with no overall change in the Tbr2-positive, intermediate progenitor population. In addition, CLASP2 knockdown produced an increase in the percentage of cells expressing the cell cycle marker Ki67 suggesting that the increased apical progenitor population may be stuck in a proliferative state. These findings correlated with an increase in the angle of the mitotic spindle following CLASP2 knockdown. Overall, our results suggest that CLASP2 has a specific role in the transition of apical progenitor cells from symmetric to asymmetric divisions affecting the population of proliferative cells at the ventricle during early brain development.

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Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01 NS20778

NIH Grant R01 EB003806

Title: Different effects of bone morphogenetic receptor type 1 subunits in neural stem and progenitor cells

Authors: J. CHEN, H. NORTH, *C.-Y. PENG, J. A. KESSLER;
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Abstract: Bone morphogenetic protein (BMP) regulates neural stem and progenitor cell maturation by signaling via its receptor subunits. BMP receptor complexes are composed of two BMP receptor II (BMPRII) subunits and two of either BMP receptor 1a (BMPR1a) or BMP receptor 1b (BMPR1b) subunits. Although BMPR1a and BMPR1b have been reported to have some overlapping functions, they appear to have divergent roles in the nervous system during development and post injury. Previous work in our lab have shown that ablation of β1-integrin in cultured neural stem cells (NSCs) results in increased BMPR1b and decreased BMPR1a recruitment to lipid rafts. Additionally, the levels of downstream BMP signaling targets phospho-P38 and phospho-SMAD1/5/8, along with Glial Fibrillary Acidic Protein (GFAP), increase in the absence of β1int. These findings suggest a potential increase in astrocytic cell fate as a result of BMPR1b signaling. *In vitro* overexpression of constitutive active and dominant negative forms of the BMPR1 receptors suggest that BMPR1b likely mediates astrocytic lineage commitment by NSCs, while BMPR1a inhibits NSC astrocytic differentiation. We hypothesize that BMPR1a and BMPR1b mediate different biologic effects on NSCs, such as quiescence or cell fate decisions, through different downstream transcriptional targets. These downstream targets are being analyzed via RNA deep sequencing.

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

Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: R01 EY015290

R01 EY012736

T32 EY013933

Fight for Sight

Title: Retinal ganglion cell genesis and subtype determination in the binocular circuit

Authors: *F. MARCUCCI[1], Q. WANG[1], T. KUWAJIMA[1], C. SOARES[1], S. KHALID[1], M. ROSS[2], C. MASON[1];
[1]Pathology, Columbia Univ., New York, NY; [2]Brain & Mind Res. Inst., Weill Med. Col. of Cornell Univ., New York, NY

Abstract: The generation of multiple cell types from progenitor pools requires mechanisms to achieve specificity of cell fate. Previous studies in the cortex and spinal cord suggest that cell subtype specification is closely related to the time of neurogenesis. In animals with binocular vision, retinal ganglion cells (RGCs) are divided into two subclasses by their laterality of projection: ipsilateral RGCs arise exclusively from the ventrotemporal (VT) retina, and contralateral RGCs arise from all retinal regions and project to the opposite side of the brain. A balanced production of ipsilaterally and contralaterally projecting RGCs is essential for generating proper connectivity. Here we explore whether ipsi and contra RGCs can be distinguished by their time of birth. We also investigated the role of a cell cycle regulatory gene, Cyclin D2, in RGC generation and axon trajectory. By using EdU birthdating in combination with subtype-specific RGC markers, we find that of the RGCs that populate the retina at E15, ipsilateral RGCs are born between E13 and E14, whereas contralateral RGCs are continuously produced during a longer interval of time that starts at E11. Moreover contralaterally projecting RGCs arising from VT retina at later stages of development and usually considered “late-born”, appear to be generated from E13 to 15, earlier than previously thought. Cyclin D2 was identified as a VT enriched gene at E11-14 (Wang and Mason, unpublished). Several lines of evidence suggest that CyclinDs can act as regulators of signaling pathways for cell fate decisions. In mice lacking Cyclin D2, fewer Zic2+ (ipsi) RGCs are apparent at E15, resulting in a smaller ipsilateral RGC projection through the optic chiasm. No compensatory increase of “late-born” contralateral RGCs, as measured by Islet2 expression, is observed in Cyclin D2 mutants; analyses are ongoing with other contralateral RGC markers. Thus, Cyclin D2, as suggested in other brain regions, may affect the timing and therefore production of specific RGC subtypes. Cyclin D2 might modulate ipsilateral RGC neurogenesis by affecting timing of birth and/or cell cycle length. To investigate theses possibilities, we are performing birthdating and cell cycle measurements in mice that lack Cyclin D2. Together, these results implicate that specification of ipsi versus contra RGC cell fate

occurs through distinct mechanisms involving neurogenic timing, leading to the expression of factors differentially regulating the phenotype of each subpopulation.

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Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NRF Grant 20110018358

BK21+ Program of Ministry of Education of Korea

Title: Anoctamin 1 regulates radial glia-like neural stem cells in mouse developing brain

Authors: G. HONG[1], J. JUNG[1], H. CHO[1], J. LEE[1], J. CHA[1], *U. OH[2,3];
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Abstract: Radial glia-like neural stem cells (RGLs) are known to play a critical role in key functions for brain development such as extension of their processes to pia mater and guidance of new born neurons to the proper region in developing cortex. Despite their significant roles, regulatory mechanisms on RGLs are not well understood. We found that a Ca2+ activated chloride channel, anoctamin 1 (ANO1) is essential for the RGL function. ANO1 was highly expressed in RGLs of mouse embryonic brain. The presence of Ca2+-activated currents were confirmed in RGLs. More importantly, knockdown of ANO1 or ANO1 inhibitors suppressed the process extension and protrusion of RGLs. Furthermore, this phenotype was reversed by overexpression of ANO1. More importantly, abnormal processes in RGLs were found in embryonic brain of Ano1-deficient mice. The size of the embryonic brain was significantly reduced compared to those of wild-type mice. BDNF is known to be essential for neurite outgrowth in RGLs. Surprisingly, BDNF activated ANO1 in RGLs. Thus, we conclude that ANO1 mediates Ca2+ dependent process extension of RGLs during embryonic cortical development.

Disclosures: **G. Hong:** None. **J. Jung:** None. **H. Cho:** None. **J. Lee:** None. **J. Cha:** None. **U. Oh:** None.

Poster

116. Embryonic Neurogenesis

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 NS032457

Paul G. Allen Family Foundation

NIH P30 HD18655

Title: Comparative analysis of cerebral cortical progenitor transcriptional heterogeneity at single-cell resolution

Authors: ***M. B. JOHNSON**[1], P. P. WANG[1], C. A. WALSH[1,2];
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Abstract: The human cerebral cortex depends for its normal development and size on a precisely controlled balance between self-renewal and differentiation of diverse neural progenitor cells. Due to their abundance in fetal human cortex compared to other species, a morphologically distinct progenitor subtype called basal or outer radial glia (ORG) has been suggested to be crucial to the evolutionary expansion of the human cortex. However, little is yet known regarding how ORG are molecularly or functionally distinct from other progenitor subtypes. We first combined progenitor subtype-specific sorting with transcriptome-wide RNA sequencing to identify genes enriched in human ORG compared to classic ventricular radial glial cells, including several transcription factors downstream of the key proneural regulator neurogenin, as well as numerous previously uncharacterized, evolutionarily dynamic long noncoding RNAs. Next, we performed single-cell transcriptional profiling of progenitors from human, mouse, and ferret, a gyrencephalic carnivore with abundant ORG, and found that the heterogeneity of progenitor transcriptional states correlates with size and complexity of the mature cerebral cortex. We find that a key feature of the diversity of human cortical progenitors is more frequent co-expression of progenitor and proneural transcriptional programs, suggestive of increased proliferation and self-renewal of neuronal lineage-committed precursors, which may contribute to increased neuronal numbers and cortical size. Finally, we have investigated the function of

human ORG-enriched genes in progenitor proliferation and differentiation using the ferret as a model system. We find that activation of the neurogenin pathway in ferret ventricular radial glial cells initiates a human ORG-like transcriptional program, and drives delamination of progenitors from the ventricular surface, a key step in the formation of basal ORG. Altogether, these studies provide new insights into the molecular drivers of progenitor diversity in human cortex and suggest genetic mechanisms that may have contributed to the evolution of human cortical complexity.

Disclosures: **M.B. Johnson:** None. **P.P. Wang:** None. **C.A. Walsh:** None.

Poster

116. Embryonic Neurogenesis

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 116.15/A15

Topic: A.01. Neurogenesis and Gliogenesis

Support: DFG: IS63/3-1

DFG: IS63/4-1

Title: Embryonic ablation of HCN/h current impedes development of the cerebral cortex by affecting stem cell proliferation and differentiation

Authors: *A. SCHLUSCHE[1], S. VAY[2], I. JAKOVCEVSKI[1], M. SCHROETER[2], A. RUEGER[2], D. ISBRANDT[1];
[1]ENP, DZNE & UzK Res. Team, Koeln, Germany; [2]Dept. of Neurology, Univ. Hosp. of Cologne, Cologne, Germany

Abstract: The development of the cerebral cortex is a complex process comprising cell division, differentiation, migration, axonal pathfinding, and synaptogenesis. Impairments in any of these steps may cause persistent structural and/or functional abnormalities. For all processes involved in cortical development the spontaneous activity including calcium waves in neurons and its precursor cells, mediated by intrinsic and extrinsic properties, is of great importance. The extrinsic properties mainly arise from stimulation of neurotransmitter receptors via synaptic or volume transmission, whereas the intrinsic biophysical properties of precursor cells and neurons are defined by their ion channel composition. The hyperpolarization-activated cyclic nucleotide-gated non-selective cation (HCN) channels mediating the h current (I_h) shape the biophysical properties of neurons throughout brain development. HCN channels consist of four subunits that

assemble into homo- or heteromeric tetramers. Our laboratory generated a transgenic mouse line expressing a dominant-negative HCN subunit (HCN-DN) that led to functional suppression of I_h independent of the endogenous subunit composition. By expressing the transgene under control of the EMX1 promoter I_h suppression starts at embryonal day 9.5 (E9.5) in a forebrain restricted manner. The functional ablation of I_h in early prenatal brain development resulted in a severe phenotype with pronounced microcephalus and reduced neonatal viability, but no alteration in cell proliferation or apoptosis at postnatal day 0 (P0) could be detected. *In utero* electroporation (IUE) of HCN-DN into the lateral ventricle performed at E15 and analyzed at E19 suggests no alteration in migration. To assess the impact of I_h blockade on differentiation *in vitro*, we pharmacologically blocked I_h using ZD7288 in rat cortical stem cells. The preliminary results show a decrease in proliferation and increased glial and reduced neuronal differentiation of stem cells upon I_h blockage. Our data support the hypothesis that I_h is an important intrinsic regulator of the proliferation and differentiation of neural progenitors, and that mutations in HCN channel genes could lead to severe brain malformations.

Disclosures: **A. Schlusche:** None. **S. Vay:** None. **I. Jakovcevski:** None. **M. Schroeter:** None. **A. Rueger:** None. **D. Isbrandt:** None.

Poster

116. Embryonic Neurogenesis

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 116.16/A16

Topic: A.01. Neurogenesis and Gliogenesis

Support: S2010-BMD-2336

RD12/0019/0013

Title: The GFAP gene involvement in neural stem cell differentiation

Authors: S. GARCIA-LOPEZ[1,2], A. GUTIERREZ-SEIJO[1,3], A. NELKE[1,2], M. P. PEREIRA[1,2], *A. MARTINEZ-SERRANO[1,2];
[1]Ctr. Mol. Biol. - S.O., Madrid, Spain; [2]Univ. Autonoma de Madrid, Madrid, Spain; [3]Univ. de Strasbourg, Strasbourg, France

Abstract: Introduction: Deep understanding of human neural stem cell (hNSC) biology is of primary interest to coax their potential into real cell replacement therapies. NSCs from several species, including humans, are limited in their proliferative capacity, entering senescence in

culture rather soon. In our lab this issue has been addressed by immortalizing the hNSC with the best immortalizing gene, viral myc (the p100gag-myc fusion protein between chicken myc and retrovial gag-pol). Still, another problem remains, the loss of their neurogenic potential with passaging. Previous work showed that this undesired drift could be overcome by the forced expression of Bcl-XL transgene. One of the consequences of the forced expression of Bcl-XL was a reduction of GFAP(+) cells during their differentiation, as determined by immunocytochemistry (ICC). Objective: The role of GFAP in hNSC differentiation has been largely underexplored. Based on previous studies, it can be postulated that the disruption of GFAP expression occurring during differentiation of hNSCs might reduce the potential for Glia generation, and, at the same time, enhance the Neurogenic potential. We examined this hypothesis in an *in vitro* model of immortalized hNSC. Methods & Results: Lentiviral particles encoding shRNAs targeting the GFAP gene have been used to silence protein expression will be monitored by WB and ICC. A MOI=5 was found to be enough to downregulate GFAP protein expression. In the differentiated hNSC samples we observed a significantly higher number of TH(+) cells (p-value < 0.05), indicating that the disruption of GFAP protein expression might promote acquisition of a neuronal fate. Conclusion: Present data on the silencing of GFAP gene expression allow to suggest that GFAP protein levels during hNSC differentiation play a role in neuronal commitment, differentiation or both.

Disclosures: S. Garcia-Lopez: None. **A. Gutierrez-Seijo:** None. **A. Nelke:** None. **M.P. Pereira:** None. **A. Martinez-Serrano:** None.

Poster

116. Embryonic Neurogenesis

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 116.17/A17

Topic: A.01. Neurogenesis and Gliogenesis

Title: Histone demethylase LSD1 controls Notch signaling and GABAergic neuronal differentiation in human neural stem cell

Authors: *K. HIRANO, M. NAMIHIRA;
Biomed. research institute, AIST, Tsukuba-Shi, Japan

Abstract: Neural stem cells (NSCs) are defined as cells that posses the ability to self renew and to differentiate into three major cell types, neurons, astrosytes and oligodendrocytes of central nerves systems (CNS). The fate specification of NSCs in cortex of embryonic mouse brain is controlled by epigenetic modifications such as DNA methylations and histone modifications.

However, the role of epigenetic modification in fetal NSCs during the development of primate cerebral cortex, which possesses more elaborate structures and functions compared with those of rodent, remains largely unknown. To address this issue, we investigate the function of epigenetic factors in human NSCs (hNSCs) that were derived from cerebral cortex of fetus and are expanded continuously in monolayer culture. We found that the inhibitor for lysine specific demethylase 1 (LSD1), which is a histone demethylase of mono- and di-methylated lysine 4 and 9 on histone H3, suppressed the GABAergic neuronal differentiation of hNSC. Furthermore, LSD1 inhibitor also upregulated several genes, including HES5 and HEYL, which are target genes of Notch signaling, at initial stage of neuronal differentiation. These results implied that histone demethylation by LSD1 play an important role in the development of GABAergic neuron through the regulation of Notch signaling in hNSCs.

Disclosures: K. Hirano: None. **M. Namihira:** None.

Poster

116. Embryonic Neurogenesis

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Program#/Poster#: 116.18/A18

Topic: A.01. Neurogenesis and Gliogenesis

Support: Swiss National Science Foundation

IGE3

Title: Becoming a new neuron in the cerebral cortex

Authors: *S. GOVINDAN, L. TELLEY, K. DEVARAJU, D. JABAUDON;
Univ. of Geneve, Geneve, Switzerland

Abstract: The cerebral cortex is composed of distinct types of neurons that assemble into specific circuits during development. While much progress has been made in understanding the type-specific gene expression of these neurons once they reach the cortex, their early post-mitotic, pre-circuit biology remains largely unexplored. To investigate these primordial differentiation processes, we developed a novel technology which allows *in vivo* labelling of select time- and phase-locked cohorts of newborn neurons right from the time of mitotic division. Through the real-time resolution provided by this approach, we were able to longitudinally characterize cell-type specific primordial transcriptional activity as it was unfolding in nascent neurons in the crucial 12 hours following cytokinesis. Our results provide a

first insight into the type-specific incipient genetic programs that direct the initial steps of neuronal differentiation and shed light on the transcriptional dynamics underlying cellular diversity and plasticity in the developing mammalian neocortex.

Disclosures: **S. Govindan:** None. **L. Telley:** None. **K. Devaraju:** None. **D. Jabaudon:** None.

Poster

116. Embryonic Neurogenesis

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Topic: A.01. Neurogenesis and Gliogenesis

Support: CONACyT Scholarship 233588

CONACyT Grant 219847

CONACyT Grant 160614

Title: Rod-enriched miRNAs and their expression during Müller glia-derived progenitors acquisition of photoreceptor phenotype

Authors: *H. QUINTERO[1], A. I. GOMEZ-MONTALVO[2], M. LAMAS[1];
[2]Farmacobiologia, [1]CINVESTAV, Ciudad DE Mexico, Mexico

Abstract: During retinal development, retinal progenitors differentiate to give rise to six neuronal and one glial type. This process requires a fine modulation in gene expression to confer identity and specific functions to each cell. MicroRNAs have the ability to regulate hundreds of genes at posttranscriptional level, arising as great candidates of differentiation regulation. Previous reports have shown the expression patterns of retinal miRNAs, however, is still necessary to identify patterns of cell-specific miRNAs in order to gain better understanding on miRNA regulation in each retinal subpopulation. Here, we identify a subset of rod-enriched miRNAs, which may be implicated in the acquisition of rod phenotype. For this purpose, we FACS-isolated CD73-positive rods and by miRNA arrays, we analyzed their profile expression; afterwards, using qPCR, we validated a subset of miRNAs as rod-enriched. Subsequently, we analyzed the expression levels of those miRNAs in a differentiation protocol towards rod phenotype. We found a preferential expression of miR-183, 182, 124, 9*, 181c and 301b* in rods. When Müller glia-derived progenitors (MGDP) are committed to early neuronal phenotypes, there is a upregulation of miR-124 expression and these levels of expression are sustained during acquisition of rod-like phenotype and are accompanied with the upregulation of

mir-9* and 181c. This work makes a progress in the establishment of miRNA signature in rods and suggests an important role of miRNAs during MGDP acquisition of rod-like phenotype

Disclosures: **H. Quintero:** None. **A.I. Gomez-Montalvo:** None. **M. Lamas:** None.

Poster

116. Embryonic Neurogenesis

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 116.20/A20

Topic: A.01. Neurogenesis and Gliogenesis

Title: Genetic disruption of O-GlcNAcase results in impaired neurogenesis in the developing mouse brain

Authors: ***C. LEE**, Y. YANG, J.-H. HUR, E.-K. KIM, P.-G. SUH;
UNIST, Ulsan, Korea, Republic of

Abstract: The monosaccharide N-acetyl-D-glucosamine can be added to serine or threonine residues of nuclear and cytoplasmic proteins to form O-linked N-acetylglucosamine (O-GlcNAc) as a post-translational modification. The addition and removal of O-GlcNAc, a dynamic cycling process, are catalyzed by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively. Importantly, it has been shown that O-GlcNAc level is closely related to a diverse physiological function such as cell division, cell signaling, metabolism, and apoptosis. O-GlcNAc is abundant in the brain and O-GlcNAc cycling is crucial for normal brain functions as well as for the etiology of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. However, the role of O-GlcNAc in the developing brain is not well known. To investigate the cellular and physiological roles of OGA associated with dysregulation of O-GlcNAcylation, we previously generated OGA homozygous null (OGA-/-) mice, which have constitutively elevated O-GlcNAc levels. Here we show that OGA gene was highly expressed in the ventricular zone (VZ) and subventricular zone (SVZ) of the developing brain. OGA-/- embryonic brain had a thinner cerebral cortex, most obvious in the cortical plate, and enlarged lateral ventricles. The number of dividing cells in the VZ was normal in the OGA-/- embryonic brain. OGA-/- mice exhibited reduced number of postmitotic neurons in the VZ of the ganglionic eminence whereas similar in the VZ of the dorsal pallium compared with WT. Together, these findings indicate that the absence of OGA leads to impaired neurogenesis in the developing brain in a region-specific manner.

Disclosures: **C. Lee:** None. **Y. Yang:** None. **J. Hur:** None. **E. Kim:** None. **P. Suh:** None.

Poster

116. Embryonic Neurogenesis

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: AHA Grant 13PRE17070078

Title: The exon junction complex controls embryonic neurogenesis through post-transcriptional gene regulation

Authors: *H. MAO[1], J. MCMAHON[2], E. MILLER[2], L.-J. PILAZ[2], D. SILVER[2];
[1]MGM, [2]Duke Univ., Durham, NC

Abstract: The cerebral cortex controls complex human traits such as memory and language. Neurons of the cerebral cortex are produced during embryonic neurogenesis. During this process, radial glia cells (RGCs) undergo self-renewal to expand their population and differentiation to produce neurons and intermediate progenitors (INPs), which also generate neurons. Disruption of embryonic neurogenesis underlies numerous neurodevelopmental disorders including microcephaly and autism. Therefore to understand the pathogenesis of these disorders it is critical to identify genes regulating neurogenesis. Our lab recently discovered that haploinsufficiency of either *Magoh* or *Rbm8a* causes microcephaly in mice. Both *Magoh* and *Rbm8a* mutants have defective RGC proliferation leading to fewer INPs and ectopic production of neurons that undergo extensive apoptosis. Magoh and Rbm8a form the exon junction complex (EJC) with Eif4a3. The EJC bind RNA and mediate RNA splicing, localization, translation, and non-sense mediated decay. Emerging evidence shows that mutations and copy number variations in EJC components are associated with human neurodevelopmental disorders. All EJC core components are highly expressed in the developing brain, indicating that these components may act together to regulate cortical development. To test this, we generated *Eif4a3* conditional mutants and found that haploinsufficiency of *Eif4a3* causes phenotypes similar to *Magoh* and *Rbm8a* mutants. Using deep RNA-sequencing of all three mutants, we identified overlapping RNA targets of the EJC, including transcription factors critical for cell fate determination, such as *Tbr2*. Using RNA immunoprecipitation (RIP) from embryonic cortices, we also discovered that core EJC components interact with RNA encoding critical neurogenesis genes. Our data argue that EJC components work together to regulate RGC proliferation and differentiation. Future proteomic, electroporation, and genomic experiments will further elucidate how EJC components impact brain development. Given the strong association of EJC mutations and human neurodevelopmental disorders, our studies are likely to be of clinical relevance.

Disclosures: **H. Mao:** None. **J. McMahon:** None. **E. Miller:** None. **L. Pilaz:** None. **D. Silver:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Packard Center for ALS Research

Travis Roy Foundation

Title: Newly identified developmental controls direct corticospinal motor neuron segmental targeting

Authors: ***V. V. SAHNI**[1], S. SHNIDER[2], J. SONG[2], J. MACKLIS[2];
[1]Stem Cell and Regenerative Biol., [2]Harvard Univ., Cambridge, MA

Abstract: In recent years, several key controls over specification and differentiation of neocortical projection neurons have been identified. These studies have focused on controls that specifically distinguish one subtype of neocortical projection neurons, e.g. corticospinal motor neurons (CSMN), from closely related corticothalamic projection neurons (CThPN) or intracortical callosal projection neurons (CPN). However, these broad subtypes of neurons exhibit known anatomical heterogeneity. For instance, CSMN somatotopically and precisely target specific segments along the rostrocaudal axis of the spinal cord, the molecular basis for which remains unknown. CSMN located rostro-laterally in the neocortex extend axons to proximal targets, such as the pons and medulla (i.e. hindbrain) and to the cervical cord (collectively "$CSMN_C$") and those located caudo-medially extend their axons more distally to the lumbar spinal cord ("$CSMN_L$"). We investigated the molecular determinants over CSMN segmental target specificity. We selectively isolated $CSMN_C$ and $CSMN_L$ at three critical developmental times, and identified differentially expressed genes between these two CSMN subpopulations during development. Using gain- and loss-of function analyses, we identified molecular controls that function to direct CSMN axon extension to appropriate levels of the spinal cord (short axon extension by $CSMN_C$ and long axon extension by $CSMN_L$). Interestingly, we find that some of these controls associate with subtype-specific forms of CSMN disease in humans. We focused our efforts on molecular controls specifically expressed by $CSMN_C$, since this population has undergone substantial expansion through evolution from rodents to primates

and humans, and potentially represents an evolutionarily "newer" CSMN sub-population. We identified a previously unstudied molecular control, that acts, at least in part, as a transcriptional regulator to suppress expression of $CSMN_L$ genes in $CSMN_C$, to limit their axon extension to the cervical cord. This provides a potential mechanism that might have allowed for the expansion of this subpopulation during evolution. Further, analysis of recently available transcriptome of the developing human brain indicates that its spatiotemporal expression is conserved in humans. Together, these newly identified controls constitute new, bi-directional mechanisms directing CSMN axonal targeting, and lay the foundation for more in-depth studies of mechanisms directing CSMN subtype-specification, as well as the development, regeneration, and evolution of precise corticospinal circuitry.

Disclosures: **V.V. Sahni:** None. **S. Shnider:** None. **J. Song:** None. **J. Macklis:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 117.02/A23

Topic: A.01. Neurogenesis and Gliogenesis

Title: D-serine accelerates neural stem cell differentiation and NMDA receptor expression

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

Abstract: Adult hippocampal neurogenesis is a process generating new functional neurons from adult neural stem/precursor cells (NSCs) that has a critical role in memory formation and cognitive functions. It is currently accepted that a combination of neurogenic niche signals and cell intrinsic programs orchestrate the transition from undifferentiated NSC state to a progenitor cell committed to the neuronal fate. In particular, the neurogenic process depends on the expression of the basic helix-loop-helix (bHLH) transcription factors, finally resulting in the activation of a neuronal gene cascade. Compelling evidence shows that among the molecules released by the neurogenic niche, D-serine represents an important co-agonist of N-methyl-D-aspartate (NMDA) receptors whose activation has been shown to enhance NSC proliferation *in vitro* and adult neurogenesis *in vivo*. Moreover, abnormal levels of D-serine have been reported in aging and Alzheimer's disease, conditions in which dysregulation of glutamatergic system is associated with impaired neurogenesis. Here we investigated the effects of exogenous administration of D-serine on cultured adult hippocampal NSCs to identify the molecular mechanisms underlying its neurogenic effect. To this aim we performed real-time RT-PCR on

cellular extracts obtained from NSC cultured under both proliferative and differentiating conditions. Our findings demonstrated that addition of D-serine to the culture medium significantly increased the proliferation of undifferentiated NSCs and this effect was associated with a marked enhancement (+217% of control cells, $p<0.05$) of the pro-proliferative Hes1 gene expression. In differentiating NSCs cultured in the presence of D-serine expression of the determination gene Mash1 was higher than controls (+50%, $p<0.05$) and it reached a peak circa 24h earlier, thus suggesting early cell commitment to the neuronal fate. It has been recently demonstrated that activation of NMDA receptors increases proliferation and differentiation of NSCs (Joo et al., 2007). In light with this evidence we evaluated whether the addition of D-serine in NSC culture medium also affects the expression of NMDA receptor subunits. Our real time PCR data demonstrated an earlier and increased expression of the NR1, NR2A and NR2B subunits (+300%, +500%, +75% respectively, $p<0.05$) in differentiating NSCs cultured in the presence of D-serine. Collectively, these results suggest that D-serine positively modulates neurogenesis by accelerating NSC commitment to the neuronal phenotype and the expression of NMDA receptor subunits.

Disclosures: **S. Barbati:** None. **L. Leone:** None. **M. D'Ascenzo:** None. **A. Mastrodonato:** None. **C. Grassi:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R15HD071799-01

Title: Role of glial cell remodeling during peripheral nerve reorganization in *Drosophila*

Authors: A. SUBRAMANIAN[1], *J. J. FERNANDES[2];
[1]Biol., [2]Miami Univ., Oxford, OH

Abstract: Remodeling of the larval nervous system during development is necessary for the development of adult specific behaviors in the fruit-fly *Drosophila* melanogaster. During metamorphosis, five pairs of abdominal nerves fuse together to form a terminal nerve trunk (TNT) as they exit the CNS. The nerves later defasciculate from the TNT at appropriate segmental levels to innervate the body wall. Glial cells are likely to play a significant role in this process, as we have determined by following the remodeling of four ensheathing layers. The

most external layer, the neural lamella and the most internal wrapping glial layer are absent during the entire process of TNT formation. However, the two other glial layers- the perineural glia (PG) and the sub-perineural glia (SPG) persist during this process. Confocal microscopy analyses have been substantiated by transmission electron microscopy (TEM) to study the ultrastructural changes that take place during peripheral nerve reorganization. The present study focuses on the role of the PG and SPG glial layers in TNT formation. A three-fold increase in the glial population was observed during the first day of metamorphosis (25% of development), when 75% of the cells comprises of perineural glia. Induction of perineural glial cell death by targeting Diphtheria Toxin (DT), results in abnormal defasciculation patterns in 100% of animals (n=15). TEM studies are currently underway to identify the nature of ultrastructural changes in these mutants. Cells of the SPG layer will also be manipulated by targeting Diphtheria Toxin (DT), and the TNT will be examined using confocal analyses and TEM. The study of glial remodeling in *Drosophila* will lay the groundwork for future studies on the role of glia- neuron communication during TNT formation, and could lead to the establishment of a model system to study gliopathies.

Disclosures: **A. Subramanian:** None. **J.J. Fernandes:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 117.04/A25

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Cholinergic neuron systems indirectly impact the growth of young mice

Authors: ***H. ZHU**[1], Y. LU[2];
[1]Phisology, Huazhong Univ. of Sci. and Technol., Wuhan, China; [2]Pathophysiology, Tongji Med. Col., WUhan, China

Abstract: abstract:cholinergic neuron systems which regulate the acetylcholine signal underlies specific aspects of cognitive functions and behaviors, and involved in the pathophosiology of some neuropsychiatric disorders. We focused on the inducible cholinergic system inhibition model based on transgenic mice and a nobel phenomenon was observed during our previous experiments: intensively inhibited individual showed smaller quantity of weight growth ranged from induction period. So we accumulate abundant (n=396) data of induced transgenic model (inductive agent tamoxifen) and we first classifying the inhibition degree as control (without inhibition gene), slight(less than 30% cholinergic neuron inhibited), medial(30%--60%inhibited

), intensive(more than 60% inhibited). There exists no differences in weight growth between those groups except intensive group which in analogous original weight with other groups manifest remarkable lower weight growth compared with them. The phenomenon showed than there might be a indirect pathway of cholinergic system participated in the growth and development of mice which might be compensatory via other neuronal system when cholinergic system is partly restricted. Yet highly restriction to the function of cholinergic system would interfere with the weight growth of young mice which indicate that cholinergic system could indirectly impact the growth of young mice. Keywords: cholinergic system, tamoxifen, weight growth

Disclosures: H. Zhu: None. **Y. Lu:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Program#/Poster#: 117.05/A26

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01 NSO41489

Title: Shh and Wnt signaling in human fetal brain development

Authors: *F. MEMI, J. ORTEGA, N. ZECEVIC;
Neurosci., Uconn Hlth. Ctr., Farmington, CT

Abstract: The embryonic patterning and development of the CNS rely on the combinatorial effect of secreted factors, such as sonic hedgehog (Shh) and wingless (Wnt) that establish gene regulatory networks which in turn, will guide the basic developmental processes including cell proliferation and specification. These molecules display a distinct expression pattern; Wnts are expressed dorsally and guide the neuronal progenitors into glutamatergic fate whereas the ventrally secreted Shh specifies GABAergic inhibitory cell population. Thus, a fine co-ordination of Wnt and Shh signaling pathways is required for the proper development of the forebrain. Despite the importance of these molecules, little is known about their expression pattern during the human fetal brain development. A possible reason is the limited availability of human embryonic tissue and the difficulty to detect secreted factors by immunohistochemistry (IHC). Thus, we used *in situ* hybridization (ISH) to determine the spatiotemporal expression pattern of Shh and Wnts in a wide range of developmental stages of human fetal brain (15-24gw). Combining ISH with IHC, we identified the Shh and Wnt-expressing cells in the human

dorsal and ventral telencephalon. We also assessed the expression pattern of the major members of Shh and Wnt signaling, such as the receptors and the downstream effectors that lead to transcriptional activation. Interestingly, we detected differences between mouse and human that can be due to the species-specific cis-regulatory elements controlling gene expression. Evolutionary changes in cis-regulatory elements have been associated with the presence of a primate-specific interneuron progenitor pool in the cortical VZ/SVZ, which we previously showed, that can be modulated by Shh (Radonjic et al., 2014). Here, to study the role of Wnt signaling in the specification of human telencephalic progenitors, we performed pharmacological *in vitro* experiments in enriched radial glial cell (RGC) cultures isolated from fetal human dorsal and ventral brain. We studied the effects of Wnt agonist (CHIR99021) and antagonist (XAV939) on cell proliferation and differentiation of human RGCs and found that, in accordance with mouse studies, activation of Wnt signaling in human RGCs mainly promotes increased cell proliferation and induction of glutamatergic neurogenesis.

Disclosures: **F. Memi:** None. **J. Ortega:** None. **N. Zecevic:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 117.06/A27

Topic: A.01. Neurogenesis and Gliogenesis

Support: UCLA Asst Prof Start Up Funds

Title: Hyaluronic acid-based hydrogels designed to direct oligodendrocyte differentiation

Authors: ***S. K. SEIDLITS**, J. LIANG, A. EHSANIPOUR, C. M. WALTHERS; Bioengineering, UCLA, Los Angeles, CA

Abstract: Introduction: Many disorders of the central nervous system (CNS) cause extensive death of highly specialized cells, such as motor neurons and oligodendrocytes. Although neural stem/progenitor cells (NS/PCs) are capable of differentiating into these specialized cells, this process is inefficient and has failed to mediate substantial repair of CNS function. In order to effectively employ cell-based therapies clinically in the CNS, it is imperative to develop strategies to direct differentiation of large numbers of NS/PCs. NS/PCs can differentiate into neurons, astrocytes and oligodendrocytes; however, this work focuses on the process of oligodendrocyte lineage (OL) differentiation. We posit that coordination of extracellular matrix and growth factor cues is necessary for OL maturation. Thus, we have developed 3D, culture

microenvironments in which the combinatorial effects of soluble and substrate-mediated cues on OL differentiation can be evaluated independently and in combination. Materials and Methods: Sodium hyaluronate (~700 kDa) was modified with thiol groups and cross-linked with 20 kDa 4-arm PEG-maleimide. Cysteine-terminated, integrin-binding peptides were added to facilitate NS/PC attachment and differentiation. NS/PCs derived from human H9 embryonic stem cells were cultured in 3D, hyaluronic acid (HA) hydrogels for 3 weeks in media formulated for stem cell proliferation or OL differentiation. After 3 weeks, cell/hydrogel constructs were fixed and characterized by immunolabling of OL markers. Results and Discussion: After culturing for 3-weeks in 3D, HA hydrogels in differentiation media, NS/PCs showed increased expression in OL markers (NG2, PDGR-alpha, CNPase) and decreased expression of markers for pluripotency (nestin, Sox2) than those cultured on laminin-coated glass coverslips in otherwise identical conditions. Addition of differentiation promoting factors to the medium further enhanced this effect. Cells grown in HA hydrogels also expressed higher levels of the HA receptor CD44 than those grown on laminin-coated coverglasses. Finally, proliferation of NS/PCs embedded in HA hydrogels was dependent on the identity of adhesive peptides incorporated. Specifically, peptides containing the RGD binding motif increased proliferation. Conclusions: These novel hydrogels mimic the HA-rich, native spinal cord environment, can be easily modified with biomolecules, such as adhesive peptides, and provide a means for biocompatible, 3D culture. Results demonstrate that 3D culture of human NS/PCs in HA-based hydrogels increases efficiency of OL differentiation compared to standard, 2D cultures.

Disclosures: **S.K. Seidlits:** None. **J. Liang:** None. **A. Ehsanipour:** None. **C.M. Walthers:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

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Title: Single-cell analysis reveals that endogenous retrotransposons generate somatic mosaicism in the neurons and non-neuronal cells

Authors: *J. A. ERWIN[1], A. C. M. PAQUOLA[1], T. SINGER[1], M. NOVOTNY[2], I. GALLINA[1], C. BUTCHER[1], J. R. HERDY[1], R. LASKEN[2], A. R. MUOTRI[3], F. GAGE[1]; [1]LOG-G, The Salk Inst. For Biol. Studies, La Jolla, CA; [2]J. Craig Venter Inst., La Jolla, CA; [3]Univ. of California San Diego, La Jolla, CA

Abstract: It has long been thought that neuronal genomes are invariable; however, recent studies have demonstrated that mobile elements actively retrotranspose during neurogenesis, thereby creating genomic diversity between neurons. In addition, mounting data demonstrate that mobile elements are misregulated in certain neurological disorders, including Rett syndrome and schizophrenia. The unique composition of genetic mosaicism present in the brain may contribute to disease and also the behavior differences observed between genetically identical organisms. Many questions remain regarding the regulation of retrotransposition, the full characterization of other mobile elements and the functional significance of retrotransposition during neurogenesis. Because each individual neuron has the potential to have a unique genome, single-cell approaches are essential to measure and observe this genomic diversity, which is obscured in bulk samples. I will present data using single-cell genome and transcriptome sequencing to characterize the nature and regulation of neuronal genome mosaicism. In order to address the question whether somatic retrotransposition occurs in other tissues and, more generally, how it impacts human development and function, we developed a targeted sequencing approach to identify Alu and L1 retrotransposition events in single cells and bulk tissues. We applied this method to cortex, hippocampus, heart and liver postmortem samples from four non-diseased young adults. We confirm that somatic L1 retrotransposition occurs in hippocampal neurons, and we also found evidence of somatic Alu retrotransposition in the liver as well as somatic L1 retrotransposition in non-neuronal cells in the cortex. We did not observe any cell-type specific difference in estimated rates of retrotransposition. These findings suggest that somatic retrotransposition is not restricted to neurons but occurs as part of the normal condition of human somatic cells. We also apply single-cell transcriptomic approaches to quantify the level of diversity present in cells with retrotransposition and identify regulators of somatic retrotransposition.

Disclosures: **J.A. Erwin:** None. **A.C.M. Paquola:** None. **T. Singer:** None. **M. Novotny:** None. **I. Gallina:** None. **C. Butcher:** None. **J.R. Herdy:** None. **R. Lasken:** None. **A.R. Muotri:** None. **F. Gage:** None.

Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIMH R01MH094653

P41 GM103533

Title: Long non-coding RNA transcriptional mechanisms in GABAergic interneuron precursors

Authors: I. CAJIGAS[1], D. LEIB[1], J. COCHRANE[2], H. LUO[1], K. SWYTER[1], S. CHEN[1], J. YATES[3], R. KINGSTON[2], *J. D. KOHTZ[1];
[1]Pediatrics/Developmental Biol., Northwestern University/Stanley Manne Children's R, Chicago, IL; [2]Mol. Biol., Harvard Univ., Boston, MA; [3]Scripps, LaJolla, CA

Abstract: Transcription-regulating long non-coding RNAs (lncRNAs) have the potential to control site-specific gene expression of thousands of targets. Previously, we showed that *Evf2*, the first described ultraconserved lncRNA, increases association of transcription activators (DLX homeodomain proteins) to key DNA enhancers, but represses gene expression. Loss of Evf2 results in GABAergic interneuron defects, reducing GABAergic synaptic inhibition in the adult hippocampus. Here, mass spectrometry shows that the *Evf2*/DLX1 ribonucleoprotein (RNP) contains SWI/SNF related chromatin-remodelers, Brahma related gene 1 (BRG1, SMARCA4) and Brahma-associated factor (BAF170, SMARCC2) in developing forebrain. The importance of BRG1/RNA and BRG1/homeodomain interactions in neurodevelopmental disorders is underscored by the finding that mutations in Coffin Siris Syndrome, a human intellectual disability disorder, localize to the BRG1 RNA binding and DLX1 binding domains. *In vitro* studies show that both RNA/BRG1 binding and RNA inhibition of BRG1 ATPase/remodeling activity is promiscuous, suggesting that context is a critical factor in RNA-dependent chromatin remodeling inhibition. Together, these experiments support a model where RNAs convert an active enhancer to a repressed enhancer by directly inhibiting chromatin-remodeling activity, revealing a novel regulatory mechanism in GABAergic interneuron precursors.

Disclosures: I. Cajigas: None. **D. Leib:** None. **J. Cochrane:** None. **H. Luo:** None. **K. Swyter:** None. **S. Chen:** None. **J. Yates:** None. **R. Kingston:** None. **J.D. Kohtz:** None.

Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: FAPESP

CAPES

CNPq

Title: Dopaminergic neuronal differentiation modifies PrPC expression

Authors: ***M. H. LUZ**, M. A. P. DA SILVA, A. M. XAVIER, I. GLEZER, K. S. LEE; Bioquímica / Biologia Mol., Univ. Federal De São Paulo - UNIFESP, Sao Paulo, Brazil

Abstract: Cellular prion protein (PrP^C) is a glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein that plays several roles in cellular homeostasis and neuroprotection. However, its conformational change may lead to loss of function and accumulation of protein aggregates. It is plausible to speculate that the conformation of PrPC can be easily altered by reactive oxygen species (ROS) due to its high expression level and the presence of repetitive octapeptide sequences (PHGGGWGQ) which are enriched of amino acids that are more susceptible to oxidation. Thus, PrPC can neutralize ROS, but the modified PrPC would be degraded. ROS are produced as byproduct in several metabolic pathways. Oxidative metabolites can also be generated from autoxidation of dopamine (DA) or from enzymatic deamination. Therefore, our purpose was to characterize the PrP^C turnover during the N2a differentiation into dopaminergic neurons. The differentiation was effectively accomplished using 1 mM dibutyryl cAMP, which induced the expression of tyrosine hydroxilase (TH) and the production of DOPAC, a dopamine metabolite. Despite the effective differentiation, we could not observe enhanced production of ROS, which was assessed by DCFDA and CellRox. However, the differentiation reduced the level of total PrPC as well as the membrane fraction. This reduction was not triggered by increased degradation, indicating that the PrPC expression was down-regulated while TH expression was induced by dibutyryl cAMP. Knockdown of PrP^C by shRNA also increased TH levels. Our data suggest that PrP^C participates in regulation of TH expression as its suppressor.

Disclosures: **M.H. Luz:** None. **M.A.P. da Silva:** None. **A.M. Xavier:** None. **I. Glezer:** None. **K.S. Lee:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Academy of Finland (EB, PH, KK)

Letten Foundation (KK)

Title: A neuron-specific carbonic anhydrase, CA VII, binds to filamentous actin and affects neuronal morphology

Authors: *E. M. RUUSUVUORI[1], E. BERTLING[2], E. KREMNEVA[3], M. VIRTANEN[4], L. VUTSKITS[4], P. LAPPALAINEN[3], P. BLAESSE[5], P. HOTULAINEN[2], K. KAILA[2]; [1]Univ. Helsinki, FIN-00014, Finland; [2]Neurosci. Ctr., FIN-00014, Finland; [3]Inst. of Biotech., Helsinki, Finland; [4]Dept. of Fundamental Neurosciences, Univ. of Geneva Med. Sch., Geneva, Switzerland; [5]Inst. of Physiol., Münster, Germany

Abstract: Accumulating evidence from work on ion-regulatory proteins (IRPs), such as ion transporters and carbonic anhydrases (CAs), show that they are of fundamental importance in a wide spectrum of molecular and cellular mechanisms controlling brain development, plasticity, and disease[1]. We have recently shown that the neuron-specific chloride transporter KCC2 is, in addition to its well-known role in chloride extrusion, a multifunctional protein which has an important structural factor in the generation of dendritic spines in cortical neurons[1,2]. The present work focuses on another neuron-specific IRP, CA isoform VII (CA VII) and demonstrates that in addition to its previously known role in neuronal pH regulation[3], and thereby in the modulation of ionic plasticity in GABAergic synapses, CA VII has a structural role via direct interaction with filamentous actin (F-actin). We have shown that mouse hippocampal neurons are endowed with two catalytically highly active cytosolic CA isoforms (VII and II) and that these isoforms are fully responsible for CA-dependent modulation of somatic and dendritic pH[3]. In the present study, the subcellular localization of CA VII and II was studied by overexpressing them in rat cortical neurons *in vitro* and *in vivo* and in cultured fibroblasts. In cell cultures, CA VII, but not CA II, showed strong co-localization with F-actin. Direct interaction of CA VII and actin was confirmed with actin pull-down assay. In cortical neurons, both in cultures and *in vivo*, CA VII localized strongly to dendritic spines. To address the CA VII-actin interaction we did molecular modelling and constructed CA VII mutants. We identified domains that were crucial for CA VII's interaction with F-actin and probably for its spine targeting. Spine density analysis of WT

and CA7 KO mouse cortical layer 2/3 neurons, performed as described before[2], showed increased spine number in the CA VII KO mice. Together with the distinctive over-expression phenotype of CA VII transfected neurons in WT mice, these results provide further support to a novel morphogenic role of CA VII in neurons. References: 1. Kaila K, Price TJ, Payne JA, Puskarjov M, Voipio J (2014) *Nat Rev Neurosci* 15:637-654 2. Puskarjov M, Seja P, Heron SE, Williams TC, Ahmad F, Iona X, Oliver KL, Grinton BE, Vutskits L, Scheffer IE, Petrou S, Blaesse P, Dibbens LM, Berkovic SF, Kaila K (2014) *EMBO Rep.* 15:723-729 3. Ruusuvuori E, Huebner AK, Kirilkin I, Yukin A, Blaesse P, Helmy M, Jung Kang H, El Muayed M, Christopher Hennings J, Voipio J, Sestan N, Hübner CA, Kaila K (2013) *EMBO J.* 32: 2275-86

Disclosures: E.M. Ruusuvuori: None. **E. Bertling:** None. **E. Kremneva:** None. **M. Virtanen:** None. **L. Vutskits:** None. **P. Lappalainen:** None. **P. Blaesse:** None. **P. Hotulainen:** None. **K. Kaila:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: IRP/NIDA/NIH

Title: Sumoylation of sigma-1 receptor chaperones at the nuclear pore complex may relate to a potential role of sigma-1 receptors in the nucleo-cytoplasmic transport of macromoleculars

Authors: *P.-T. LEE, T.-P. SU;
Cell. Pathobiology Section, Integrative Neurosci. Res. Br., IRP/NIDA/NIH, Baltimore, MD

Abstract: Sigma-1 receptors (Sig-1Rs) are ligand-regulated membrane proteins which function as molecular chaperones in the endoplasmic reticulum (ER) that plays important roles in the proper folding and quality control of proteins. Our recent works indicate that Sig-1Rs not only exist at the ER and plasma membrane but also exist in the nuclear envelope (NE). NE is a contiguous structure of the ER network that provides bidirectional nucleo-cytoplasmic transport of macromolecules through the nuclear pore complex (NPC). The NPC is comprised of multiple copies of 30 different proteins that facilitate transport of karyopherin-cargo complexes through the nuclear pore. We test the hypothesis that Sig-1Rs at the NE may at least in part associate with the NPC and thus participate in the functioning of NPC. We found that Sig-1Rs exist in the isolated fractions of NE and NPC by using nuclei sub-fractionation procedures. Sig-1Rs also

partly co-localize and interact with NPC as seen from the immunostaining and immunoprecipitation assays. Sig-1R depletion in HeLa cells slightly attenuates the nuclear import of the nuclear factor of activated T cell (NFAT), suggesting that Sig-1Rs are involved in the control of nuclear transport. The post-translational modifications (PTMs) of proteins may also occur as they are translocated between the cytoplasm and the nucleus. The modification includes SUMOylation which covalently adds small ubiquitin-related modifier (SUMO) to lysine residue of its substrates by an enzymatic cascade. It has been shown that SUMO-conjugating enzyme UBC9 as well as the de-SUMO enzyme sentrin-specific proteases (SENPs) is also localized in the cytoplasmic filaments of NPC and NE. The results from our immmunoprecipitation assay and *in vitro* pull-down assay revealed that Sig-1Rs interact with UBC9. Those results together suggest that Sig-1R may be modified by SUMO. To investigate the PTM of Sig-1R by SUMO, we generate recombinant glutathione S-transferase (GST)-tagged proteins including full-length and three truncated fragments of mouse Sig-1R. The *in vitro* SUMOylation assay revealed that full-length (amino acids 1 to 223) and truncated (amino acids 80 to 173) of recombinant Sig-1Rs can be conjugated by SUMO-1, which belongs to a subtype of SUMO family. In addition, mutation of the lysine residue of Sig-1R at amino acid 142 to arginine completely abolished the conjugation by SUMO-1 using either *in vivo* or *in vitro* SUMOylation assay. In this study, we demonstrated a novel PTM of Sig-1R chaperone that may occur at the NPC. Our results suggest that SUMOylation status of Sig-1R at the NPC may play potential roles in the nucleo-cytoplasmic transport of macromolecules.

Disclosures: **P. Lee:** None. **T. Su:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Support: KAKENHI 23115102

KAKENHI 23111516

KAKENHI 23700447

JST CREST

Title: DNA polymerase β activity in neural progenitors is required for postmitotic neuronal survival in the developing cortex

Authors: K. ONISHI, N. SUGO, S. TOYODA, T. HIRAYAMA, T. YAGI, *N. YAMAMOTO; Osaka Univ, Grad Sch. Frontier Biosci, Suita, Osaka, Japan

Abstract: DNA repair is essential to maintain the genome stability in all cell types including neuronal cells. It has been demonstrated that lack of DNA polymerase β (Polβ), an enzyme of the base excision repair (BER) pathway, results in neuronal apoptosis in the developing cortex (Sugo et al., *EMBO J*, **19**, 1397-1402, 2000). However, how Polβ functions on cortical cells during development is totally unknown. We addressed this issue by investigating phenotypic differences between two distinct forebrain-specific Polβ conditional knockout mice, Emx1-CRE/Polβ$^{fl/fl}$ and Nex-CRE/Polβ$^{fl/fl}$ mice. In Emx1-CRE/Polβ$^{fl/fl}$ embryos, in which Polβ function is inactivated in all excitatory cortical cells including progenitor cells, the majority of cortical plate (CP) cells but not ventricular zone (VZ) cells showed cleaved caspase3-positive apoptosis in E14.5, similarly to the phenotype of Polβ$^{-/-}$ embryos. In Emx1-CRE/Polβ$^{fl/fl}$ embryos, γH2AX-foci immunopositivity was also increased in the majority of both CP and VZ cells, indicating the occurrence of DNA double-strand breaks (DSBs). In contrast, E14.5 Nex-CRE/Polβ$^{fl/fl}$ embryos, in which Polβ becomes inactivated after the final mitosis, showed such abnormalities in neither CP nor VZ cells. To further investigate the influence by the lack of the BER pathway, cultured Polβ-deficient cortical progenitors were treated with a base-damaging agent, methyl methanesulfornate. This treatment increased occurrence of not only DNA single-strand breaks but also DSBs during S phase. Taken together, our results suggest that Polβ contributes to neuronal survival in the developing cortex by reducing the catastrophic risk of conversion from a base damage into DSB in neural progenitors.

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Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: National Research Foundation grant of Korea (No. 2011-0028317)

Title: Overexpression of human gata-1 and gata-2 interferes with spine formation and produces depressive behavior in rats

Authors: *S. KO, ESQ[1], M. CHOI[2], S. WANG[1], S. LEE[1], R. S. DUMAN[3], H. SON[2]; [1]Hanyang Univ., Seoul, Korea, Republic of; [2]Col. of Medicine, Hanyang Univ., Seoul, Korea, Republic of; [3]Yale Univ. Sch. of Med., New Haven, CT

Abstract: Functional consequences to which vertebrate GATA transcription factors contribute in the adult brain remain largely an open question. The present study examines how human GATA-1 and GATA-2 (hGATA-1 and hGATA-2) are linked to neuronal differentiation and depressive behaviors in rats. We investigated the effects of adeno-associated viral expression of hGATA-1 and hGATA-2 (AAV-hGATA1 and AAV-hGATA2) in the dentate gyrus (DG) of the dorsal hippocampus on dendrite branching and spine number. We also examined the influence of AAV-hGATA1 and AAV-hGATA2 infusions into the dorsal hippocampus on rodent behavior in models of depression. Viral expression of hGATA-1 and hGATA-2 cDNA in rat hippocampal neurons impaired dendritic outgrowth and spine formation. Moreover, viral-mediated expression of hGATA-1 and hGATA-2 in the dorsal hippocampus caused depressive-like deficits in the forced swim test and learned helplessness models of depression, and decreased the expression of several synapse-related genes as well as spine number in hippocampal neurons. Conversely, shRNA knockdown of GATA-2 increased synapse-related gene expression, spine number, and dendrite branching. The results demonstrate that hGATA-1 and hGATA-2 expression in hippocampus is sufficient to cause depressive like behaviors that are associated with reduction in spine synapse density and expression of synapse- related genes.

Disclosures: S. Ko: None. **M. Choi:** None. **S. Wang:** None. **S. Lee:** None. **R.S. Duman:** None. **H. Son:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: WELFund Grant

University of Hartford

Title: The Erk5 signaling cascade and its possible role in modulating nurr1 transcriptional activity in the mouse developing midbrain

Authors: *P. SACCHETTI[1], E. KARHAN[2], G. DOWNING[2], C. CONNELLY[1], Q. LI[3]; [1]Biol. Sci., [2]Master's Program in Neurosciences, Univ. of Hartford, West Hartford, CT; [3]Mount Holyoke Col., South Hadley, MA

Abstract: The nuclear receptor nurr1 (NRA4A2) is an essential player in the development and maintenance of gene expression in developing midbrain dopamine neurons and seems to confer neuroprotection to these neurons. Differently from other members of the nuclear receptor superfamily of transcription factors, nurr1 transcriptional activity does not seem to be under the control of a ligand. In addition, the quest for nurr1 modulators has provided no specific insights into the mechanisms governing the activation and inactivation of this important developmental transcription factor. However, the identification of regulators would be extremely useful to clarify the mechanisms by which nurr1 confers neuroprotection to dopamine neurons. The Extracellular Signal-Regulated Kinase 5 (Erk5) signaling pathway is implicated in neuronal differentiation and cell survival in response to environmental stressors in different cell types. Interestingly, Erk5 can interact and enhance the activity of nurr1 *in vitro*. However, no clear evidence exists of the presence of Erk5 in midbrain neurons. In the present study, we wanted to address the possible implication of the Erk5 signaling cascade in the development of midbrain dopamine neurons in mice. We have undertaken the systematic analysis of mRNA and protein expression of Erk5 and the main components of the signaling cascade in the midbrain during dopamine neuronal development. Preliminary data suggests that some components of the Erk5 signaling cascade are expressed during midbrain development. The goal is to determine if nurr1 and Erk5 are coexpressed in developing dopamine neurons to further explore the role of this signaling cascade in regulating nurr1 transcription activity and mediating neuroprotection.

Disclosures: **P. Sacchetti:** None. **E. Karhan:** None. **G. Downing:** None. **C. Connelly:** None. **Q. Li:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: CONACYT 179234

PAPIIT-DGAPA, UNAM IN206213

Title: Role of reactive oxygen species and NADPH oxidases in the action of NMDA and high potassium in developing cerebellar granule neurons

Authors: *J. MORAN, M. OLGUIN-ALBUERNE, F. GOMEZ-FERNANDEZ, R. IBARRA-GARCIA PADILLA, S. GONZALEZ-MARTINEZ;
Natl. Univ. of Mexico, Apdo. Postal 70-253, Mexico

Abstract: Increasing evidence reveals a fundamental role of reactive oxygen species (ROS) in physiological and developmental processes. However, the mechanisms underlying such influence remain unclear. NADPH oxidases (NOX) are thought to be one of the main sources of these ROS, and the inhibition of NOX negatively affects neural differentiation, axon growth, and neurogenesis. On the other hand, in previous studies we demonstrated that depolarizing conditions (25 mM KCl, K25) and N-methyl-D-aspartate (NMDA) promote neuronal survival and maturation of cultured rat cerebellar granule neurons (CGN). It has also been shown that NMDA receptors are implicated in neuronal migration of CGN. Besides, it has been proposed that the activation of the NMDA receptor produces ROS from NOX2. Thus, all these evidences suggest that ROS and NOX may play an important role as mediators of some trophic conditions such as potassium depolarization and NMDA receptor activation during cerebellar development. In this work, we evaluated whether ROS and NOX are involved in the trophic actions of NMDA and K25 in CGN. In order to evaluate this possibility, we cultured CGN from 0 to 7 days *in vitro* (DIV) under physiologic concentrations of KCl (5 mM, K5) with or without 150 μM NMDA (NMDA) or depolarizing concentrations of KCl (25 mM, K25) that are believed to mimic the presynaptic trophic action on CGN. Cells were treated also with the antioxidants MnTMPyP and EuK-134, as well as the NOX inhibitors apocynin and AEBSF, and cell ROS levels, cell viability and neuronal migration were measured. Under these conditions, we observed that CGN cultured under all three conditions showed a marked increase in the ROS levels during 0-2 DIV, but from 3-5 DIV, CGN in K25 reduced ROS levels, while K5 and NMDA maintained a relatively high concentration of ROS. Under these conditions, K25 markedly reduced cell death that was observed at 3-7 DIV under K5 or NMDA conditions. Both, MnTMPyP and apocynin, reduced the effect of K25 on CGN survival. In addition, only NMDA significantly increased the rate of neuronal migration and all antioxidants and NOX inhibitors tested markedly reduced CGN migration. These results suggest that ROS could play a critical role in some developmental processes of CGN and that the trophic actions of potassium depolarization and NMDA receptor activation seem to be mediated by ROS probably produced by a NOX. At early times of development, the production of ROS seems to be critical for neuronal maturation. During a second stage of CGN development, ROS levels reach a low basal state to allow cell survival, otherwise this would lead to the activation of the programmed cell death

Disclosures: J. Moran: None. **M. Olguin-albuerne:** None. **F. Gomez-fernandez:** None. **R. Ibarra-garcia padilla:** None. **S. Gonzalez-martinez:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Title: Isoform specific regulation of GABAergic motor neuron development in *C. elegans*

Authors: *R. CAMPBELL[1], W. W. WALTHALL[2];
[2]Biol., [1]Georgia State Univ., Atlanta, GA

Abstract: The generation of complexity and diversity in the nervous system throughout evolution has been a fundamental area of research in neuroscience. It has been proposed that differences in gene regulation (epigenetic, transcriptional, post transcriptional, translational and post translational) could be responsible for the complexity and diversity in nervous systems. More recently evidence has shown that there is a trend in increased splice variants in the nervous system from reptiles to primates during development. Suggesting that splice variants may allow for increased complexity and diversity during evolution. However, due to the complexity of the nervous system, testing these hypotheses is difficult. Using the nematode, Caenorhabditis elegans, these hypotheses can be examined at a single neuron level. Previous studies have shown that evolutionarily conserved transcription factors are both necessary and sufficient for the post mitotic differentiation of different motor neurons. By examining the transcriptional regulation of one such factor, the COUP-TFII homolog, UNC-55, mechanisms of both differential transcription and isoform specific regulation of the expression of UNC-55 can be examined between motor neuron subtypes. Utilizing bioinformatics, putative transcription factor binding sites (cis sites) of the MEIS domain transcription factor, UNC-62 were found on the unc-55 promoter. Different classical mutant alleles and newly generated CRISPR/Cas9 alleles of UNC-62 repress, activate or have no effect on UNC-55::GFP expression. Dissection of the unc-55 promoter identified multiple regions that correlate with the UNC-62 mutant expression patterns. Using epistasis experiments, we found further evidence that different isoforms of UNC-62 work through different regions of the unc-55 promoter. Furthermore we provide evidence that the differentiation of GABAergic motor neurons is required in part by different UNC-62 isoforms in different sub classes. MEIS/UNC-62 and COUP-TFII/UNC-55 both appear to have conserved roles in development of the nervous system in other model systems. Thus, isoform specific regulation of gene expression and differentiation via conserved genes could provide a mechanism throughout evolution to generate diversity and complexity. Isoform specific transcriptional regulation of differentiating neurons is a relatively unexplored area of research.

Further research into this new exciting area could help to answer fundamental questions about evolution of the nervous system.

Disclosures: R. Campbell: None. **W.W. Walthall:** None.

Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant EY023665

Title: Rainbow enhancer family for restrictive gene expression in the zebrafish green, red and blue cone photoreceptors

Authors: *X. WEI;
Univ. Pittsburgh, Pittsburgh, PA

Abstract: Specific interactions between cis- and trans-transcriptional regulatory factors underlie distinct gene expression profiles, which in turn dictate the morphogenesis and functionality of various cell types. It is, however, challenging to study such interactions because many regulatory factors are yet to be identified and because the principles that govern these interactions are complex and remain elusive. Here, we report the identification of three members of the rainbow enhancer family that control restrictive gene expression in the red, green, and blue (RGB) cone photoreceptors in the zebrafish retina: the enhancers of apicobasal polarity genes *ponli* and *crb2b* and the enhancer of an unknown gene. These rainbow enhancers share teleost-conserved sequence motifs that are critical for their RGB cone specific transcriptional regulation. We propose that a common or similar cis-regulatory mechanism(s) may underlie restrictive expression of a set of genes in RGB cones and consequently underlie proper RGB cone development and functions.

Disclosures: X. Wei: None.

Poster

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Support: NCTR/FDA Grant E0752801

Title: Ketamine alters the expression of cytochrome p450 (cyp) genes in zebrafish larvae

Authors: *J. KANUNGO, B. L. ROBINSON, S. F. ALI, M. G. PAULE, M. DUMAS; Neurotoxicology, Natl. Ctr. For Toxicological Research/Food and Drug Admin., Jefferson, AR

Abstract: Ketamine, an antagonist of the N-methyl-d-aspartate (NMDA)-type glutamate receptors, is a pediatric anesthetic. Cytochrome P450 (CYP) mixed-function mono-oxygenase superfamily members include enzymes that not only metabolize endogenous molecules such as hormones, retinoids and lipids, but also biotransform xenobiotics. Deregulated activation/suppression of these CYP enzymes can cause physiological and developmental complications. Our studies show that ketamine causes developmental toxicity (neurotoxicity and cardiotoxicity) in zebrafish embryos. We have also shown that ketamine alters steroid hormone levels (endocrine disruption) and down-regulates CYP aromatase (CYP19A1a) gene expression in zebrafish larvae. In mammals, xenobiotics are known to induce the expression of their own metabolic enzymes. In this study, we explored whether ketamine alters the expression of its own metabolic enzyme CYP3A65 (zebrafish ortholog of the mammalian CYP3A4) gene in a dose-dependent manner in zebrafish larvae. Additionally, we also assessed ketamine-induced changes in the expression of *CYP1A*. CYP1A is induced by the carcinogenic polyaromatic hydrocarbons and halogenated aromatic hydrocarbons, compounds metabolically activated by this enzyme. Zebrafish larvae at three days post-fertilization (3 dpf) were exposed to ketamine for 24 h. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) analyses revealed that ketamine, at various concentrations (including doses equivalent to human sub-anesthetic and anesthetic doses), significantly induced CYP3A65 mRNA expression in a dose-dependent manner. In contrast, a sub-anesthetic dose of ketamine did not change CYP1A mRNA expression, whereas anesthetic doses significantly reduced its expression. These results, compliant with reported mammalian data, reinforce the suitability of modeling anesthetic-induced toxicity in zebrafish larvae *in vivo.*

Disclosures: J. Kanungo: None. **B.L. Robinson:** None. **S.F. Ali:** None. **M.G. Paule:** None. **M. Dumas:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: R01NS082283

P20 GM103620

P20 GM103548

R01GM110373

Title: Role of CLN6 in neurite outgrowth and vesicle trafficking

Authors: *S. KOH[1], J. CAIN[1], H. MAGEE[1], K. WHITE[1], D. TIMM[1], K. HENSLEY[2], J. WEIMER[1];
[1]Sanford Hlth., Sioux Falls, SD; [2]The Univ. of TOLEDO, Toledo, OH

Abstract: Neurons become polarized as they develop specialized structures called axons and dendrites. These structures, or neurites, are made up of a network of microtubules which are used to transport cargo containing vesicles from the neuron soma. The cargo transported in these vesicles is necessary for the polarization and survival of the neuron. CRMP2 is a cytosolic phosphoprotein involved in the specification of neurites and regulates vesicular transport through an array of additional binding proteins. Disruption in CRMP2 signaling has been implicated in a host of neurological disorders, including variant late infantile neuronal ceroid lipofuscinoses (vLINCLs). vLINCL is a childhood neurodegenerative disease with age of onset between 2 and 6 years and results in seizures, motor dysfunction, retinopathy, and premature death. vLINCL results from mutations in *CLN6,* an ER-membrane and vesicular associated protein found to complex with CRMP2 and Kinesin light chain 4 (KLC4). We hypothesize that CLN6 forms a complex with CRMP2, a microtubule binding protein, and KLC4, a kinesin microtubule motor protein, to form the CCK complex which works to shuttle vesicular cargo along the network of microtubules in axons and dendrites. The trimeric CCK complex regulates initial neuronal outgrowth and delivery of vital cargo to the distal end of the growing neurite using CLN6 on an ER-derived vesicle, serving as a "molecular tag" for cargo transport. Our lab has shown that mutations in Cln6 can disrupt the formation of the CCK complex, affecting both neurite outgrowth and vesicular transport. To assess the role of Cln6 in neuritogenesis and vesicle transport, primary neuronal cultures were prepared from E15.5 timed pregnant wild type and *Cln6* mutant mice. Early neurite outgrowth was measured and the number of ER vesicles was counted in the presence and absence of Lanthionine ketimine *ethyl ester* (LKE), a molecule shown to stabilize CRMP2 interactions and promote neurite outgrowth. Live images were captured and the velocity of ER vesicles was measured. Disruption of the CCK complex in the *Cln6* mutant mice resulted in reduced initial neurite outgrowth, which was rescued with LKE

treatment. *Cln6* mice had fewer ER derived vesicles in their processes, which was also recovered with LKE treatment. Our findings suggest that mutations in Cln6 can alter the stability of the CCK complex, reducing the proper transport of ER-derived vesicles from the cell soma to the distal portion of the neuron and that treatment with CRMP2 stabilizing compounds are able to partially restore these defects in intracellular transport and may provide therapeutic benefit in vLINCL patients.

Disclosures: S. Koh: None. **J. Cain:** None. **H. Magee:** None. **K. White:** None. **D. Timm:** None. **K. Hensley:** None. **J. Weimer:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 117.20/A41

Topic: A.01. Neurogenesis and Gliogenesis

Title: Functional Maturation of neuronal networks *in vitro* is accelerated by combination of bioactive whey protein, natural bovine complex lipids and DHA

Authors: B. M. BADER[1], C. KUANG[2], C. EHNERT[1], K. JUEGELT[1], A. GRAMOWSKI-VOSS[1], Y. XIAO[2], R. MCMAHON[2], *O. H.-U. SCHROEDER[1], D. HONDMANN[3]; [1]NeuroProof GmbH, Rostock, Germany; [2]Mead Johnson Pediatric Nutr. Inst., Evansville, IL; [3]Mead Johnson Pediatric Nutr. Inst., Glenview, IL

Abstract: Optimal and adequate nutrition early in life is important for healthy growth and development. Especially brain development depends on supply of lipids and fatty acids. Studies have shown that Docosahexaenoic Acid (DHA), for example, plays an important role in infant brain development and it has been added to infant formula. It is of great interest to explore whether additional nutrients, in concert with DHA, are beneficial for the early brain development. In this context, we developed a brain-on-chip platform integrating multielectrode array (MEA) chips and neuronal tissue culture to dissect the nutritional impact on the neuronal network maturation, and asked whether the synergistically beneficial effects of nutritional combination of a bioactive whey protein (BWP), natural bovine complex lipids and DHA on neuronal maturation. Our newly developed "EARLY BRAIN DEVELOPMENT INDEX" is the measure for compound effects on early neuronal network maturation into complex neuronal circuits. We used primary neuronal cell cultures from mice (E15 cortex) which form actively communicating networks within a week when growing on micro electrode arrays (MEA). This spontaneous activity maintains for at least four weeks *in vitro*. We used the MEA technology

(Axion12 well MEAs, Maestro recording system, Axion Biosystems, Inc.) to record extra-cellular action potentials (i.e. spike trains) of single neurons of the network which were recorded at 7, 14, 21 and 28 days *in vitro*. These spike trains were analyzed by proprietary multi-parametric data analysis methods computing over 200 functional parameters describing the activity patterns for each recording day. This in-depth analysis allows following functional neuronal maturation from neurons of embryonic status to mature neuronal cells for 4 weeks *in vitro* and investigating of compound-induced effects on this maturation. The results show that combining DHA, BWP and natural bovine complex lipids together accelerates functional neuronal maturation above all other combinations and does not exhibit the late decline shown for the single components. In conclusion, natural bovine complex lipids as well as BWP in combination with DHA significantly accelerate functional neuronal maturation *in vitro*.

Disclosures: **B.M. Bader:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. F. Consulting Fees (e.g., advisory boards); Mead Johnson. **C. Kuang:** A. Employment/Salary (full or part-time):; Mead Johnson. **C. Ehnert:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. **K. Juegelt:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. **A. Gramowski-Voss:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH, Rostock, Germany. **Y. Xiao:** A. Employment/Salary (full or part-time):; Mead Johnson. **R. McMahon:** A. Employment/Salary (full or part-time):; Mead Johnson. **O.H. Schroeder:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH, Rostock, Germany. **D. Hondmann:** A. Employment/Salary (full or part-time):; Mead Johnson.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

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Program#/Poster#: 117.21/A42

Topic: A.01. Neurogenesis and Gliogenesis

Support: Grant-in-Aid for Scientific Research on Innovative Areas, Grant Number 22123001

Grant-in-Aid for JSPS Fellows, Grant Number 12J08000

Title: Dmrt genes differentially participate in Cajal-Retzius cell development of the cerebral cortex

Authors: *T. KIKKAWA[1], N. SAKAYORI[3], H. YUUKI[1], Y. KATSUYAMA[2], N. OSUMI[1]; [1]Dept. of Developmental Neurosci., Tohoku Univ. Sch. of Med., Sendai, Miyagi, Japan; [2]Dept. of Organ Anat., Tohoku Univ. Sch. of Med., Sendai, Japan; [3]Dept. of Mol. Genet., Inst. of Biomed. Sciences, Fukushima Med. Univ., Fukushima, Japan

Abstract: For the development of the central nervous system, a large variety of neuronal cell types are needed to be generated at defined times and locations. Cajal-Retzius (CR) cells are the first neurons generated during corticogenesis and are essential pioneer neurons that control neuronal migration in the cortex. CR cells are derived from specific regions within the cortex, i.e., the pallial septum (PS), pallial-subpallial boundary (PSB), and cortical hem (CH). However, the molecular mechanism underlying the generation of CR cell subtype in distinct CR cell origins is poorly understood. We have previously shown that *Dmrta1* (*double-sex* and *mab-3* related transcription factor like family A1) is expressed in neural stem/progenitor cells in the neocortex and regulates proneural genes downstream of Pax6 (Kikkawa et al., 2013). In this study, we found that Dmrta1 was expressed in the PS, PSB, and a part of the CH, whereas Dmrt3 was strongly expressed in the CH. To reveal functions of *Dmrta1* and *Dmrt3* in the production of CR cells from distinct origins, we observed CR cells in *Dmrta1* and *Dmrt3* knockout (KO) mice (Konno et al., 2012). We found that *Dmrt3* ablation decreased the number of p73-positive CR cells derived from the CH compared with wild type (WT) mice, which may be reflection of the abnormal CH structure of *Dmrt3* KO mice. However, there were no differences between WT and *Dmrta1* KO mice in the numbers of p73-positive CR cells in the caudal cortex. These results suggest that *Dmrt3* is involved in the development of the CH-derived CR subtype. To further explore differential functions among Dmrt family members in CR cell development, we are currently analyzing the PS- and PSB-derived CR subtypes using *Dmrta1* and *Dmrt3* KO mice.

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

Poster

117. Molecular Mechanisms of Neural Differentiation

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: CIRM Training Grant TG2-01158

Title: Studying the function of human hippocampal neurons in a dish

Authors: *A. SARKAR, H. J. KIM, C. BARDY, A. MEI, R. JAPPELLI, F. H. GAGE;
Lab. of Genetics-Gage, The Salk Inst. For Biol. Studies, La Jolla, CA

Abstract: Hippocampus is the site of learning and memory in the brain and a growing body of evidences suggest some dysfunctions in hippocampal circuitry in the pathophysiology of many psychiatric and neurodegenerative diseases. More specifically, deficits in synaptic transmission in the hippocampal neurons have already been implicated in schizophrenia, major depression disorder and bipolar disorder. The availability of human ES and pluripotent stem cells (hPSCs) offers the opportunity to generate lineage-specific cells to investigate mechanisms of human diseases in brain regions such as the hippocampus. In this study, we investigated the development and functioning of a human neuronal circuitry *in vitro* consisting of hippocampal neurons derived from human ES cells. We utilized a two-step human stem cell based, directed differentiation protocol to generate hippocampal cultures. In the first step, we have used HuES6 cells to generate neuronal progenitor cells (NPC) with forebrain specific identities. In the second step, we further differentiated these NPCs to generate a neuronal culture enriched in mature hippocampal neurons. These neurons express appropriate cell fate and synaptic markers, and proved to be functionally active by the presence of mature spontaneous and evoked action potentials. We then used a microfluidic device based compartmentalized platform to study the synaptic connectivity between neurons. To identify the hippocampal neurons, we have also been able to utilize a promoter specific lentiviral reporter for live cell imaging. We demonstrated the establishment of functional neuronal network with time-lapse calcium imaging and extracellular electrophysiology recordings on these neurons at multiple time-points during differentiation. We demonstrate that this model recapitulates many of the features of hippocampal neuronal circuitry. The ability to mimic the developmental process of disease-relevant cell types in an *in vitro* setting is important for providing insights into the mechanisms of neurodevelopmental disorders. Furthermore, this lineage-specific model may provide important insights into the functioning of human hippocampal neurons from patients with various neurological disorders.

Disclosures: A. Sarkar: None. **H.J. Kim:** None. **C. Bardy:** None. **A. Mei:** None. **R. Jappelli:** None. **F.H. Gage:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 117.23/A44

Topic: A.01. Neurogenesis and Gliogenesis

Title: Akirin2: A novel regulator of cortical development

Authors: P. J. BOSCH, L. C. FULLER, *J. A. WEINER;
Dept. of Biol., The Univ. of Iowa, Iowa City, IA

Abstract: Corticogenesis relies on proper temporal and spatial regulation of progenitor cell division to create a highly organized six-layer cortex. Premature differentiation of cells, disruption of cell cycle timing, excessive apoptosis, or incorrect migration signals can have devastating effects, resulting in a number of congenital disorders. Though genes encoding many key players in cortical development have been identified, our understanding remains incomplete. We found that Akirin2, a small nuclear protein with no known protein domains, DNA/RNA binding motifs or catalytic activity, is expressed in the embryonic telencephalon. Akirin2 has been reported to bind to 14-3-3 proteins, to regulate the NF-κB pathway, and to play roles in immune function, skeletal myogenesis, and meiosis. Little else is known, though Akirins have been postulated to control gene expression by acting as an interface between transcription factors and chromatin remodeling machinery. To study the role of Akirin2 in cortical development, we generated cortex-restricted knockout mice by crossing Emx1-Cre and floxed Akirin2 (Goto et al., Nature Immunol., 2008) lines. Most mutants do not survive past birth, and exhibit a massive reduction in cortex size, with very little dorsomedial telencephalon present. At embryonic day (E)12, when neuronal differentiation has just begun, the mutant telencephalon is already noticeably reduced in size. As the Emx1-Cre driver is active at E9, it appears that Akirin2 is required for the proliferation or survival of progenitors, or for prevention of premature neuronal differentiation. In support of this, we find disorganized and reduced EdU staining, following a 2 hour exposure, in the cortical ventricular zone of E12-14 mutants compared to controls. Interestingly, the Emx1-Cre transgene is also active in the limb epithelium, and we observe a soft-tissue syndactyly in Akirin2 mutant paws. Cleaved caspase-3 staining is decreased, and EdU staining is increased, in interdigital tissue at E13-14, implying that disrupted developmental apoptosis and/or proliferation leads to fusion of digits in Akirin2 mutants. We are currently analyzing cell-type specific markers during early corticogenesis and using a TdTomato Cre-reporter allele to directly visualize mutant progenitors and their behavior. We are also generating Akirin2/Bax double knockout mice to genetically block apoptosis and assess whether Akirin2 is required for survival of cortical progenitors. Elucidating Akirin2's mechanism of action will contribute to our overall understanding of cortical development and shed light on the cellular roles of this still-enigmatic protein.

Disclosures: P.J. Bosch: None. **L.C. Fuller:** None. **J.A. Weiner:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: National Creative Research Initiative (2010-0018272, I.S.)

Basic Science Research programs (2012R1A1A1040142, N.W.) (NRF).

Title: Imidazole-based small molecules that promote neurogenesis in pluripotent cells

Authors: *D. HALDER;
Dept. of Chemistry, Yonsei Univ., Ctr. For Biofunctional Molecules Res., Seoul, Korea, Republic of

Abstract: Imidazole-based small molecules that promote neurogenesis in pluripotent cells** *Debasish Halder, Jinhong Park, Wan Namkung and Injae Shin** **Abstract**: Several lines of evidence suggest that P19 cells are appropriate *in vitro* models for studies of neurogenesis. Herein we describe two small molecules termed neurodazine (Nz) and neurodazole (Nzl), which induce neuronal differentiation of P19 cells. Their ability to induce neurogenesis of P19 cells is comparable to that of retinoic acid. However, Nz and Nzl were found to be more selective neurogenesis inducers than retinoic acid owing to their unique ability to suppress astrocyte differentiation of P19 cells. Our results show that Nz and Nzl promote production of physiologically active neurons because P19 cell-derived neurons induced by these substances have functional glutamate responsiveness. The present study suggest that Nz and Nzl could serve as important tools to induce formation of specific populations of neuronal cell types from P19 cells as part of studies aimed at uncovering the molecular mechanisms that determine cell fate and developing new cell therapies. * Corresponding author e-mail: injae@yonsei.ac.kr ** This work was supported financially by grants from National Creative Research Initiative (2010-0018272, I.S.) and Basic Science Research programs (2012R1A1A1040142, N.W.) (NRF).

Disclosures: D. Halder: None.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

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Program#/Poster#: 117.25/A46

Topic: A.01. Neurogenesis and Gliogenesis

Support: ACHRI/HBI

Title: Pten regulates amacrine cell number by reducing responsiveness to Tgfb negative feedback signaling

Authors: *N. TACHIBANA, R. CANTRUP, R. DIXIT, D. ZINYK, S. MCFARLANE, C. SCHUURMANS;
Univ. of Calgary, Calgary, AB, Canada

Abstract: The retina is comprised of one glial and six neuronal cell types that are all derived from a common pool of multipotent retinal progenitors during development. Generating proper numbers of each of these cell-types is essential to establish a functional neural network. Pten is a protein and lipid phosphatase that negatively regulates PI3K signaling and controls progenitor cell proliferation as well as cell differentiation in several cell lineages. Here we asked whether Pten plays a role in controlling cell proliferation and differentiation in the developing retina. To better understand how Pten might function in the developing retina, we first performed a detailed spatio and temporal analysis of its expression, comparing the distribution of Pten to that of phosphorylated, activated forms of Akt, which are reduced when Pten is active. We found a general trend towards higher pAkt levels over developmental time, with Pten and Akt expressed at low levels in retinal progenitors, and at higher levels in postmitotic retinal cells in the inner and ganglion cell layers. To assess Pten function, we generated a retinal-specific conditional knock-out. The loss of Pten resulted in a transient increase followed by a decrease in progenitor cell proliferation. Consequently, fewer amacrine cells and rod photoreceptors were produced. We further pursued Pten function in regulating amacrine cell production, demonstrating that Pten is not required in amacrine cells to regulate negative feedback signaling. Instead, we found that Pten is required in retinal progenitor cells, where it regulates pSmad2/3 levels and responsiveness to Tgfb2 negative feedback signaling. Taken together, this study has revealed that Pten is a key regulator of proliferation and differentiation in the retina, acting in part by controlling responsiveness to Tgfb2 negative feedback signaling.

Disclosures: **N. Tachibana:** None. **R. Cantrup:** None. **R. Dixit:** None. **D. Zinyk:** None. **S. McFarlane:** None. **C. Schuurmans:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: C.06. Developmental Disorders

Support: NIMH R01MH087660

UL1 TR000041

Title: Comparative analysis of DNA methylation in saliva and brain of children with and without autism spectrum disorder (ASD)

Authors: *J. MARTINEZ[1], A. DELORA[1], F. MENNEN[2], J. A. STEPHEN[3], E. L. BEARER[1]; [1]Pathology, Univ. of New Mexico, Albuquerque, NM; [2]USC, Los Angeles, CA; [3]The Mind Res. Network, Albuquerque, NM

Abstract: Adverse childhood experience leads to adult mental and physical health problems. Our hypothesis is that these outcomes are in part due to epigenetic changes that alter patterns of expression in the brain and impact brain circuitry. Yet there are no existing biomarkers to witness these epigenetic events. Our over-arching goal is to discover and develop biomarkers to detect and measure such epigenetic changes. For epigenetic analysis, we focus on DNA methylation because basic science has developed the tools for detection and analysis, and because clinical studies of specific methylation sites correlate with emotional outcomes. DNA methylation is currently detected by three approaches: 1) bisulfite conversion and sequencing, with or without reduced representation; 2) Infinium HM450K BeadChip; and 3) Methyl binding domain (MBD) pulldown followed by sequence analysis of DNA fragments. For analysis of methylation events in the brain, we are limited to either peripheral tissue in the living subject or post-mortem brain. Hence correlation of peripheral methylation patterns with brain is a necessary step to identify biomarkers for diagnosis. Here we report whole genome methylation patterns in children with and without ASD, compare methods for analysis, and compare DNA methylation patterns in saliva with post-mortem brains. We found a subset of specific genes with significant changes in both brain and saliva compared to normal controls. These studies identified a subset of methylation sites in saliva that correlate with those brain in these children with ASD. We are now applying these techniques to children referred for therapy after documented maltreatment. As a first pass to determine chronic stress objectively, we measure hair cortisol, and for impact we analyze whole genome DNA methylation patterns. We expect that methylation patterns will be a useful biomarker to identify children at risk for future psychosocial disorders, facilitating earlier interventions than are now possible. Supported by NIMH R01MH087660 and UL1 TR000041 pilot project (ELB).

Disclosures: J. Martinez: None. **A. DeLora:** None. **F. Mennen:** None. **J.A. Stephen:** None. **E.L. Bearer:** None.

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Werner Otto Foundation Grant 8/84

Title: Absence of the serine protease inhibitor neuroserpin alters neurogenesis, neuronal morphology and leads to behavioural abnormalities

Authors: *G. GALLICIOTTI[1], M. NEUMANN[2], R. REUMANN[2], B. SZALAY[2], M. SCHWEIZER[3], F. MORELLINI[3], M. GLATZEL[2];
[1]Univ. Med. Ctr. Hamburg-Eppendorf, [2]Neuropathology, [3]Ctr. for Mol. Neurobio., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Neuroserpin, a functional inhibitor of tissue plasminogen activator, is involved in the pathophysiology of stroke, schizophrenia, depression and fear disorders. Neuroserpin expression is restricted to the central and peripheral nervous system, mainly in hippocampus, amygdala, olfactory bulb and neocortex, and is particularly high at late developmental stages and perinatally. In neurons and neuroendocrine cells neuroserpin is stored in large dense core vesicles from where it is secreted in a regulated manner. Neuroserpin's expression suggests a function in development and maintenance of the nervous system. Indeed, experiments performed with cultured cells demonstrate a role for neuroserpin in neuritogenesis and synaptogenesis. In order to elucidate the function of neuroserpin *in vivo*, we analysed neuroserpin-deficient mice. In particular, we investigated neuroserpin's function in neurogenesis, neuritogenesis and synaptogenesis, and the behaviour of neuroserpin-knock out mice. We found that absence of neuroserpin leads to reduced neuronal precursor cell proliferation and premature neuronal differentiation in the neurogenic subgranular zone of the hippocampus during developmental neurogenesis, resulting in decreased cellularity in the dentate gyrus of adult mice. Golgi impregnation and diolistic labeling of brains from neuroserpin-deficient mice show differences in neuronal morphology in knock out animals. Behavioural testing indicates impairment in social behavior, spatial learning and memory and contextual memory in neuroserpin-deficient mice. In conclusion, we found that absence of neuroserpin impairs developmental neurogenesis and alters neuronal morphology *in vivo*. Neuroserpin's role in neurodevelopment may account for the behavioural changes observed in neuroserpin deficient mice, pointing to a role for neuroserpin in development of neuropsychiatric diseases.

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Poster

117. Molecular Mechanisms of Neural Differentiation

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Program#/Poster#: 117.28/A49

Topic: A.01. Neurogenesis and Gliogenesis

Title: Magnetic nanoparticles for manipulating neuronal growth and differentiation

Authors: *M. MARCUS, K. BARANES, M. KARNI, N. ALON, I. LEVY, S. MARGEL, A. SHARONI, O. SHEFI;
Bar Ilan Univ., Ramat Gan, Israel

Abstract: The ability to manipulate neuronal organization and growth has extensive implications in neuronal regeneration and tissue engineering. It has been shown that physical forces play a key role in shaping neuronal structure and in interactions between neurons and their vicinity. In the present study we use magnetic nanoparticles (maghemite, γ-Fe2O3) as mediators to apply physical forces locally and as carriers of neuronal growth factors. We use these nano-complexes in order to locate cells, promote neuronal growth and affect growth orientation. We designed and generated magnetic fields with controlled magnetic flux densities at multiple scales of size and strength. In addition to strong permanent bar magnets and electromagnets we fabricated a unique device, embedded with micro-patterned pads that can be magnetized selectively. First, we incubated, prior to plating, the treated cells, PC12 cells and primary neurons, in medium enriched with iron oxide nanoparticles conjugated to fluorescent tag. Both types of cells uptake the nanoparticles and turned sensitive to the magnetic stimulation with no cytotoxic effect. We successfully improved the cells motility and attracted the cells to one magnetic pole or the other or towards magnetic 'hot spots'. Plating PC12 cells atop the micro-patterned device has led to an organized network of clusters of cells. After cells adhered to the plate the magnetic field affected the neuronal outgrowth orientation, combining the normal chemical signaling with the applied physical forces. Currently we are mathematically modelling nanoparticles uptake by cells and the organization of magnetized cells in response to various external magnetic fields. In addition, we found that covalent conjugation of the magnetic nanoparticles to Nerve growth factor (β-NGF) which is a critical component in nerve tissue development and repair enhanced the typical effect of NGF. Morphometric and molecular measurements revealed that treatment with the nanoparticle-NGF complex leads to a promoted differentiation progression and to more complex dendritic trees. Even low doses were sufficient to trigger the promoted differentiation process. Moreover, stability and signaling pathway assays suggest conjugation to NPs as a method to extend the half-life of NGF, thereby increasing its availability and efficiency. Our study presents

an emerging magneto-chemical method for the manipulation of neuronal migration and growth opening new directions in non-invasive neuronal repair.

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Poster

117. Molecular Mechanisms of Neural Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Pritzker Neuropsychiatric Research Consortium

Title: FGF2-mediated signal transduction in human SH-SY5Y neuroblastoma cells involves cross-talk between the ERK1/2 pathway and protein kinase C

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

Abstract: The SH-SY5Y human neuroblastoma cell line is an appropriate model system in which to examine FGF2-mediated signal transduction. Coupling of the FGF receptor (FGFR) to the extracellular signal-regulated kinase (ERK1/2) pathway in these cells results in rapid ERK1/2 phosphorylation in response to FGF2 that is blocked by the FGFR inhibitor, PD 173074. SH-SY5Y cells are of the N (neuron)-type and they can be further differentiated to a mature catecholaminergic phenotype by the combination of FGF2 and a phorbol ester that activates protein kinase C (PKC). Cells differentiated in this manner assume a phenotype characterized by increased expression of tyrosine hydroxylase (TH) and of GAP-43, a neuron-specific protein that is a major PKC substrate. Induced morphological changes include elaboration of long processes with varicosities and growth cone-like terminals. Increased GAP-43 expression is primarily caused by activation of PKC while that of TH is more responsive to FGF2. Thus, it is of interest to determine whether and how the ERK1/2 and PKC pathways interact to mediate the cell signaling underlying such effects and to compare these interactions in undifferentiated and differentiated cells. Either FGF2 or the phorbol ester, phorbol 12, 13-dibutyrate (PDB), increases ERK1/2 phosphorylation in SH-SY5Y cells but not in an additive manner. Preincubation of cells with the PKC inhibitor, GF 109203X, not only reduces PDB-stimulated ERK1/2 phosphorylation to basal levels as expected, but also causes a nearly-complete inhibition of ERK1/2 phosphorylation elicited by FGF2. These results suggest a considerable degree of cross-talk

between the ERK1/2 and PKC pathways. Cells differentiated with FGF2 and PDB contain higher levels of phosphorylated ERK1/2 than control cells. However, once differentiated, SH-SY5Y cells lose the ability to activate ERK1/2 phosphorylation upon retesting with FGF2, an alteration that is only seen when cells are differentiated with the combination of PDB and FGF2. The overall conclusion derived from these experiments is that both ERK1/2 and PKC activities are essential components of normal FGF2-mediated signaling in SH-SY5Y neuroblastoma cells and during the differentiation process.

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

Poster

117. Molecular Mechanisms of Neural Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 117.30/A51

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant DC008955

Burke Medical Research Institute

Title: A shared molecular mechanism regulates transcription of tyrosine hydroxylase and glutamate decarboxylase 1 in the olfactory bulb

Authors: ***M. WANG**[1], E. CAI[1], N. FUJIWARA[1], H. BAKER[1,2], J. W. CAVE[1,2];
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Abstract: The molecular mechanisms responsible for the preferential co-expression of tyrosine hydroxylase (Th) and glutamate decarboxylase 1 (Gad1) in glomerular layer interneurons of olfactory bulb are not understood. In this study, we show that transcription of Th and Gad1 in the olfactory bulb is regulated, in part, by a shared mechanism mediated by conserved G:C-rich regions within the proximal promoter. These G:C-rich regions form single-stranded DNA secondary structures. In addition, they also recruit heterogeneous nuclear ribonucleoproteins (hnRNP) K and/or LL, which preferentially bind C-rich single stranded DNA and up-regulate promoter activity. Stabilization of DNA secondary structures with small molecules, such as TMPyP4, block hnRNP protein binding and modulate gene promoter activity. These findings suggests that G:C-rich region secondary structures are novel targets for pharmacological modulation of the dopaminergic and GABAergic phenotypes. We also describe studies that test

if either DNA secondary structure formation or recruitment of hnRNP proteins is modulated by synaptic activity, which is known to regulate Th and Gad1 transcription levels in the olfactory bulb. Together, these findings show that conserved G:C-rich regions in Th and Gad1 proximal promoters mediate similar transcription regulatory functions and provide novel insight into the molecular mechanisms underlying the specification and potential modulation of dopaminergic and GABAergic phenotypes.

Disclosures: **M. Wang:** None. **E. Cai:** None. **N. Fujiwara:** None. **H. Baker:** None. **J.W. Cave:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.01/A52

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH intramural research program

Title: Cholinergic regulation of the hippocampal output to entorhinal cortex

Authors: ***J. HAAM**, J. L. YAKEL;
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Abstract: The hippocampus is the major brain structure that plays an important role in memory formation; it receives sensory inputs from the cortex during memory encoding while transferring the temporary hippocampal information back to the cortex during memory consolidation. It has been shown that acetylcholine (ACh) stimulates memory encoding while inhibiting the memory consolidation process, but the underlying mechanism is yet unclear. Given that memory encoding and consolidation are mutually exclusive processes, we hypothesized that ACh suppresses the hippocampus to the entorhinal cortex (EC) circuit, which is the gateway to the memory consolidation pathway. The CA1 pyramidal neurons in the hippocampus are the final output of the hippocampus and receive feedback inhibition from the GABAergic interneurons oriens lacunosum-moleculare (OLM) cells which express high levels of ACh receptors (AChRs). Our previous study has shown that cholinergic inputs stimulate OLM interneurons, which increases GABAergic inputs to CA1 pyramidal neurons. To study how ACh regulates the circuits, we used electrophysiological recordings with optogenetics. The blue light-sensitive channelrhodopsin ChR2 was selectively expressed in cholinergic neurons by injecting AAV9-dfloxed hChR2 to cultured slices from the choline acetyltransferase (ChAT)-cre mice.

Photostimulation of cholinergic neurons caused a decrease in depolarizing step-induced firing in CA1 pyramidal neurons. To examine whether the inhibition of CA1 pyramidal neurons modulates the hippocampal output to the EC, we recorded from the layer V EC (ECV) neurons with stimulation of CA1 pyramidal neurons. We used the retrograde monosynaptic tracing method using G-deleted rabies viruses. For this experiment, the red-shifted channelrhodopsin C1V1 was expressed in ChAT neurons to allow the expression of ChR2 specifically in CA1 pyramidal neurons that are presynaptic to ECV neurons. Red light-stimulation of cholinergic neurons caused a decrease in blue-light evoked currents in ECV. The cholinergic suppression of the evoked currents in ECV was blocked by the M1 muscarinic receptor pirenzepine. We also tested whether cholinergic inputs affect hippocampal theta modulation of ECV neurons. Theta burst stimulation in the CA1 pyramidal layer caused an increase in firing activity in ECV neurons. Photostimulation of cholinergic neurons suppressed the theta burst stimulation-induced increase in firing in ECV neurons. Our data demonstrate that ACh may inhibit the memory consolidation process by suppressing the hippocampal output via the OLM-mediated feedback inhibition onto CA1 pyramidal neurons.

Disclosures: J. Haam: None. **J.L. Yakel:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.02/A53

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Therapeutic hypothermia in neonatal hypoxia-ischemia: neuroprotection through alteration in acetylated metabolites

Authors: T. TAKENOUCHI[1], Y. SUGIURA[1], T. MORIKAWA[1], T. NAKANISHI[2], Y. NAGAHATA[1], T. SUGIOKA[1], K. HONDA[1], *A. KUBO[1], T. HISHIKI[1], T. MATSUURA[1], T. HOSHINO[1], T. TAKAHASHI[1], M. SUEMATSU[1], M. KAJIMURA[1];
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Abstract: Mild hypothermia following intrapartum asphyxia has been widely used as a standard therapeutic intervention. However, the exact molecular mechanisms whereby hypothermia improves outcomes remain unclear. To investigate underlying mechanisms responding to the hypothermic intervention, we employed a two-step approach; metabolomics to target metabolic pathways responding to lowering temperature, and quantitative imaging mass spectrometry to reveal spatial alterations in targeted metabolites at specific regions of the brain. Seven-day male

Sprague-Dawley rats underwent surgical ligation of left common carotid artery, followed by systemic hypoxia with 8% oxygen for 2.5 hours. Subsequently, rats were returned to 21% oxygen at either 38°C (normothermia) or 30°C (hypothermia) for 3 hours. Brain metabolic states were rapidly fixed by in-situ freezing. Neurobehavioral outcome was assessed using objective scale of posturing during the reoxygenation period. Non-targeted metabolomics of 107 metabolites showed that hypothermia causes not only decreases but also increases in metabolites. Specifically, hypothermia diminishes the carbon biomass related to acetyl-moieties such as pyruvate and acetyl-CoA. Conversely, it increases deacetylated metabolites such as carnitine and choline. Quantitative imaging mass spectrometry showed that hypothermia decreases acetylcholine contents in hippocampus and amygdala. In the same anatomical regions, there was an inverse increase in carnitine. Collectively, these findings suggests that hypothermia after hypoxia-ischemia exhibits neuroprotection by altering cellular acetylation status with coordinated suppression of acetyl-CoA which resides at metabolic crossroads of glycolysis, amino acid catabolism and ketolysis.

Disclosures: **T. Takenouchi:** None. **Y. Sugiura:** None. **T. Morikawa:** None. **T. Nakanishi:** None. **Y. Nagahata:** None. **T. Sugioka:** None. **K. Honda:** None. **A. Kubo:** None. **T. Hishiki:** None. **T. Matsuura:** None. **T. Hoshino:** None. **T. Takahashi:** None. **M. Suematsu:** None. **M. Kajimura:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.03/A54

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH / NIDA R01 DA033811

Title: Insulin increases striatal cholinergic interneuron excitability and enhances dopamine release via nAChRs

Authors: *J. C. PATEL[1], C. R. LEE[1], M. A. STOUFFER[1], P. WITKOVSKY[2], R. P. MACHOLD[3], M. E. RICE[1];
[1]Dept. of Neurosurg., [2]Dept. of Ophthalmology, [3]Smilow Neurosci. Program, NYU Sch. of Med., New York, NY

Abstract: Insulin in the brain can act on reward-related circuitry to regulate feeding behavior. Previous studies have focused on insulin actions at the level of mesolimbic dopamine (DA)

neurons via insulin receptors (InsRs) that enhance DA transporter activity and induce long-term depression of excitatory inputs. We reported previously that insulin increases evoked extracellular DA concentration $[DA]_o$ detected by fast-scan cyclic voltammetry in the striatal complex by enhancing DA release (Stouffer et al. SFN, 2011, 732.16). However, whether this is a direct effect on DA axons or indirect via local circuitry is not known. Using immunohistochemistry on rat striatal sections we indeed found striatal InsRs on DA axons. Strikingly, we also found abundant InsR expression on large cell bodies identified as cholinergic interneurons (ChIs) by co-immunostaining for choline acetyltransferase (ChAT), the primary acetylcholine (ACh) synthesizing enzyme. Using whole-cell current-clamp recording in striatal slices we examined the effect of insulin on ChI excitability using a series of 3 s depolarizing current pulses (200, 300 and 400 pA). Each pulse elicited a train of action potentials that exhibited spike frequency adaptation, often with loss of spiking by the end of the current pulse. In the absence of insulin, ChIs showed little change in the number of action potentials evoked by each stimulus over time (n = 12 stimulus pairs, 4 cells; $p > 0.05$). By contrast, insulin (30 nM) attenuated spike frequency adaptation, resulting in an increase in spike number for each current step (from 12.7 ± 3.1 to 19.1 ± 4.6 at 200 pA; 15.6 ± 3.9 to 23.7 ± 5.6 at 300 pA; 18.7 ± 5.2 to 27.3 ± 6.7 at 400 pA; n = 7 stimulus pairs each; $p < 0.05$), with an overall increase in spike number from 15.7 ± 2.3 to 23.4 ± 3.2 (n = 21 stimulus pairs; $p < 0.001$). Importantly, the effect of insulin on spike number was prevented by HNMPA (5 μM), a selective InsR inhibitor, but not by picropodophyllin (1 μM), an inhibitor of IGF-1R (insulin-like growth factor 1 receptor). Given that ChIs potently regulate striatal DA release via nicotinic ACh receptors (nAChRs) on DA axons, we tested their role in the effect of insulin on evoked $[DA]_o$. Indeed, the effect of insulin on elevating evoked $[DA]_o$ in rat striatum was prevented by nAChR antagonists, mecamylamine (5 μM) or DHβE (1 μM). Moreover, insulin failed to enhance evoked $[DA]_o$ in mice with genetic ablation of forebrain ChAT *vs*. controls. Thus, abundant InsR expression on ChIs, enhancement of ChI excitability with acute insulin, and the nAChR-dependence of insulin-enhanced DA release implicate ChIs as novel insulin targets that can enhance striatal DA release, and thereby influence food reward.

Disclosures: **J.C. Patel:** None. **C.R. Lee:** None. **M.A. Stouffer:** None. **P. Witkovsky:** None. **R.P. Machold:** None. **M.E. Rice:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.04/A55

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH/NIGMS COBRE Grant

Title: Point mutations in the vesicular acetylcholine transporter uncover key roles for central acetylcholine release in organismal survival and behavior

Authors: *S. BOPPANA, A. C. BLAKE, Jr., O. AKINRINSOLA, H. LAWAL;
Biol. Sci., Delaware State Univ., Dover, DE

Abstract: Decline in cholinergic neurotransmission is associated with normal and pathological aging. However, the precise role of changes in acetylcholine (ACh) release in mediating behaviors, such as locomotion, and learning and memory remain poorly understood. The vesicular acetylcholine transporter (VAChT), found in many organisms including worms, flies and humans, is responsible for the packaging and transport of acetylcholine for exocytotic release. A complete loss of VAChT function is lethal, while severe mutations cause decreased locomotor performance in *Drosophila*. To understand the effect of cholinergic release on behavior, we are studying four developmentally lethal point mutation alleles (Vacht1, Vacht2, Vacht4 and Vacht8) of varying severity. Here we report an adult rescue of this developmental lethality through the expression of a <50% expression of wildtype VAChT in each of the mutations analyzed. Analysis of locomotive behavior in the rescued adults shows a differential effect corresponding to the severity of these point mutations. In addition, this genetic rescue analysis reveals a distinction in the level of ACh required for the rescue of lethality and that needed for the restoration of normal locomotion behavior. To further determine the mechanism through which these mutations disrupt the function of the transporter, we generated the point mutant alleles described above *in vitro* and assessed the effects of these mutations on trafficking of VAChT and the transport of ACh by VAChT in the *Drosophila* S2 cell line. Together these studies reveal the importance of central acetylcholine release in regulating survival and synaptic activity and underscore the role of point mutations in dissecting neurotransmitter transporter function.

Disclosures: **S. Boppana:** None. **A.C. Blake:** None. **O. Akinrinsola:** None. **H. Lawal:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.05/A56

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant 1 R01 DA038966

Title: The distribution within the mouse striatum and specificity of transgenic expression of opsins in cholinergic interneurons

Authors: A.-C. SIENA, N. CHUHMA, *S. RAYPORT, S. MINGOTE;
Psychiatry / Molec Therapeut., Columbia Univ., New York, NY

Abstract: Striatal cholinergic interneurons (ChIs) are involved in motor coordination, cognition, motivated behavior, as well as the pathophysiology of several neuropsychiatric disorders. They make up about 1% of the striatal neuron population, and are the source of all striatal acetylcholine. Despite their integral role in striatal function, a modern stereological analysis of the number and their density has not been performed in the mouse, and there is a lack of knowledge regarding the regional density of ChIs within striatal subdomains. To gain a more comprehensive understanding of neuronal function, researchers are now utilizing optogenetics to selectively drive action potential firing with channelrhodopsin (ChR2) or inhibit action potential firing with halorhodopsin (NpHR). Crucial to optogenetic experiments, we need to know what percent of target cells express the desired opsins and are therefore subject to photostimulation. Here, using unbiased stereological methods, we found that the regional density of ChIs was relatively uniform across striatal subdomains, specifically the nucleus accumbens (NAc), medial dorsal striatum (medial Str), and lateral dorsal striatum (lateral Str). Within NAc subregion, ChI density may be higher in the NAc shell than the NAc core. To address the potential for optogenetic modulation of ChI's, we bred conditional transgenic mice choline acetyl transferase (ChAT) cre driver mice to express ChR2 or NpHR in ChIs (Ai32 mice and Ai39 mcie respectively). We found that transgenic expression of ChR2 or NpHR in ChIs was highly specific and efficient in the NAc, medial Str, and lateral Str. In summary, ChIs are relatively uniformly distributed in the mouse striatum, reliably express ChR2 or NpHR in conditional transgenic mice and should thus be susceptible to reliable optogenetic modulation.

Disclosures: **A. Siena:** None. **N. Chuhma:** None. **S. Rayport:** None. **S. Mingote:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.06/A57

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIMH 1R01MH086530

Dystonia Medical Research Foundation

Alzheimer´s Foundation - NF AVAST in Czech Republic

Title: Negative modulation of choline transporter (CHT) function reveals superior cholinergic capacity of CHT-overexpressors

Authors: *P. VALUSKOVA[1,2], A. KOSHY CHERIAN[1], K. PITCHERS[1], Y. KIM[1], C. W. LINDSLEY[3], E. A. ENNIS[3], R. D. BLAKELY[3], M. SARTER[1];
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Abstract: The uptake of choline by the hemicholinium-3 (HC-3)-sensitive high affinity choline transporter (CHT) has been described as the rate-limiting step for acetylcholine (ACh) synthesis. We previously demonstrated that the capacity of the CHT to sustain ACh synthesis and release primarily reflects the density of CHTs in synaptosomal plasma membrane. Limitations in the intracellular availability of CHTs for mobilization and surface expression, such as modeled by CHT heterozygosity, diminish the capacity of cholinergic neurons and cause associated impairments in attentional performance. As decline in cholinergic functions greatly contributes to the severity of cognitive impairments we have been exploring the potential usefulness of therapies designed to enhance CHT function. To this end, we demonstrated that cholinergic neurons of mice overexpressing the CHT (CHT-OXP) show a 2 to 3-fold elevation of CHT densities in synaptic plasma membrane and intracellular domains and that CHT-mediated choline uptake is more than 2-fold higher than in wild type (WT) mice (Holmstrand et al., 2013). Here we employed choline-sensitive microelectrodes to measure choline currents in the presence of the CHT blocker HC-3 or the negative modulator ML352 (Ennis et al., 2015). To evoke presynaptic ACh release events ("transients"), we pressure-ejected an α4β2* nAChR agonist into the recording area (see Parikh et al., 2008, 2010). In the absence of a CHT ligands, the amplitudes of evoked transients in CHT-OXP were smaller than in WT mice, reflecting the superior rate of clearance of choline and the underlying, relatively high density of surface CHTs in CHT-OXP. Three pressure ejections of one of the CHT ligands were followed by repeated assessment of evoked currents over the next 40 min. In WT mice, HC-3 and ML352 both reduced the amplitude of evoked transients. This finding is consistent with the pharmacological classification of HC-3 or ML352, indicating that these compounds interfered with choline uptake and thus ACh synthesis and eventually release. In contrast, evoked transient amplitudes in CHT-OXP remained initially unaffected by the CHT ligands but later increased over baseline. These results are consistent with the hypothesis that CHT-OXP models a gain in cholinergic function and suggest that interference with CHT function enhances CHT surface density in over-expressors.

Disclosures: **P. Valuskova:** None. **A. Koshy Cherian:** None. **K. Pitchers:** None. **Y. Kim:** None. **C.W. Lindsley:** None. **E.A. Ennis:** None. **R.D. Blakely:** None. **M. Sarter:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.07/A58

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Cholinergic system regulation of behavior in *Drosophila* melanogaster larvae

Authors: *C. ENGLISH, C. MALLOY, R. L. COOPER;
Univ. of Kentucky, Lexington, KY

Abstract: We investigated the role of acetylcholine in the *Drosophila* melanogaster larval CNS to identify how this neuromodulator regulates locomotion and feeding behaviors. We combined pharmacological and genetic approaches in order to deduce acute changes in behaviors upon altering the activity of the cholinergic system. Genomic screens have revealed that there are ten receptors in *Drosophila* that are very similar to the nicotinic acetylcholine receptors (nAchRs) of mammals. In *Drosophila*, acetylcholine is a neurotransmitter within the CNS and is the excitatory neurotransmitter for sensory neurons but not motor neurons, as in mammals. It has been found that this system is important in regulating specific behaviors including medial fiber escape response; however, the integration of cholinergic signaling within the CNS and regulation of motor output in this model organism is not well-known. Thus, we are investigating the role of cholinergic neuronal activity in regulating distinct motor behaviors. In addition, a distinctive advantage of *Drosophila* larvae is the short developmental time (~4 days) in which the development of the CNS can be investigated. Genetic and pharmacological techniques will be employed, including temperature-sensitive silencing and activation of cholinergic neurons in transgenic flies and acute and long-term feeding of cholinergic agonists and antagonists in order to investigate activity based modulation of specific behaviors. Analysis has shown that silencing cholinergic neurons, genetically, decreases body wall contractions significantly, reducing crawling speed. Preliminary pharmacological analysis shows that *Drosophila* larvae fed high concentrations of nicotine for 20 minute displayed a decrease in body wall contractions. Thus, it is evident that this system is integral in regulating two distinct motor behaviors. In addition, the proposed experimental design allows for a multitude of options for future experimentation including investigation of regulation of olfaction and response to light upon altering the cholinergic system. All of these are testable for proof of concept and will provide the degree of

inhibition in sensory-motor responses. This study will help to establish the role of acetylcholine in regulating simple motor behaviors.

Disclosures: C. English: None. **C. Malloy:** None. **R.L. Cooper:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.08/A59

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Grant-in-Aid for Scientific Research (C) #25463100

Grant-in-Aid for Young Scientists (B) #25861763 and #15K20563

Nihon University Multidisciplinary Research Grant for 2014-2015

Title: Intra-accumbal infusion of endomorphin-1 and endomorphin-2 decreases accumbal acetylcholine efflux via mu2 receptors in the nucleus accumbens of freely moving rats

Authors: *T. SAIGUSA, Y. AONO;
Nihon Univ. Sch. of Dent. at Matsudo, Chiba, Japan

Abstract: Background: Nucleus accumbens (NAc) is a terminal area of mesolimbic dopaminergic neurons that project from the ventral tegmental area. We have shown that local administration of the putative endogenous mu receptor agonists endomorphin (EM)-1 and EM-2 into NAc increased accumbal dopamine (DA) efflux with and without stimulating accumbal mu receptors, respectively (Okutsu et al., 2006). Accumbal cholinergic neurons express mu receptors that are thought to inhibit neural activity. We have shown that intra-accumbal administration of EM-1 and EM-2 reduces accumbal acetylcholine (ACh) efflux. Mu receptors are divided into mu1 and mu2 receptors based on sensitivity to the mu receptor antagonist naloxonazine. In order to study involvement of mu receptor subtypes in regulation of accumbal cholinergic activity, we analysed effects of the mu receptor antagonist CTOP and selective mu1 receptor antagonist naloxonaize on EM-1- and EM-2-induced reduction of accumbal ACh efflux of freely moving rats using *in vivo* microdialysis. We also investigated antagonist effects on EM-1- and EM-2-induced accumbal DA efflux as the perfusate contains a low concentration of physostigmine (50 nM) that may affect EM's effects on accumbal DA efflux. Methods: Male Sprague-Dawley rats were used. ACh and DA levels in accumbal perfusates, taken every 15 and 20 min, were determined by HPLC-ECD, respectively. CTOP, EM-1 and EM-2 were administered

intracerebrally through the dialysis probe. Doses of these compounds indicate total amount (mol) over a 30-min infusion time. CTOP was infused just before and naloxonazine given intraperitoneally 24h before local administration of EM-1 and EM-2, respectively. Results and Discussion: CTOP (3 nmol) inhibited EM-1 (30 nmol)- and EM-2 (30 nmol)-induced reduction of ACh levels. CTOP (3 nmol) also inhibited EM-1 (15 nmol)-induced DA efflux, but failed to alter EM-2 (30 nmol)-induced DA efflux. While naloxonazine (15 mg/kg ip) did not alter EM-1 (30 nmol)- and EM-2 (30 nmol)-induced reduction of ACh levels, it inhibited EM-1 (15 nmol)-induced of DA efflux. The present study shows that locally administered EM-1 and EM-2 into NAc reduce accumbal ACh efflux through mu2 receptor activation. The present results support our reports that EM-1 increases accumbal DA efflux by mu1 receptor stimulation and EM-2 enhances accumbal DA efflux through mechanisms independent from opioid receptor activation (Okutsu et al., 2006). The present study also implies that mu1 receptors play a stimulatory role in regulating accumbal dopaminergic activity and mu2 receptors play an inhibitory role in regulating accumbal cholinergic activity.

Disclosures: T. Saigusa: None. **Y. Aono:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.09/A60

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Raman spectroscopy for observe acetylcholine activity during normal sleep and REM sleep deprivation in Balb/C mice

Authors: *R. BELTRAN-RAMIREZ[1,2], J. CHAVEZ-GARCIA[3], I. FLORES-MUNGUIA[3], J. ESPINOZA, Jr[3], C. VENTURA-MEJIA[3], F. DIAZ-HURTADO[3], L. STOKES[3], C. GONZALEZ-SANDOVAL[3], C. GONZALEZ-SANDOVAL[3], C. GONZALEZ-SANDOVAL[3], C. GONZALEZ-SANDOVAL[3], G. LOPEZ-ARMAS[3];
[1]Sistemas de Informacion, Univ. De Guadalajara, Zapopan, Mexico; [2]CENTRO DE ENSEÑANZA TECNICO INDUSTRIAL, Guadalajara, Mexico; [3]CENTRO DE ENSEÑANZA TECNICO INDUSTRIAL, GUADALAJARA, Mexico

Abstract: Introduction: Insomnia is one of the most common sleep disorders in humans. Due to ethical and methodological difficulties involved in human research we use mice models to evaluate what has been called Paradoxical Sleep Deprivation (PSD) which is selective for Rapid eye movement (REM) sleep. By other hand brainstem cholinergic neurotransmission is involved

in the generation and maintenance of rapid eye movement (REM) sleep. There is strong evidence that pontine cholinergic and cholinoceptive neurons, interacting in coordination, trigger and maintain REM sleep. So, for investigate Acetylcholine activity we used Raman spectroscopy which is a technique who involves shining a monochromatic light source on a sample and detecting the scattered light with automated system to manipulate the Raman and locate each sample to bring the same focal length with the intention of bringing the same conditions in each of the samples, this procedure allows to know the active molecules in sleep vs PSD offering the opportunity to know the mechanism of neuronal functioning under these conditions. Thus, Raman spectroscopy provides information about molecular vibrations that can be used for sample identification and quantitation. Objective: Measure the content of acetylcholine and serotonin by Raman spectrophotometry and western blot technique in cerebral cortex. Material and Methods: Adult male Balb-C mice, bred in in the animal house of University Center of Health Sciences of the University of Guadalajara, weighting 23-26g. Mice were housed in groups of 5 and kept in a room with a controlled light-dark cycle (lights on from 8 a.m. to 8 p.m.) and temperature (22 ± 2oC). Groups were 1. Control, 2. PSD. REM sleep deprivation started at 9 a.m. Animals were placed individually in water tanks on a small flower-pot for 96 h. The control mice were kept individually in their home cages at the same room. Results: We found by Raman spectroscopy and western blot technique major content of acetylcholine neurotransmitter in brain cortex of animal exposed to PSD.

Disclosures: **R. Beltran-Ramirez:** None. **J. Chavez-garcia:** None. **I. Flores-Munguia:** None. **J. Espinoza:** None. **C. Ventura-mejia:** None. **F. Diaz-hurtado:** None. **L. Stokes:** None. **C. Gonzalez-sandoval:** None. **C. Gonzalez-sandoval:** None. **C. Gonzalez-sandoval:** None. **C. Gonzalez-sandoval:** None. **G. Lopez-armas:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.10/A61

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant 1P20GM103653-01A1

Title: Deficits in central acetylcholine release impairs induced locomotion in *Drosophila*

Authors: A. C. BLAKE, Jr[1], M. HEREDIA[1], *H. O. LAWAL[2];
[1]Biol. Sci., [2]Biol., Delaware State Univ., Dover, DE

Abstract: The role of cholinergic dysfunction in the cognitive decline associated with neurological diseases such as Alzheimer's disease is well known. The key elements required for presynaptic acetylcholine (ACh) release and post-synaptic signaling have also been described in detail. However, the precise relationship between alterations in ACh neurotransmission and downstream changes in behavior in the nervous system remain poorly understood. One critical element of cholinergic signaling is the vesicular acetylcholine transporter (VAChT) which is required for the transport of ACh from the cytoplasm into synaptic vesicles for exocytotic release. A complete loss of Vacht is lethal in flies, worms and mammals. Here we hypothesize that subtle changes in Vacht will uncover important roles of ACh release in behavior. Using point mutations to disrupt VAChT function, we report that a series of Vacht mutations impair two locomotion circuits, baseline and touch response locomotion. In particular, Vacht mutants display a deficit in the timing of the response to touch stimulus in a manner corresponding to severity of the mutant alleles studied. We categorized the resulting allelic series as mild, moderate and severe with respect to acetylcholine release. We further report the genetic rescue of Vacht mutant deficits using wildtype VAChT. Interestingly, here too, we find that the expression of the same level of wildtype VAChT in the different mutant backgrounds produces a differential effect on locomotion behavior corresponding to the severity of those mutations. In addition, we conducted a pharmacological rescue study using agonists of the dopaminergic and cholinergic pathways, using dopamine and brucine respectively, and found that both drugs partly rescue the locomotion deficits in the mutants. Together, this report supports a key role for acetylcholine signaling in regulating the timing of the response to a mechanical stimulus and underscores the utility of point mutations that compromise VAChT activity *in vivo* as tools to elucidate the complex relationship between altered ACh release and behavioral deficits.

Disclosures: **A.C. Blake:** None. **M. Heredia:** None. **H.O. Lawal:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.11/A62

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Support: DFG grant GO 2334/1-1

DFG grant AH67/7-1

NIH/NIDA grant 1P30 35 DA035756-01

HHMI

Helmholtz Association

Title: An essential role of acetylcholine-glutamate synergy at habenular synapses in nicotine dependence

Authors: *A. GORLICH[1], S. FRAHM[2], B. ANTOLIN-FONTES[1], J.-F. ZANDER[2], J. SANTOS-TORRES[3], G. AHNERT-HILGER[2], I. IBAÑEZ-TALLON[1];
[1]Lab. of Mol. Biol., Rockefeller Univ., New York, NY; [2]Charité - Universitätsmedizin Berlin, Berlin, Germany; [3]Max Delbrück Ctr. for Mol. Med., Berlin, Germany

Abstract: Disturbances in transmitter co-release may contribute to a variety of disorders, including drug addiction. Despite the evidence that medial habenula (MHb) neurons regulate nicotine craving and intake, and co-release acetylcholine (ACh) and glutamate, the contribution of ACh to nicotine dependence is not well understood. Here we conditionally deleted the ACh-synthesizing enzyme choline-acetyltransferase (ChAT) in MHb neurons. Postsynaptic recordings revealed that the absence of ACh reduces the glutamate content of synaptic vesicles and eliminates presynaptic facilitation. Electron microscopy and immuno-isolation analyses demonstrated colocalization of ACh and glutamate vesicular transporters. Glutamate reuptake was stimulated in the presence of ACh, indicating vesicular synergy. Mice lacking CHAT in habenular neurons were insensitive to nicotine-conditioned reward and withdrawal. These data demonstrate that ACh controls the quantal size and release frequency of glutamate at habenular synapses, and suggest that the synergistic functions of these neurotransmitters are generally important for modulation of cholinergic circuit function and behavior.

Disclosures: **A. Gorlich:** None. **S. Frahm:** None. **B. Antolin-Fontes:** None. **J. Zander:** None. **J. Santos-Torres:** None. **G. Ahnert-Hilger:** None. **I. Ibañez-Tallon:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 118.12/A63

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Texas Biomedical Device Center

Title: Vagus nerve stimulation drives neural activity in the locus coeruleus and nucleus basalis

Authors: *D. HULSEY, J. RILEY, M. IYENGAR, R. RENNAKER, S. HAYS, M. KILGARD; The Univ. of Texas At Dallas, Richardson, TX

Abstract: Acetylcholine and Norepinephrine modulate neural plasticity. The Locus Coeruleus (LC) and Nucleus Basalis (NB) produce these neuromodulators and their projections supply the cortex. Vagus Nerve Stimulation (VNS) paired with motor training has been shown to drive robust, specific neural plasticity in the motor cortex. Eliminating cholinergic input to the cortex blocks the plasticity-enhancing effects of VNS. VNS may act to enhance cortical plasticity by engaging a neuromodulatory system associated with plasticity. Indirect evidence suggests that VNS acts through noradrenergic and cholinergic function. The plasticity-enhancing effects of VNS have an inverted U function based on stimulation intensity, suggesting that the systems engaged by VNS may display a non-linear response to stimulation. Here we directly measure activity in the LC and NB with simultaneous neurophysiological recordings in response to VNS. In addition we vary the amplitude, duration, frequency, and inter stimulus interval of the stimulations. We find that VNS directly drives neural activity in both the LC and NB. Varying the parameters of stimulation drives differential activity patterns. Understanding the dynamics of neural responses in these brain regions will allow optimizing stimulation parameters for therapeutic purposes.

Disclosures: **D. Hulsey:** None. **J. Riley:** None. **M. Iyengar:** None. **R. Rennaker:** None. **S. Hays:** None. **M. Kilgard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MicroTransponder Inc..

Poster

118. Brain Cholinergic Mechanisms

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant R37-NS008174

Crill Endowed Professorship

Title: Analysis of calcium signaling after activation of muscarinic acetylcholine receptors in sympathetic neurons by measurements and mathematical modeling

Authors: *M. KRUSE, O. VIVAS, B. HILLE;
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Abstract: Activation of M_1 muscarinic acetylcholine receptors leads to activation of phospholipase C and hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), generating the signaling molecules diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). Binding of IP_3 to IP_3 receptors activates these ion channels and causes release of Ca^{2+} from intracellular stores into the cytoplasm. In many cell types cytoplasmic Ca^{2+} concentrations rise significantly due to this mechanism, however, in superior cervical ganglion (SCG) neurons only small rises in cytoplasmic Ca^{2+} levels have been observed upon muscarinic stimulation. Here, we analyzed the dynamics of $PI(4,5)P_2$ depletion, IP_3 generation and Ca^{2+} release from intracellular stores in SCG neurons by FRET measurements, Ca^{2+} photometry, and mathematical modeling. We found that upon depletion of $PI(4,5)P_2$ by activation of muscarinic receptors, high levels of IP_3 are generated, but again only small increases in cytoplasmic Ca^{2+} levels were observed. Measurements of IP_3 receptor levels by Western blot showed strong expression of the proteins, ruling out low expression levels of the receptors as the reason for the small Ca^{2+} signals. We used the kinetics of IP_3 generation and degradation measured with the FRET probe LIBRAvIII to adapt a kinetic model of phosphoinositide metabolism upon muscarinic stimulation, which we had previously developed for tsA201 cells, to study the diffusion of IP_3 in the soma of SCG neurons. This new three-dimensional spatial model showed rapid diffusion of IP_3 throughout the entire soma, demonstrating the ability of IP_3 to reach IP_3 receptors before its degradation. Our findings led us to postulate a modulation of IP_3 receptor activity by IP_3R-binding proteins like IRBIT (IP_3R binding protein released with inositol 1,4,5-trisphosphate). IRBIT has been shown previously to be expressed in SCG neurons by immunofluorescence analysis, and to modulate IP_3 receptor activity in these cells. We found expression of IRBIT in SCG neurons by Western blot and included modulation of IP_3 receptor availability for IP_3 binding by a protein with IRBIT's properties into our kinetic model. This allowed for successful reproduction of observed kinetics and amplitudes of Ca^{2+} signals in SCG neurons. Our model for phosphoinositide metabolism in SCG neurons indicates that interaction of proteins like IRBIT with IP_3 receptors will restrict release of Ca^{2+} from intracellular stores despite generation of high levels of IP_3. The extended model can be used to analyze the effects of muscarinic stimulation on Ca^{2+} signaling in SCG neurons.

Disclosures: **M. Kruse:** None. **O. Vivas:** None. **B. Hille:** None.

Poster

118. Brain Cholinergic Mechanisms

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Support: CIHR MOP-89825 (EKL)

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CIHR MOP-102467 (JM)

Canada Research Chairs Program (EKL)

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Alzheimer's Society of Ontario (PF)

Cryptic Rite Charitable Foundation (JM)

Title: Calcium regulation of sustained cholinergic excitation of prefrontal executive circuitry in the TgCRND8 mouse model of Alzheimer's disease

Authors: *E. PROULX[1], L. KANG[1], P. FRASER[2], J. MCLAURIN[3], E. K. LAMBE[1]; [1]Physiol, Univ. Toronto, Toronto, ON, Canada; [2]Med. Biophysics, [3]Lab. Med. and Pathobiology, Univ. of Toronto, Toronto, ON, Canada

Abstract: The cholinergic modulation of the prefrontal cortex is essential to working memory and attention. In particular, acetylcholine exerts robust excitation of prefrontal layer 6 pyramidal neurons to achieve optimal performance on challenging attention tasks. From an integrated approach of electrophysiology, pharmacology and multi-photon calcium imaging, we demonstrate that nicotinic and muscarinic acetylcholine receptors work together to sculpt the excitation of these prefrontal output neurons in a calcium-dependent manner. We further characterize and pharmacologically reverse a disruption of this signaling in the TgCRND8 mouse model of Alzheimer's Disease. Taken together, our results point to a trade-off between excitation and spiking fidelity in pyramidal cells of the major corticothalamic layer of prefrontal cortex, a compromise that may ultimately have important ramifications for the regulation of excitability of prefrontal executive circuits.

Disclosures: E. Proulx: None. **L. Kang:** None. **P. Fraser:** None. **J. McLaurin:** None. **E.K. Lambe:** None.

Poster

118. Brain Cholinergic Mechanisms

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Program#/Poster#: 118.15/A66

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Canadian Institute of Health Research (CIHR) grant #211687

Title: PET imaging with [18F]FEOBV following lesion of cholinergic pedunculopontine tegmental neurons in rat

Authors: *M. CYR[1,2], M. J. PARENT[1,2], N. MECHAWAR[2], P. ROSA-NETO[2,3], J.-P. SOUCY[3], S. CLARK[4], M.-A. BÉDARD[1,2];
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Abstract: Background: [18F]fluoroethoxybenzovesamicol ([18F]FEOBV) is a PET radiotracer with high selectivity and specificity to the vesicular acetylcholine transporter (VAChT). It has been shown to be a sensitive *in vivo* measurement of changes of cholinergic innervation densities following lesion of the nucleus basalis of Meynert (NBM) in rat. The current study used [18F]FEOBV with PET imaging to detect the effect of a highly selective lesion of the pedunculopontine (PPTg) nucleus in rats. Methods: Twenty-five male Long-Evans rats (body weight: 250 - 500 g) were used for this study. Eighteen rats underwent stereotaxic surgery to induce either a selective lesion of the PPTg cholinergic neurons (Lesion group; N = 12) or no lesion (Sham group; N = 6). Another group of rats (Control group; N = 7) was used for comparison with the two experimental (Lesion & Sham) groups. After bilateral and selective lesions of the PPTg cholinergic neurons, rats were scanned using [18F]FEOBV, then sacrificed, and their brain tissues collected for immunostaining and quantification of the VAChT. Results: Comparisons with control rats revealed that cholinergic losses can be detected in the brainstem, lateral thalamus, and pallidum by using *in vivo* imaging methods with [18F]FEOBV. Similarily, comparisons with shams revealed that comparable losses can be detected in the same regions using standard *ex vivo* measurements. In the brainstem PPTg area, significant correlations were observed between *in vivo* and *ex vivo* measurements, while this was not the case in the thalamic and pallidal projection sites. Conclusions: These findings support PET imaging with [18F]FEOBV as a reliable *in vivo* method for the detection of neuronal terminal losses resulting from lesion of the PPTg. Moreover, quantification of cholinergic losses using [18F]FEOBV with PET was compared here with ex-vivo immunocytochemistry, allowing to corroborate cholinergic losses in the same brain regions. Useful applications can be found in the study of neurodegenerative diseases in human, such as Parkinson's disease, multiple system atrophy, progressive supranuclear palsy, or dementia with Lewy bodies.

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Poster

118. Brain Cholinergic Mechanisms

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Wellcome Trust Grant WT102561/Z/13/Z

Title: Receptor pharmacology of gamma oscillations induced in the avian hippocampal formation *in vitro*

Authors: ***P. DHEERENDRA**[1], N. M. LYNCH[2], M. O. CUNNINGHAM[1], T. V. SMULDERS[1];
[1]Inst. of Neurosci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [2]Univ. of Louisville, Louisville, KY

Abstract: Introduction: Gamma rhythms are a physiological feature of the mammalian hippocampus and play an important role in memory processing. However, such oscillations have not been explored in the avian hippocampal formation (HF) whose neuroanatomy is unlike its mammalian counterpart. We therefore investigate how divergent structures perform convergent functions. If similar microcircuitry underlies the avian HF, then we would predict that similar network properties should be detectable. Aims: We investigate the existence of gamma oscillations in avian HF, the underlying mechanisms of rhythmogenesis and the role of different receptors in this activity. Methods: We euthanized newly hatched chicks by cervical dislocation. We employed *in vitro* electrophysiology to record local field potentials in chick brain slices (400 μm). Bath application of various agonists and antagonists allowed us to elucidate the receptor pharmacology of avian hippocampal gamma oscillations *in vitro*. Results: In P0 - P4 chick HF brain slices, persistent gamma frequency oscillations (peak power: 64 ± 24.8 μV2/Hz; peak frequency: 36 ± 1.4 Hz; n = 27 slices) were induced by the bath application of the cholinergic agonist, carbachol (10 μM). However, the bath application of kainate (50 - 800 nM), a glutamate receptor agonist, did not elicit gamma. Similar to other species, carbachol-evoked gamma oscillations were sensitive to GABAA, AMPA/kainate and muscarinic (M1) receptor antagonism. Conclusions: We conclude that in juvenile chick HF, gamma rhythmogenesis is cholinergic in nature. This is unlike in adult mammals where both cholinergic and glutamatergic mechanisms are known to exist. However, similar to mammalian species, muscarinic

acetylcholine receptor (mAChR) activated avian HF gamma oscillations are likely to arise via a pyramidal-interneuron gamma (PING) based mechanism.

Disclosures: P. Dheerendra: None. **N.M. Lynch:** None. **M.O. Cunningham:** None. **T.V. Smulders:** None.

Poster

118. Brain Cholinergic Mechanisms

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Great Ape Aging Project USPHS/NIH Grant 14380

Title: A comparative study of the cholinergic innervation of the basal ganglia among human and nonhuman primate species

Authors: *A. STEPHENSON[1], M. K. EDLER[1], L. J. WILSON[1], J. M. ERWIN[2], W. D. HOPKINS[3], B. JACOBS[4], P. R. HOF[5], C. C. SHERWOOD[2], M. A. RAGHANTI[1]; [1]Dept. of Anthrop., Kent State Univ., Kent, OH; [2]Dept. of Anthrop., The George Washington Univ., Washington DC, DC; [3]Emory Univ., Atlanta, GA; [4]Dept. of Psychology, Colorado Col., Colorado Springs, CO; [5]Fishberg Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Cholinergic innervation of the basal ganglia is important in learning and memory, and striatal cholinergic neurons have been implicated in the integration of cognitive and motivational states with behavior. Further, deficits in acetylcholine have been correlated with loss of cognitive function in Alzheimer's disease and schizophrenia. These lines of evidence suggest a potentially important role for this subcortical innervation in the evolution of human cognitive functions. The present study quantified axons and interneurons immunoreactive for choline acetyltransferase (ChAT) in regions of the executive and motor loops of the basal ganglia of humans, great apes

(chimpanzee and gorilla), Old World monkeys (macaque and baboon), and one New World monkey (capuchin). Stereologic methods were used to quantify ChAT-ir axon length density to neuron density (ALv/Nv) and the percentage of cholinergic neurons in striatal regions. The repeated-measures ANOVA for ChAT-ir ALv/Nv revealed significant main effects of species ($F_{5, 23} = 40.26$, $p < 0.05$) and area ($F_{3, 23} = 248.92$, $p < 0.05$), with a significant interaction ($F_{15, 23} = 31.65$, $p > 0.05$). Post hoc tests showed that apes had the highest ALv/Nv in both executive and motor loops. For the percentage of ChAT-ir neurons within the striatum, there was a significant main effect of species ($F_{5, 25} = 35.19$, $p < 0.05$), with gorillas displaying the highest percentage, followed by capuchins. Interestingly, humans did not possess the highest or lowest density of cholinergic innervation, as expressed by axons or neurons. The phylogenetic differences observed were unexpected and indicated that a relative increase in cholinergic innervation was not required to support human cognitive abilities.

Disclosures: **A. Stephenson:** None. **M.K. Edler:** None. **L.J. Wilson:** None. **J.M. Erwin:** None. **W.D. Hopkins:** None. **B. Jacobs:** None. **P.R. Hof:** None. **C.C. Sherwood:** None. **M.A. Raghanti:** None.

Poster

118. Brain Cholinergic Mechanisms

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Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Acetylcholine, dopamine and exploration of uncertain outcomes

Authors: ***J. NAUDE**[1,2], S. TOLU[2], M. DONGELMANS[2], N. TORQUET[2], U. MASKOS[3], P. FAURE[2];
[1]CNRS, Paris, France; [2]Univ. Pierre et Marie Curie / CNRS, Paris, France, Paris, France; [3]Inst. Pasteur, Paris, France

Abstract: Making a choice among alternatives requires assigning them a value. This exploitation of known reward sources is under control of dopaminergic (DA) transmission. The firing of DA neurons is tuned by nicotinic acetylcholine receptors (nAChRs), in particular by β2-containing receptors, yet their contribution in value-based decisions remains unclear. Experimental data have implicated VTA β2*nAChRs in slow exploratory locomotion in open-fields without rewards. In the context of decision-making, exploration is opposed to the exploitation of a known reward source. We thus tested whether β2*-nAChRs expressed at the level of the VTA are implicated in the motivation to explore. We developed a mice-adapted multi-armed bandit task,

in which mice had to explore three locations associated with intracranial stimulation (ICSS). We compared the behavior of mice under different settings of the ICSS-bandit task: a certain setting where all locations deliver an ICSS, an uncertain setting where the rewarding locations are associated with different ICSS probabilities, and a dynamic setting where the places delivering ICSS change over time. Two forms of exploration were monitored: exploratory choices, expressed as a proportion of choices between the ICSS-associated locations; and exploratory locomotion, expressed as the time needed to reach the rewarding goals. We used computational models of reinforcement learning and decision-making to assess exploratory strategies in these different settings. We found that both exploratory choices and locomotion were affected by the expected uncertainty of the reward locations. This suggests that mice assigned a positive motivational value to expected uncertainty. Model-based analysis showed that mice lacking the nAChR β2-subunit lacked uncertainty-driven exploration, resulting in lesser adaptive strategies in a dynamic environment. Re-expression of β2*-nAChRs in the ventral tegmental area (VTA) restored uncertainty-driven exploration. Finally, we used electrophysiological recordings to correlate exploratory decisions with DA activity. Overall, our results implicate the nicotinic regulation of the VTA in the translation of expected uncertainty into motivation to explore.

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Poster

118. Brain Cholinergic Mechanisms

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Danish Strategic Research Council (COGNITO)

Title: Donepezil-induced c-Fos expression in the rat prefrontal cortex and nucleus accumbens is modulated by acute scopolamine pre-treatment

Authors: *F. WICHERN[1], F. ESCLASSAN[2], G. GILMOUR[2], J. D. MIKKELSEN[1];
[1]Neurobio. Res. Unit, Rigshospitalet, Copenhagen Univ. Hosp., Kobenhavn O, Denmark; [2]Eli Lilly and Co., Lilly Res. Labs., Windlesham, United Kingdom

Abstract: Cholinergic pathways play an important role in cognitive function, and impaired cholinergic neurotransmission is considered to underlie the pathogenesis of schizophrenia and Alzheimer's disease. Lesions of cholinergic neurons in the forebrain or pharmacological

blockade of muscarinic acetylcholine receptors have been shown to impair cognitive function in humans and animals. Administration of donepezil, a reversible acetylcholinesterase inhibitor, increases cholinergic neurotransmission and improves cognitive function and the drug is approved for the symptomatic treatment of mild and moderate cognitive impairments in Alzheimer's disease. In this study, we investigated the impact of acute administration of donepezil on c-Fos expression, a surrogate biomarker for neuronal excitation, in specific forebrain regions known to be involved in cognition. **Furthermore, we investigated to what extent this activation was mediated via muscarinic receptors by pre-administration of muscarinic receptor antagonists**. Adult male Wistar rats were treated with donepezil hydrochloride (5 mg/kg; p.o.) alone or pre-treated with either the non-selective muscarinic antagonist scopolamine hydrobromide (0.1 mg/kg; s.c.), or the scopolamine analogue methylscopolamine (0.1 mg/kg; s.c.). Methylscopolamine is also a non-selective muscarinic receptor antagonist, but is in contrast to scolopamine unable to cross the blood-brain barrier. One hour after donepezil treatment, the animals were perfused and sections of the forebrain were processed for c-Fos-immunohistochemistry. A single dose of donepezil significantly increased the number of c-Fos positive neurons in the medial prefrontal cortex and nucleus accumbens (both shell and core region). While scopolamine pre-treatment prevented the donepezil-stimulated c-Fos induction in the prefrontal cortex and the nucleus accumbens, methylscopolamine pre-treatment blocked donepezil-induced c-Fos expression only in the nucleus accumbens. These studies further support that cholinergic neurotransmission is increased in the prefrontal cortex responsible for improvement of cognitive function. Because scopolamine and methylscopolamine administration differently inhibited donepezil-stimulated c-Fos induction in the brain, this suggests both indirect and direct effects of scopolamine on cholinergic activity in the brain.

Disclosures: F. Wichern: None. **F. Esclassan:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **G. Gilmour:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **J.D. Mikkelsen:** None.

Poster

118. Brain Cholinergic Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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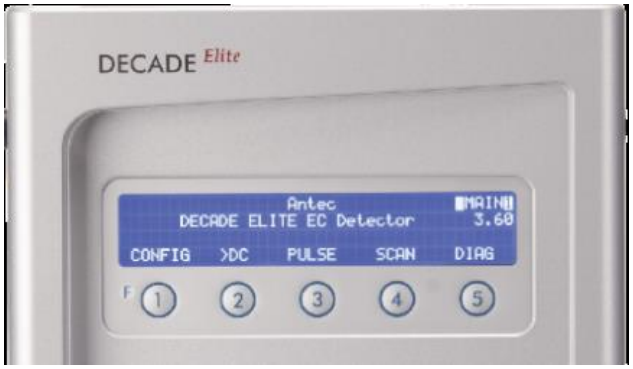
Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Sensitive analysis of acetylcholine in brain microdialysates using the ALEXYS (U)HPLC-ECD system

Authors: *M. EYSBERG, L. M. VAN HEERWAARDEN, H.-J. BROUWER, N. J. REINHOUD;
Antec BV, Zoeterwoude, Netherlands

Abstract: In the central nervous system, acetylcholine levels are associated with attention, learning, memory, consciousness, sleep, and control of voluntary movements. Detection of basal (sub-nanomolar) levels of acetylcholine in brain microdialysate samples, requires an optimized method. The recent release of the new DECADE Elite (electrochemical detector) in combination with the ultra-sensitive SenCell with a platinum working electrode make it possible to quantify basal acetylcholine levels in brain microdialysates. For the measurement of acetylcholine an Immobilized Enzyme Reactor (IMER) was used to convert acetylcholine into hydrogenperoxide, which can be detected either on a glassy carbon electrode coated with Horse Radish Peroxydase (HRP) or directly on a Pt working electrode. The HRP method needs laborious regular preparation of electrodes. The application of a Pt electrode is more user friendly. The robust method is shown to be sensitive and applicable to measure acetylcholine levels in brain microdialysates.



Disclosures: **M. Eysberg:** None. **L.M. van Heerwaarden:** None. **H. Brouwer:** None. **N.J. Reinhoud:** None.

Poster

119. GPCR I

Location: Hall A

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Program#/Poster#: 119.01/A72

Topic: B.03. G-Protein Coupled Receptors

Support: DMRF Foundation

Title: Post-synaptic interaction of D1R and M4 mAChR signaling pathways in striatonigral spiny projection neurons

Authors: *T. PANCANI[1], D. GUIMARAES[2], M. EHRLICH[3], P. CONN[1];
[1]Vanderbilt Ctr. for Neurosci. Drug Discovery, Vanderbilt Univ. Med. Ctr., Nashville, TN;
[2]Federaal Univ. of Minas Gerais, Belo Horizonte, Brazil; [3]Icahn Sch. of Med. at Mount Sinai, New Yourk, NY

Abstract: The striatum serves as a major relay unit for the basal ganglia, and receives dopaminergic inputs from the substantia nigra pars compacta and glutamatergic inputs from the cerebral cortex and thalamus. The interaction between dopamine (DA) and glutamate (Glu) strongly determines striatal output and regulation of motor function. Reversal of hyperlocomotion induced by the dopaminergic stimulants (i.e. amphetamine), is a widely used animal model of for assessing antipsychotic-like activity of pharmacological agents. D1 receptor (D1R)-mediated activation of the Gαolf/adenylyl cyclase (AC) and protein kinase A (PKA) in striatonigral SPNs (D1-SPNs) is involved in the hyperlocomotor response to psychostimulants, through increase in DARPP-32 phosphorylation and potentiation of NMDA receptor currents (INMDA). The M4 subtype of muscarinic acetylcholine receptor (mAChR), is known to play an important role in the modulation of DA activity in striatum. We have shown that M4 positive allosteric modulators (PAMs) play a pivotal role in the inhibition of *in vivo* responses to amphetamine, suggesting that M4 could represent a novel therapeutic target for the treatment of schizophrenia. However, while these behavioral effects are likely to be partially due to M4-mediated inhibition of DA release, we now show that the novel M4 PAM VU0467154 reverses the increase in locomotor activity induced by the direct-acting D1R agonist SKF82958. This suggests that M4 PAMs could also reduce DA signaling acting downstream from inhibition of DA release. Interestingly, M4 couples to Gαi/o, which inhibits AC and could directly antagonize the effects of D1R activation on the Gαolf/AC/PKA pathway. Consistent with this, mAChR agonists inhibit D1R-mediated activation of striatal AC and phosphorylation of DARPP-32. Furthermore, by monitoring INMDA in SPNs from acute slices we found that M4 activation can prevent the D1-mediated enhancement of INMDA in D1-SPNs. We are also investigating the effects of treatment with VU0456154 on D1R agonist-mediated hyperlocomotion in mice lacking M4 selectively on D1-SPNs. These results demonstrate that M4-mediated antipsychotic effects could also result from direct postsynaptic actions of M4 onto D1- SPNs.

Disclosures: **T. Pancani:** None. **D. Guimaraes:** None. **M. Ehrlich:** None. **P. Conn:** None.

Poster

119. GPCR I

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Support: NIMH Grant R01 MH073676

NIMH Grant 2R01 MH082867

Title: Role of GABAA receptors and mGlu5 signaling in M1-dependent long-term depression in prefrontal cortex

Authors: *A. GHOSHAL, S. P. MORAN, J. M. ROOK, J. W. DICKERSON, Z. XIANG, C. W. LINDSLEY, P. J. CONN;
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Abstract: Application of carbachol (CCh) can lead to concentration dependent long-term depression (LTD) in the layer 2/3 to layer 5 excitatory synaptic transmission of the mouse prefrontal cortex (PFC). PFC LTD induced by 50μM CCh (mLTD) can be completely blocked by the M1 orthosteric antagonist VU0255035, is absent in M1-KO mice, and can be potentiated by an M1-selective positive allosteric modulator (PAM) when a lower threshold concentration of CCh is used. These data indicate that this mLTD is mediated by the M1 muscarinic subtype. In addition, we demonstrated that mLTD is absent in a mouse model of schizophrenia, achieved by repeated administration of phencyclidine (NMDA receptor antagonist). In the present study we further elucidated the mechanism of mLTD in the mouse PFC. Using slice electrophysiology, we recorded changes of field excitatory post synaptic potentials (fEPSPs) in layer 5 PFC following CCh application under different conditions. When fEPSPs were recorded in presence of GABAA antagonist bicuculline (20μM), 50μM CCh failed to induce any mLTD in the drug-naïve mice, indicating that GABAA receptors play an important role in the induction of mLTD. In contrast, pretreatment with NMDA receptor antagonist AP-V (50μM) failed to block CCh-induced mLTD, suggesting that mLTD is NMDA receptor independent. However, pretreatment with the metabotropic glutamate receptor 5 (mGlu5) negative allosteric modulator, MPEP (30μM) 10 min before and during CCh application completely blocked the induction of mLTD. The involvement of mGlu5 in mLTD was further evident in studies that included the mGlu5 positive allosteric modulator (PAM) VU0409551. 10μM VU0409551 was able to potentiate a threshold form of mLTD (induced by 10μM CCh) into a robust mLTD in the mouse PFC of drug-naïve mice.

Additionally, mGlu5 PAMs were also able to fully rescue mLTD deficits in the PCP-treated mice. Thus, acute pretreatment of 10μM VU0409551 prior to 50μM CCh led to robust LTD in the PCP-treated mice. These studies, taken together, convincingly show that mLTD is NMDA receptor independent but is dependent on inhibitory neurotransmission as well as concurrent activation of mGlu5 receptors. We are currently performing additional whole cell electrophysiology experiments to determine the nature of interactions between muscarinic, metabotropic glutamatergic and GABA receptors in inducing mLTD in the PFC.

Disclosures: **A. Ghoshal:** None. **S.P. Moran:** None. **J.M. Rook:** None. **J.W. Dickerson:** None. **Z. Xiang:** None. **C.W. Lindsley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Astrazeneca, Bristol-Myers Squibb, Janssen Therapeutics. **P.J. Conn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Astrazeneca, Bristol-Myers Squibb, Janssen Therapeutics.

Poster

119. GPCR I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 119.03/A74

Topic: B.03. G-Protein Coupled Receptors

Support: R01 MH073676

P50 NS071669

F32 MH095285

Title: M4-muscarinic receptors attenuate striatal dopamine release via production of a local messenger in direct pathway medium spiny neurons

Authors: *D. J. FOSTER[1], J. WILSON[1], T. PANCANI[1], J. WESS[2], C. K. JONES[1], Z. XIANG[1], P. J. CONN[1];
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Abstract: Two seminal clinical studies have demonstrated that the M1/M4 preferring agonist xanomeline can provide significant therapeutic efficacy in treating psychosis in patients with Alzheimer's disease or schizophrenia. Unfortunately gastrointestinal side-effects, likely mediated by M2/M3 receptors, have removed xanomeline from consideration for clinical use. However, these studies suggest that compounds that selectively modulate M1 and/or M4 could be therapeutically beneficial in treating psychosis. Recent reports utilizing novel subtype-selective

M4 positive allosteric modulators (PAMs) have demonstrated efficacy in several preclinical models of psychosis. However, the mechanism whereby M4 can mediate these antipsychotic-like effects is not well understood. Here, we utilize a novel M4 PAM (VU0467154) and genetically modified mice to elucidate the role of M4 in regulating striatal dopamine release and dopamine-dependent behaviors. Application of the non-selective muscarinic receptor agonist Oxo-M decreased electrically-evoked striatal dopamine release in a concentration-dependent manner as monitored via fast scan cyclic voltammetry in acute brain slices. The inhibition of dopamine release induced by sub-maximal Oxo-M concentrations was potentiated by inclusion of VU0467154, demonstrating that M4 activation can negatively regulate dopaminergic signaling. In the striatum M4 is found primarily on direct pathway medium spiny neurons but is also expressed on cholinergic interneurons and cortical afferents. Interestingly, VU0467154-mediated reductions in dopamine release were completely absent in conditional knock-out mice in which M4 was selectively deleted from D1-expressing neurons. Additionally, the effects of VU0467154 were blocked by pretreatment with the CB2 antagonist AM630. Collectively, these results suggest that M4 activation attenuates dopamine release via endocannabinoid production in direct pathway medium spiny neurons, a signaling pathway that may be of therapeutic interest to developing novel treatments for schizophrenia.

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Poster

119. GPCR I

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 119.04/A75

Topic: B.03. G-Protein Coupled Receptors

Support: Conacyt 220448

Title: Functional interaction between histamine H_3 and adenosine A_{2A} receptors in rat striato-pallidal nerve terminals

Authors: *G. E. MORALES FIGUEROA, R. GONZÁLEZ-PANTOJA, J. ESCAMILLA-SÁNCHEZ, J.-A. ARIAS-MONTAÑO;
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Abstract: In the central nervous system histamine H_3 receptors (H_3Rs) regulate the synthesis and release of histamine, and the release of other neurotransmitters and neuromodulators. The globus pallidus (GP) belongs to the basal ganglia, a rich neural network involved in the control of motor behavior. The high expression of H_3Rs and the very low levels of the corresponding mRNA in GP strongly suggest that H_3Rs are located on the synaptic afferents to the nucleus, mainly on the terminals of striato-pallidal neurons. These neurons also express high levels of adenosine A_{2A} receptors (A_{2A}Rs), coupled to Gs proteins and whose stimulation modulates GABA release from GP synaptosomes and slices. H_3Rs couple to Gαi/o proteins, and in slices from rat striatum and substantia nigra pars reticulata their activation decreases K^+-evoked [^{3}H]-GABA release by opposing the stimulatory action of dopamine D_1 receptors, coupled to Gs proteins. The high expression of the H_3Rs and A_{2A}Rs on GP synaptosomal membranes (1327 and 454 fmol/mg protein, respectively) was confirmed with radioligand binding assays. A_{2A}R activation with the selective agonist CGS-21680 (3 nM) enhanced K^+-evoked [^{3}H]-GABA release from perfused GP synaptosomes (153 ± 20% of controls), and this effect was reduced by the H_3R selective agonist immepip in a concentration-dependent manner (EC_{50} 5.2 nM). In turn, the inhibitory effect of immepip (100 nM) was prevented by the H_3R antagonist/agonist inverse clobenpropit (3 μM). These results indicate that through H_3Rs histamine modulates GABA release from striato-pallidal neurons and that this effect is selectively exerted on the component of release regulated by adenosine A_{2A}Rs. A_{2A}R-mediated facilitation of GABA release depends on the cAMP/PKA pathway, and one plausible explanation for the H_3R selective effect is thus an action on the same signaling pathway. In GP slices A_{2A}R activation increased cAMP accumulation (295% of basal, EC_{50} 3.2 nM) and H_3R activation reduced by 50% this effect in a concentration-dependent manner (IC_{50} 5 nM). Further, direct activation of the cAMP/PKA pathway by forskolin (10 μM) mimicked the effect of A_{2A}R activation on K^+-evoked [^{3}H]-GABA release. An alternative explanation for the H_3R effect is the formation of H_3R-A_{2A}R heteromers, and preliminary experiments with rat GP synaptosomal membranes showed the H_3R agonist immepip to reduce in a modest (2-fold) but significant manner the A_{2A}R affinity for the agonist CGS-21680, whereas CGS-21680 increased the H_3R affinity for immepip, suggesting that H_3R/A_{2A}R dimerization does take place and modulates the affinity of these receptors for their respective agonists.

Disclosures: **G.E. Morales Figueroa:** None. **R. González-Pantoja:** None. **J. Escamilla-Sánchez:** None. **J. Arias-Montaño:** None.

Poster

119. GPCR I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 119.05/A76

Topic: B.03. G-Protein Coupled Receptors

Support: Conacyt 220448

Title: Adenosine A2A and histamine H3 receptors gather to modulate intra-striatal GABAergic transmission

Authors: *R. MÁRQUEZ GÓMEZ, C. GUTIERREZ-RODELO, J.-A. OLIVARES-REYES, J.-A. ARIAS-MONTAÑO;
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Abstract: In the central nervous system histamine modulates the synthesis and the release of several neurotransmitters through the activation of pre-synaptic H3 receptors (H3Rs). These receptors are widely expressed in the striatum, the main input nucleus of the basal ganglia, and in striato-pallidal neurons and cortico-striatal afferents H3Rs are co-expressed with adenosine A2A receptors (A2ARs), which increase glutamatergic cortico-striatal transmission and reduce GABAergic intra-striatal transmission. Although A2ARs and H3Rs canonically activate antagonistic signaling pathways, their modulatory role on assembled striatal synaptic transmission has remained largely unassessed. In this work we set out to determine the effect of A2AR and H3R co-activation on the function of collaterals of GABAergic striato-pallidal neurons. We first studied GABA uptake by striatal isolated nerve terminals (synaptosomes) and found that activation of either A2ARs or H3Rs reduced in a modest but significant manner GABA transport (-8 ± 2% and -12 ± 3%, respectively), and that their co-activation results in additive inhibition (-22 ± 3%). In order to explain these results we have addressed the possibility for these receptors to form a functional heterodimer, and herein we show that H3R activation diminishes A2AR affinity for the selective agonist CGS-21680 (increase in Ki value from 9 to 20 nM) and prevents cAMP accumulation induced by the same agonist. In contrast, A2AR activation had no effect on H3R affinity for the agonist RAMH. Further, co-immunoprecipitation assays suggest that A2AR/H3R interaction may occur on the striatal synapses, presumably on both the collaterals of GABAergic projection neurons and cortico-striatal terminals. A2A and H3 receptors have been proposed as new targets for the treatment of cognitive and motor disorders, and our results suggest an interaction that might bear relevance for the regulation of basal ganglia transmission and therefore for the design of drugs useful for the treatment of some of such disorders.

Disclosures: R. Márquez Gómez: None. **C. Gutierrez-Rodelo:** None. **J. Olivares-Reyes:** None. **J. Arias-Montaño:** None.

Poster

119. GPCR I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 119.06/A77

Topic: B.03. G-Protein Coupled Receptors

Support: Conacyt 220448

Title: Differential homologous desensitization of the human histamine H3 receptors of 445 and 365 amino acids

Authors: *A.-M. GARCÍA-GÁLVEZ, C. FLORES-CLEMENTE, R. GONZÁLEZ-PANTOJA, J. ESCAMILLA-SÁNCHEZ, J.-A. ARIAS-MONTAÑO;
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Abstract: Histamine H_3 receptors (H_3R) couple to Gi/o proteins and are primarily found as pre-synaptic receptors in the central nervous system, where they regulate the synthesis and release of histamine and the release of other neurotransmitters. Alternative splicing of the human H_3R (hH_3R) originates 20 isoforms, and in addition to the receptor of 445 amino acids (hH_3R_{445}), an isoform of 365 aa (hH_3R_{365}) is abundantly expressed in several brain regions. Desensitization is a major mechanism to regulate the functional response of G protein-coupled receptors (GPCRs). Homologous desensitization is triggered by the phosphorylation of activated receptors by GPCR kinases (GRKs), and we previously showed that the hH_3R_{445} stably expressed in CHO-K1 cells experiences homologous desensitization. The hH_3R_{365} lacks 80 residues in the third intracellular loop (i3), an important region for GPCR coupling to G proteins, as well as for the phosphorylation by GRKs and the subsequent binding of β-arrestins in the homologous desensitization mechanism. In this work we therefore set to study whether the hH_3R_{365} also experiences homologous desensitization and the possible differences with the hH_3R_{445}. Both hH_3R isoforms were stably expressed in CHO-K1 cells, and receptor levels were determined by [^{3}H]-N-α-methyl-histamine binding assays in membranes and whole cells. Functional desensitization was evaluated in cells pre-incubated with the H_3R agonist R-α-methyl-histamine (RAMH). The lack of 80 aa in the hH_3R_{365} had no effect on the expression by CHO-K1 cells, the affinity for selective ligands or the presence in the cell surface. Pre-incubation for 30 min with *RAMH* (1 μM) reduced by 58 ± 8% hH_3R_{365} signaling through Gi/o proteins and resulted in the loss of receptors from the cellular surface (-64 ± 10%). In contrast to the 445 aa isoform, the hH_3R_{365} affinity for the agonist immepip (pKi 8.65 ± 0.23) was not significantly reduced by RAMH pretreatment (pKi values 8.10 ± 0.15 and 8.20 ± 0.13 for pre-incubation with agonist for 30 and 60 min, respectively). Maximal desensitization differed in both the extent (96 15% and 58 ± 8% for hH_3R_{445} and hH_3R_{365}, respectively) and length of exposure required (60 and 30 min). Further, at 60 and 120 min of RAMH pre-incubation the hH_3R_{365} showed partial re-sensitization, whereas the hH_3R_{445} remained desensitized. These results suggest that the hH_3R_{365} experiences

homologous desensitization, but the process differs from the hH_3R_{445} in time-course, magnitude and re-sensitization.

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Poster

119. GPCR I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 119.07/A78

Topic: B.03. G-Protein Coupled Receptors

Title: Functional cross-talk between α1-adrenergic receptors and mGlu7 metabotropic glutamate receptors in heterologous expression systems and brain tissue: possible relevance in stress-relates psychiatric disorders

Authors: L. IACOVELLI[1], L. DI MENNA[2], N. USCHOLD-SCHMIDT[3], D. PETERLIK[3], C. STANGL[3], G. MOLINARO[2], R. ORLANDO[1], P. J. FLOR[3], A. DE BLASI[1], *G. BATTAGLIA[2], V. BRUNO[1,2], F. NICOLETTI[1,2];
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Abstract: Increasing evidence suggests that metabotropic glutamate (mGlu) receptors including mGlu7 receptor are important in the pathophysiology of stress-related psychiatric disorders such as anxiety and major depression. It has also been shown that mice deficient in mGlu7 receptors have an antidepressant-like behaviour and altered stress response. On the other hand, the role of adrenergic signalling in the modulation of mood and depression-related behaviour has also been pointed out both in clinical studies and in animal models. It has been shown that α1A-adrenergic (AR) signalling, but not α1B-AR signalling, produces antidepressant-like behaviour in the mouse and that prolonged stimulation of α1A-AR induces a reduction in anxious and depressive behaviour in animals (Doze et al, Brain Res, 2009, 1285:148-57; Doze et al, Mol Pharmacol, 2011 80:747-58). In the present study, we investigated the cross-talk between α1A-AR and mGlu7 receptors essentially using 3 different approaches. First, we performed experiments in HEK293 cells that were transiently transfected to express α1A-AR and mGlu7. We demonstrated that phenylephrine (PE)-induced polyphosphoinositide (PI)-hydrolysis was significantly reduced by both L-2-amino-4-phosphonobutanoate (L-AP4) and L-serine-O-phosphate (L-SOP), which activate mGlu7 receptors at high concentrations. Co-expression of the 2 receptors with the GRK2

C-terminal tail, completely prevented the mGlu7 effect. This demonstrates that mGlu7 effect on α1A-AR signalling is mediated by Gβγ. Accordingly, we further show that the mechanism of mGlu7 inhibition of α1A-AR-signalling requires MAPK activation because both L-AP4 and L-SOP inhibition of PE-induced PI hydrolysis was reduced in the presence of both UO126 and PD98059. Second, we used brain slices from the mouse cerebral cortex, in which we fully confirmed the ability of high concentrations of L-AP4 and L-SOP to inhibit noradrenaline-stimulated PI hydrolysis. Third, biochemical experiments have been integrated by behavioural and endocrinological experiments. We demonstrated that i.c.v. PE injection induced an anti-depressant behaviour in rats, as measured by the forced swimming test, and this effect was reduced by L-SOP administration. To study the responsiveness of the HPA axis, we collected trunk blood to measure plasma corticosterone levels, 30 min after i.c.v. injection of PE or L-SOP + PE. L-SOP strongly reduced the increase in corticosterone levels induced by PE. Our data demonstrate that mGlu7 receptors negatively modulate α1A-AR signalling and that the interplay between these 2 receptor systems may play a role in the pathophysiology of mood disorders.

Disclosures: **L. Iacovelli:** None. **L. Di Menna:** None. **N. Uschold-Schmidt:** None. **D. Peterlik:** None. **C. Stangl:** None. **G. Molinaro:** None. **R. Orlando:** None. **P.J. Flor:** None. **A. De Blasi:** None. **G. Battaglia:** None. **V. Bruno:** None. **F. Nicoletti:** None.

Poster

119. GPCR I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 119.08/A79

Topic: B.03. G-Protein Coupled Receptors

Title: Metabotropic glutamate receptor 5 upregulates surface NMDA receptor expression in striatal neurons via CaMKII

Authors: *D. JIN[1], B. XUE[2], L. MAO[2], J. WANG[3];
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Abstract: Metabotropic and ionotropic glutamate receptors are closely clustered in postsynaptic membranes and are believed to interact actively with each other to control excitatory synaptic transmission. Metabotropic glutamate receptor 5 (mGluR5), for example, has been well documented to potentiate ionotropic NMDA receptor activity, although underlying mechanisms

are poorly understood. In this study, we investigated the role of mGluR5 in regulating trafficking and subcellular distribution of NMDA receptors in adult rat striatal neurons. We found that the mGluR1/5 agonist DHPG concentration-dependently increased NMDA receptor GluN1 and GluN2B subunit expression in the surface membrane. Meanwhile, DHPG reduced GluN1 and GluN2B levels in the intracellular compartment. The effect of DHPG was blocked by an mGluR5 selective antagonist MTEP but not by an mGluR1 selective antagonist 3-MATIDA. Pretreatment with an inhibitor or a specific inhibitory peptide for synapse-enriched Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) also blocked the DHPG-stimulated redistribution of GluN1 and GluN2B. In addition, DHPG enhanced CaMKIIα activity and elevated GluN2B phosphorylation at a CaMKII-sensitive site (serine 1303). These results demonstrate that mGluR5 regulates trafficking of NMDA receptors in striatal neurons. Activation of mGluR5 appears to induce rapid trafficking of GluN1 and GluN2B to surface membranes through a signaling pathway involving CaMKII.

Disclosures: **D. Jin:** None. **B. Xue:** None. **L. Mao:** None. **J. Wang:** None.

Poster

119. GPCR I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 119.09/A80

Topic: B.03. G-Protein Coupled Receptors

Title: Colocalization of mu-opioid receptors and metabotropic glutamate receptors in rat spinal cord and in dorsal root ganglia

Authors: *A. E. KALYUZHNY, M. GRAHEK, A. PTAK, R. ZIMMERMAN, J. HAGEN, J. HOUCHINS, S. STOESZ, K. REAGAN;
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Abstract: Glutamate is the major excitatory neurotransmitter in CNS acting via ionotropic (NMDA, Kainate and AMPA) and metabotropic glutamate receptors (mGlu). During neuropathic pain, glutamatergic neurotransmission becomes elevated and impairs the antinociceptive effects of opioid drugs. It has been reported that spinal mGluRs can modulate nociception and mGluR antagonists can potentiate effects of morphine. To analyze the anatomical substrate underlying the interactions between mGluRs and mu-opioid receptors, we have employed multi-color immunofluorescence histochemistry on tissue sections of rat spinal cord and dorsal root ganglia (DRG). Polyclonal and mouse monoclonal antibodies were raised against recombinant mGluR1

(Group I), mGluR2/3 and mGluR2 (Group II), and mGluR8 (Group III) proteins. Rabbit monoclonal antibodies against the rat mu-opioid receptor (OPRM1) were raised against a peptide immunogen from the N-terminal portion of the receptor. There was an overlap in distribution of mGluRs and mu-opioid receptors in spinal cord dorsal horn and we observed co-localization in some punctate profiles. In spinal cord dorsal horn, mGluRs were distributed in lamina I - III with more intense labeling in lamina 2, whereas mu-opioid receptors were mostly in lamina I - II with higher density in lamina I. Labeling for mGluR1, mGluR2, mGluR2/3 and mGluR8 in the DRG was more abundant in medium and large size neurons rather than in small-sized neurons, whereas labeling for mu-opioid receptors was detected predominantly in small-sized neurons. In DRG we observed colocalization of mu-opioid receptors with mGluRs in small-sized neurons but the frequency of colocalization was different depending on the type of mGluR. Our data indicate that there is an anatomical overlap in distribution of mu-opioid receptors and mGluRs in spinal cord. Colocalization of mu-opioid and mGluRs in small-sized DRG neurons suggest direct interactions between mu-opioid and metabotropic glutamate receptors.

Disclosures: **A.E. Kalyuzhny:** None. **M. Grahek:** None. **A. Ptak:** None. **R. Zimmerman:** None. **J. Hagen:** None. **J. Houchins:** None. **S. Stoesz:** None. **K. Reagan:** None.

Poster

119. GPCR I

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Program#/Poster#: 119.10/A81

Topic: B.03. G-Protein Coupled Receptors

Title: Functional cross-talk between group-I and group-II metabotropic glutamate receptors in heterologous expression systems and brain tissue

Authors: L. DI MENNA[1], L. IACOVELLI[2], V. BRUNO[2,1], G. BATTAGLIA[1], *F. NICOLETTI[2,1];
[1]Mol. Pathology, I.R.C.C.S. Neuromed, Pozzilli, Italy; [2]Univ. Sapienza, Rome, Italy

Abstract: One of the dogmas in the field of metabotropic glutamate (mGlu) receptors is that mGlu1 and mGlu5 receptors are coupled to polyphosphoinositide (PI) hydrolysis via a Gq/11 protein, whereas mGlu2 and mGlu3 receptors are negatively coupled to adenylyl cyclase activity via a Gi/o protein. This general belief has conditioned functional studies carried out in cultured neurons and brain tissue. However, an intriguing observation is that the prototypical mGlu1/5 receptor agonist, 3,5-dihydroxyphenylglycine (DHPG), is less efficacious than (1S,3R,4S)-1-aminocyclo-pentane-1,3,4-tricarboxylic acid (1S,3R-ACPD) in stimulating PI hydrolysis in brain

slices. This difference does not reflect a greater intrinsic efficacy of 1S,3R-ACPD at mGlu1 and mGlu5 receptors, but rather the ability of the compound to recruit either mGlu2 or mGlu3receptors. If combined with selective mGlu2/3 receptor agonists, which are inactive on their own, DHPG stimulates PI hydrolysis to the same extent as 1S,3R-ACPD in hippocampal slices (Genazzani et al 1994 Brain Res 659:10-16; Schoepp et al 1996 Neuropharmacology 35:1661-1672). This suggests that a functional cross-talk between group-I and group-II mGlu receptors exists, but the molecular nature of this cross-talk is unknown. In HEK-293 cells co-expressing mGlu1 receptors with either mGlu2 or mGlu3 receptors, DHPG-stimulated PI hydrolysis was amplified by the mGlu2/3 receptor agonist, (-)-2-oxa-4-aminocyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268). A similar potentiation was observed when the mGlu5 receptor was co-expressed with either mGlu2 or mGlu3 receptors. In cortical slices prepared from adult mice, LY379268 was able to potentiate DHPG-stimulated PI hydrolysis, as expected. However, potentiation was lost in slices prepared from mGlu3-/- mice and was unaffected in slices prepared from mGlu2-/- mice. This suggested that native mGlu3, but not mGlu2, receptors are functionally linked to mGlu1/5 receptors and play a permissive role on mGlu1/5 receptor-mediated PI hydrolysis. This hypothesis was supported by data obtained in cortical slices prepared from mice at postnatal day 14 (PND14). mGlu5 and mGlu3 receptors are known to be highly expressed in the early postnatal life, and all group-I mGlu receptor agonists are known to cause large stimulations of PI hydrolysis at this age. Interestingly, DHPG-stimulated PI hydrolysis was largely reduced in cortical slices prepared from PND14 mGlu3-/- mice as compared to age-matched wild-type or mGlu2-/- mice. Thus, it appears that endogenous activation of mGlu3 receptors largely contributes to mGlu1/5 receptor-mediated PI hydrolysis during early brain development.

Disclosures: **L. Di Menna:** None. **L. Iacovelli:** None. **V. Bruno:** None. **G. Battaglia:** None. **F. Nicoletti:** None.

Poster

119. GPCR I

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Topic: B.03. G-Protein Coupled Receptors

Support: KAKENHI 23700390

KAKENHI 26460707

MEXT S1311011

Title: Bidirectional interaction between adenosine A1 receptor and type-1 metabotropic glutamate receptor

Authors: *Y. KAMIKUBO[1], T. TABATA[2], T. SAKURAI[1];
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Abstract: Adenosine A1 receptor (A1R) is a G protein-coupled receptor (GPCR) for the ubiquitous neuromodulator adenosine and is known to play roles in regulation of neuronal excitability, arousal level, and pain sensitivity. We previously reported a new type of neuronal action of A1R; in cerebellar Purkinje cells, A1R activation leads to the attenuation of neuronal responses involving type-1 metabotropic glutamate receptor (mGluR1), a GPCR for the excitable neurotransmitter. Such a response includes long-term depression of postsynaptic glutamate-responsiveness, a cellular basis for cerebellar motor learning. Here we explore in more detail interaction between A1R and mGluR1 using non-neuronal heterologous expression cells. Our co-immunoprecipitation and FRET analysis revealed that A1R and mGluR1 form complexes even in a non-neuronal cell type. We used cAMP production assay to evaluate the activity of the signaling cascade coupled to A1R and found that mGluR1 activation leads to inhibition of this cascade. These findings demonstrate that the two classes of GPCRs have a cellular environment-independent intrinsic property to form heteromeric complexes and mutually modulate their signaling. This modulation may enable the GPCR complex as a whole to mediate intriguing cellular responses.

Disclosures: **Y. Kamikubo:** None. **T. Tabata:** None. **T. Sakurai:** None.

Poster

119. GPCR I

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Topic: B.03. G-Protein Coupled Receptors

Support: CONACYT México 152326 to B.F.

Title: Cannabinoid control of GABAergic transmission in the globus pallidus is switched during dopamine D2 receptor (D2Rs) activation

Authors: *R. N. CABALLERO[1], I. CONDE ROJAS[1], R. SÁNCHEZ-ZAVALETA[1], F. PAZ-BERMÚDEZ[1], B. FLORÁN[2], D. ERLIJ[3];

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Abstract: Neurochemical evidence shows that activation of CB1 cannabinoid (CB1Rs) with exogenous cannabinoid agonists in the basal ganglia is switched from an inhibitory effect into a stimulatory response by activating D2Rs. This effect has been attributed to restricted Gi/o protein coupling because it is mimicked by pretreatment with pertussis toxin. In the current experiments we use electrophysiological techniques to determine the significance of these effects in the control of pallidal synaptic transmission. Paired pulse technique recorded with whole patch technique in GPe cells showed that the CB1 agonist ACEA depressed GABA release initiated by stimulation of striato-pallidal terminals. The inhibition of release was transformed into stimulation by treatment with either the D2R antagonist quinpirole or pertussis toxin. This stimulation was blocked by treatment with the PKA inhibitor H89. Depolarization of the postsynaptic membrane (DSI) induced a reduction of spontaneous inhibitory currents that was blocked by pretreatment with the cannabinoid antagonist AM 251. Activation of D2R with quinpirole converted the DSI into a stimulatory response. This stimulatory response disappeared when PKA was blocked with H89. These experiments show that cannabinoid effects on GABA transmission in the GPe are modulated by dopamine. The switch in cannabinoid effects appears to be mediated by restricted Gi/o protein coupling because it is mimicked by pertussis toxin treatment. The stimulation of release also appears to be mediated by coupling to Gs protein because it disappears when activation of PKA is blocked with H89. The switch in the effects of depolarization shows that the action of endogenous cannabinoids is controlled by activation of D2Rs.

Disclosures: R.N. Caballero: None. **I. Conde Rojas:** None. **R. Sánchez-Zavaleta:** None. **F. Paz-Bermúdez:** None. **B. Florán:** None. **D. Erlij:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.01/A84

Topic: B.03. G-Protein Coupled Receptors

Support: NINDS R21 NS088916-01 to GTS

Title: Group I mGluR-β-arrestin signaling in hippocampal synaptic plasticity

Authors: *A. G. ENG[1,2], D. A. KELVER[1], T. P. HEDRICK[1], G. T. SWANSON[1];
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Abstract: A novel signaling mode mediated by the β-arrestin (βarr 1 and βarr 2) proteins has been observed in numerous family A and family B seven transmembrane receptors (7TMRs). Far less is known about βarr signaling by family C 7TMRs such as the group I metabotropic glutamate receptors (mGluR1 and mGluR5), which are viewed as promising targets in the treatment of neuropsychiatric disorders including epilepsy, Fragile X syndrome, cocaine addiction, and neuropathic pain. Accordingly, our objectives are to determine whether group I mGluR-βarr signaling underlies synaptic plasticity and to identify downstream effectors of a putative mGluR-βarr signaling pathway. We tested the hypothesis that βarr $1^{-/-}$ or βarr $2^{-/-}$ mice possess selective deficits in mGluR-dependent plasticity by conducting blinded studies comparing hippocampal slice recordings obtained from knockout mice and wildtype littermates. We observed that mGluR1-dependent potentiation of EPSCs induced by low frequency, paired stimulation of hippocampal mossy fiber inputs to CA3 pyramidal neurons was absent in mice lacking βarr 2 (post-train amplitudes were 94 ± 9% of baseline, n=13 for βarr $2^{-/-}$, compared to 131 ± 7%, n=11 for βarr $2^{+/+}$, p=0.004) but intact in mice lacking βarr 1 (137 ± 14% n=14 for βarr $1^{-/-}$, 143 ± 6% n=10 for βarr $1^{+/+}$, p=0.66). In contrast, long-term potentiation of mossy fiber-CA3 synapses induced by high frequency stimulation was unaffected by gene targeting of either βarr isoform (190 ± 23% n=9 for βarr $1^{-/-}$, 163 ± 16% n=7 for βarr $1^{+/+}$, p=0.34; 165 ± 16% n=8 for βarr $2^{-/-}$, 167 ± 14% n=6 for βarr $2^{+/+}$, p=0.94). mGluR-dependent long-term depression of Schaffer collateral-CA1 synapses elicited by low frequency stimulation is also unaffected in βarr $1^{-/-}$ (74 ± 9% n=10 for βarr $1^{-/-}$, 80 ± 8% n=15 for βarr $1^{+/+}$, p=0.6) and βarr $2^{-/-}$ mice (93 ± 11% n=14 for βarr $2^{-/-}$, 72 ± 5% n=18 for βarr $2^{+/+}$, p=0.1). Finally, we tested whether MAPK or Src family tyrosine kinases underlie mGluR1-βarr 2-mediated plasticity of mossy fiber-CA3 synapses. Pharmacological antagonism of MEK1/2 prevented induction (102 ± 7% n=7 for u0126-treated slices, 137 ± 9% n=17 for vehicle slices, p=0.004), as did inhibition of Src family tyrosine kinases (111 ± 8% n=10 for PP2-treated slices, p=0.03 compared to same vehicle group), but inhibition of cRaf-1 kinase had no effect (137 ± 20% n=9 for GW5074-treated slices, p=0.98 compared to vehicle). Together, these data delineate a new role for the βarr 2 isoform in conjunction with mGlu1 receptors, support the involvement of βarrs in learning and memory processes, and suggest that pathway selection could be viable in mGluR-targeted therapeutics.

Disclosures: **A.G. Eng:** None. **D.A. Kelver:** None. **T.P. Hedrick:** None. **G.T. Swanson:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.03. G-Protein Coupled Receptors

Support: UGC, India

IISER Mohali, India

DBT, India

Title: Group I metabotropic glutamate receptors (mGluRs) regulation in the central nervous system

Authors: *P. K. MAHATO, S. PANDEY, S. BHATTACHARYYA;
Dept. of Biol. Sci., Indian Inst. of Sci. Educ. & Res., Mohali, India

Abstract: Receptor trafficking in a cell is a crucial cellular process which ensures delivery of the receptors to proper destination in the cell membrane either by lateral movement on plasma membrane or direct insertion at the specific site at the neuronal surface. Glutamate is a major excitatory neurotransmitter in the central nervous system and acts through Ionotropic Glutamate Receptors and Metabotropic Glutamate Receptors (mGluRs). Glutamate receptors are localized as clusters at the neuronal membrane through interactions with intracellular scaffolding proteins but are also reported to be found dispersed in other regions of the membrane. There is a dynamic equilibrium between the different pools of receptors and mechanism of the process is poorly understood. Group I mGluRs belong to a family of G-protein coupled receptors (GPCRs) that show widespread distribution in the brain and they primarily localize at the post-synaptic sites. mGluR5, a Group I mGluR family member, is positively coupled to the IP3/DAG pathway. It has been reported that mGluR5 gets desensitized on ligand binding. The objective of our study was to investigate the post-desensitization events of mGluR5. Our finding suggests that the receptor gets internalized on ligand application and subsequent to the internalization mGluR5 enters the recycling compartment followed by the recycling of the receptor to the cell surface. We have also observed that inhibition of specific protein phosphatases either pharmacologically or by endogenous knockdown leads to the blocking of mGluR5 recycling differentially. In addition, we are also exploring the role of Homer proteins in the regulation of Group I mGluRs in the CNS. Homer is a scaffolding protein that has been reported to regulate the surface expression and function of Group I mGluRs. We are addressing these questions using Lentiviral mediated “molecular replacement” approach. This strategy involves knockdown of endogenous Homer protein and simultaneous expression of various forms of Homer for a possible rescue. We are studying the structure-function analysis of Homer in Group I mGluR regulation using this elegant approach.

Disclosures: **P.K. Mahato:** None. **S. Pandey:** None. **S. Bhattacharyya:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.03/A86

Topic: B.03. G-Protein Coupled Receptors

Support: 2RO1NS053398

Title: Regulation of cerebrocortical neuron spontaneous Ca^{2+} oscillations by metabotropic glutamate receptors

Authors: *S. MEHROTRA, T. F. MURRAY;
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Abstract: Metabotropic glutamate receptors (mGluRs) are a family of G-protein coupled receptors activated by the neurotransmitter glutamate. These receptors are classified into three groups based on sequence homology, signal transduction mechanism and pharmacological profile. The Group I (mGluR 1 and 5) receptors are mainly postsynaptic, whereas Group II (mGluR 2 and 3) and Group III (mGluR 4, 6, 7 and 8) are primarily presynaptic. Dissociated primary cultures of cerebrocortical cortical neurons exhibit spontaneous Ca^{2+} oscillations in the presence of physiological [Mg^{2+}]; these oscillations are thought to correspond to synchronized bursts of action potentials with attendant release of glutamate. This study was designed to characterize individual contribution of each of the three groups of mGluRs towards spontaneous Ca^{2+} oscillation in murine cerebrocortical neurons. Cerebrocortical primary cultures from Swiss-Webster mice (E17) were used in this study. Cultures maintained for 10 days *in vitro* (DIV) were used. Primary neuronal cultures were loaded with Fluo-3, incubated at 37°C for 1 hour and transferred to a fluorescence plate reader (FLIPR). The emitted fluorescence signals were recorded at 515–575 nm after excitation at 488 nm. We found that the Group I agonist (*RS*)-3,5-dihydroxyphenylglycine, DHPG (EC_{50}= 266 nM, 95% CI=61-1144 nM) produced a concentration-dependent increase in the frequency of spontaneous Ca^{2+} oscillations. In contrast the mixed Group I/II agonist (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid, trans-ACPD suppressed Ca^{2+} oscillations with an IC_{50} of 715 nM (95% CI=410-1,248). This response to trans-ACPD was reversed by the mixed Group I/II antagonist (*RS*)-α-methyl-4-carboxyphenylglycine, (RS)-MCPG. The selective group II agonist (1*R*,4*R*,5*S*,6*R*)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid, LY379268 (IC_{50}= 3 nM, 95% CI=1.8-4.9 nM) mimicked the response to trans-ACPD in producing a concentration-dependent complete inhibition of Ca^{2+} oscillations. This inhibitory response was reversed by the selective group II mGluR antagonist (2*S*)-2-amino-2-[(1*S*, 2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic

acid, LY341495 (K_B= 0.9 nM). The group III agonist L-(+)-2-amino-4-phosphonobutyric acid, L-AP4 (IC_{50}= 29 nM, 95% CI=29-293 nM) produced a partial inhibition of Ca^{2+} oscillations, which was reversed by the antagonist of group III mGluRs, (*RS*)-α-methylserine-*O*-phosphate, MSOP (EC_{50}= 3.8 μM, 95% CI=0.5-25 μM). These data collectively demonstrate the predominance of inhibitory group II mGluRs in controlling synchronized Ca^{2+} oscillations in murine cerebrocortical neurons.

Disclosures: S. Mehrotra: None. **T.F. Murray:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

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Program#/Poster#: 120.04/A87

Topic: B.03. G-Protein Coupled Receptors

Support: CSIR Senior Research Fellowship

IISER Mohali

DBT, India

Title: Cellular and molecular mechanisms of group I mGluR trafficking in the central nervous system

Authors: *S. PANDEY, P. K. MAHATO, S. BHATTACHARYYA;
Dept. of Biol. Sci., Indian Inst. of Sci. Educ. and Res., Mohali, India

Abstract: Central nervous system (CNS) is composed of millions of neurons, which are connected through each other via synapse. Synapse is the junction where preceding neuron transfers its information to later neuron. Proper release of neurotransmitters in the pre-synaptic terminus and trafficking of neurotransmitter receptors in the post-synaptic terminus play crucial role in normal synaptic transmission and any alteration in these regulations could lead to pathological symptoms. mGluR1 is a GPCR that belongs to the group I family of metabotropic glutamate receptors (mGluRs). This receptor is positively coupled to Gαq/11 and activates protein kinase C pathway upon activation. mGluR1 is distributed throughout the CNS and its role has been implicated in various forms of synaptic plasticity including learning and memory along with its role in various neurological disorders like Fragile X syndrome and Autism. In the last few years this receptor has emerged as a potential therapeutic target for various neurological and psychiatric diseases. While studying trafficking of mGluR1, we observed that upon agonist

stimulation mGluR1 gets endocytosed and recycled back to the surface in both non-neuronal and neuronal cells. We also found a crucial role of Protein phosphatase 2A (PP2A) in the recycling of mGluR1. Inhibition of PP2A by various means (pharmacologically, over-expression of dominant negative PP2A and endogenous knockdown of PP2A) resulted in the inhibition of the recycling of mGluR1. Presently we are studying the role of Tamalin/GRASP, a postsynaptic scaffolding protein in the trafficking and signaling of mGluR1. Tamalin has been reported to interact with mGluR1 and regulates its surface stability. We are exploring the role of Tamalin in group I mGluR trafficking using lentiviral mediated "molecular replacement" approach. This approach involves acute knockdown of endogenous protein and simultaneous expression of various forms of that protein for possible rescue. We are currently using this elegant approach to unravel the structure-function analyses of Tamalin in the trafficking of group I mGluRs in the central nervous system.

Disclosures: **S. Pandey:** None. **P.K. Mahato:** None. **S. Bhattacharyya:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.05/A88

Topic: B.03. G-Protein Coupled Receptors

Title: Regulation of KCC2 expression by group-I metabotropic glutamate receptors in the cerebellum

Authors: *S. NOTARTOMASO[1], G. MASCIO[1], P. SCARSELLI[1], K. MARTINELLO[1], S. FUCILE[1], G. BATTAGLIA[1], F. NICOLETTI[1,2];
[1]I.R.C.C.S. Neuromed, Pozzilli, Italy; [2]Univ. "Sapienza", Rome, Italy

Abstract: The mGlu1 metabotropic glutamate receptor is found in large amounts in cerebellar Purkinje cells, where it is a key player in mechanisms of activity-dependent synaptic plasticity underlying motor learning. The cognate mGlu5 receptor is instead nearly absent in Purkinje cells of adult mice or rats, but is found in Purkinje cells during the first ten days of postnatal life. Interestingly, the mGlu5 receptor is re-expressed in adult Purkinje cells in response to pharmacological blockade of mGlu1 receptors or under pathological conditions, such as type-1 spinocerebellar ataxia or experimental autoimmune encephalomyelitis (Notartomaso et al., Mol. Brain, 2013; Fazio et al., Neuropharmacology, 2012). Here, we examined whether mGlu1 and mGlu5 receptors have any role in regulating the expression of the K+-Cl− co-transporter, KCC2. KCC2 is expressed by mature neurons, where it lowers the Cl- equilibrium potential to more

negative values than the resting potential, thereby allowing the inhibitory action of GABAA receptors. GABAA receptors are instead excitatory in immature neurons, which do not express KCC2. In Purkinje cells, KCC2 is localized at climbing fiber synapses during postnatal development, and at basket cell GABAergic synapses in the adult life (Kawakita et al., Eur. J. Neurosci., 2012). We have found that expression of KCC2 in Purkinje cells is affected by drugs that positively or negatively modulate mGlu1 receptors, and show substantial changes in mice with genetic deletion of mGlu5 receptors. We are currently investigating whether pharmacological modulation of mGlu1 receptors or the lack of mGlu5 receptors cause changes in the Cl- equilibrium potential in Purkinje cells and affect the behavioural response to GABAA receptor activation in mice.

Disclosures: S. Notartomaso: None. **G. Mascio:** None. **P. Scarselli:** None. **K. Martinello:** None. **S. Fucile:** None. **G. Battaglia:** None. **F. Nicoletti:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

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Program#/Poster#: 120.06/A89

Topic: B.03. G-Protein Coupled Receptors

Title: DHPG-stimulated polyphosphoinositide hydrolysis is entirely mediated by mGlu1 receptors in the retina

Authors: M. ROMANO[1], G. MASCIO[1], L. DI MENNA[1], P. SCARSELLI[1], F. BIAGIONI[1], M. MADONNA[1], R. GRADINI[2,1], G. BATTAGLIA[1], *V. BRUNO[2,1], F. NICOLETTI[2,1]; [1]I.R.C.C.S. Neuromed, Pozzilli, Italy; [2]Univ. Sapienza, Rome, Italy

Abstract: Group-I metabotropic glutamate receptors (mGlu1 and mGlu5 subtypes) are coupled to Gq/11 and their activation leads to polyphosphoinositide (PI) hydrolysis with ensuing mobilization of intracellular Ca2+ and activation of protein kinase C. Stimulation of PI hydrolysis by the group-I mGlu receptor agonist, 3,5-dihydroxyphenylglycine (DHPG), is largely mediated by the mGlu5 receptor in most brain regions (e.g., hippocampus, cerebral cortex, and corpus striatum), with the exception of the developing cerebellum in which both receptors equally contribute to the stimulation of PI hydrolysis. Both mGlu1 and mGlu5 receptors are found in the retina, where their precise function is largely unknown. We first measured DHPG-stimulated PI hydrolysis in slices prepared from the bovine retina. We were surprised to find that the PI response to DHPG was abrogated by the mGlu1 receptor negative allosteric modulator (NAM), 3,4-dihydro-2H-pyrano[2,3]b quinolin-7-yl) (cis-4-

methoxycyclohexyl) methanone (JNJ6259685), but was only minimally affected by the mGlu5 receptor NAM, 2-methyl-6-(phenylethynyl)pyridine (MPEP). In contrast, MPEP was more efficient in reducing DHPG-stimulated PI hydrolysis in the bovine hippocampus, as expected. We then measured DHPG-stimulated PI hydrolysis in the mouse retina by incubating one entire retina per single test tube in the PI assay. Individual mouse retinas pre-labeled with [3H]-myo-inositol responded to DHPG with an increased [3H]-inositol phosphate formation. Again, this response was highly sensitive to inhibition by JNJ6259685 but not to MPEP, although both mGlu1 and mGlu5 receptor proteins could be detected in the mouse retina by Western blot analysis and immunohistochemistry. These data show for the first time that excitatory amino acid-stimulated PI hydrolysis in the retina is almost entirely mediated by the mGlu1 receptor. This may facilitate the study of mGlu1 receptor-coupled signal transduction in a native environment, and suggests that the mGlu1 receptor might be a better drug target than the mGlu5 receptor in the experimental treatment of degenerative disorders of the retina.

Disclosures: **M. Romano:** None. **G. Mascio:** None. **L. Di Menna:** None. **P. Scarselli:** None. **F. Biagioni:** None. **M. Madonna:** None. **R. Gradini:** None. **G. Battaglia:** None. **V. Bruno:** None. **F. Nicoletti:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

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Topic: B.03. G-Protein Coupled Receptors

Support: NRF grant 2011-0011694

Title: Regulation of mGluR7 trafficking by SUMOylation in neurons

Authors: ***Y. SUH**[1,2,3], J.-H. CHOI[3], J.-Y. PARK[3], S. PARK[1];
[1]Dept. of Biomed. Sci., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [2]Neurosci. Res. Institute, Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [3]Dept. of Pharmacology, Ajou Univ. Sch. of Med., Suwon, Korea, Republic of

Abstract: Protein SUMOylation is a post-translational modification by which Small Ubiquitin-like MOdifier (SUMO) proteins are covalently linked to the lysine residues of target proteins via an enzymatic cascade. SUMOylation of synaptic proteins plays important regulatory roles in synapse formation, axonal mRNA transport, channel activity, and receptor endocytosis. The metabotropic glutamate receptor type 7 (mGluR7), a presynaptic G protein-coupled receptor

modulates excitatory neurotransmission and synaptic plasticity by inhibiting neurotransmitter release. MGluR7 at Lys889 has been shown to be modified by SUMO proteins *in vitro* assays, and a consensus motif for SUMO conjugation is conserved in the C-terminus of mGluR7. However, recent studies have failed to demonstrate the SUMO conjugation of full-length mGluR7 in mammalian cells and neurons. Here we have explored whether mGluR7 is a target of SUMOylation. Using biochemical approaches coupled with confocal imaging, we find that mGluR7 at Lys889, the sole SUMOylation site on the C-terminus of mGluR7 is a target of SUMO conjugation both by SUMO-1 and SUMO-2/3 in HEK293T cells. The SUMOylation of mGluR7 is prevented by SUMO-specific isopeptidase SENP-1. Although we have failed to detect SUMOylation of mGluR7 in cultured cortical neurons, SUMOylated form of mGluR7 is present in brain lysate. Furthermore, we find that mutation of mGluR7 at Lys889 to Arg markedly increases mGluR7 internalization in hippocampal neurons. Overexpression of SENP-1 leads to increased internalization of mGluR7, whereas SENP-1 Cys603Ser, a catalytic inactive mutant has no effects, suggesting that endocytosis of mGluR7 is enhanced by reduced SUMO conjugation of mGluR7. Taken together, these data support a model in which SUMOylation of mGluR7 at Lys889 is critical for stable surface expression of mGluR7 in neurons.

Disclosures: **Y. Suh:** None. **J. Choi:** None. **J. Park:** None. **S. Park:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

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Topic: B.03. G-Protein Coupled Receptors

Support: the National Program of Basic Research of China (2013CB835100)

the National Natural Science Foundation of China (31271173)

Title: Group I metabotropic glutamate receptors regulate the excitability of rat retinal ganglion cells

Authors: ***Q. LI**, P. CUI, X.-Y. LI, F. LI, F. GAO, L.-Z. LI, X.-L. YANG, Z. WANG;
Fudan Univ., Shanghai, China

Abstract: Retinal ganglion cells (RGCs), output neurons of the retina, receive inhibitory inputs from amacrine cells and excitatory inputs directly from cone bipolar cells. Changes in RGC excitability may directly influence visual processing in the retina. Group I metabotropic

glutamate receptors (mGluR I) were extensively expressed on retinal neurons. However, the effects of activating mGluR I on excitability of RGCs are largely unknown. Here, we showed that DHPG (10 microM), a mGluR I agonist, significantly increased the firing and caused a depolarization of the membrane potential of the cells, which could be reversed by LY367385 (10 microM), a selective mGluR1 antagonist, but not by MPEP (10 microM), a selective mGluR5 antagonist, suggestive of the involvement of mGluR1 in the DHPG-induced effect. Intracellular dialysis of either U73122 (10 microM), a phosphatidylinositol (PI)-PLC inhibitor or bisindolylmaleimide IV (10 microM), a protein kinase C inhibitor blocked the DHPG effect. Furthermore, intracellular dialysis of BAPTA (10 milliM), a calcium chelator or CaM kinaseIIinhibitor KN-93 (10 microM) showed the similar effects. In the presence of cocktail synaptic blockers (CNQX, D-AP5, bicuculline and strychnine), with spontaneous firing being disappeared, DHPG persisted to depolarize the membrane potential of RGCs and induce the cells to fire action potentials, suggesting that activation of mGluR I directly regulates the excitability of RGCs. The DHPG-induced depolarization of RGCs could not be blocked by TTX, indicating no involvement of Na^+ channels. In contrast, DHPG suppressed hyperpolarization-activated currents. The reverse potential of these currents was approximately -70 mV, very close to potassium equilibrium potential, implying that K^+ channels were mediated the DHPG-induced effects. Further experiments showed that inwardly rectifying potassium (Kir) currents and hyperpolarization-activated cation currents (I_h) could be recorded in RGCs, and extracellular application of DHPG indeed induced suppression on these two channels. Our results suggest that activation of mGluR1 regulates the excitability of rat RGCs by inhibiting Kir and Ih currents, which is mediated by intracellular Ca^{2+}-dependent PLC-PKC and CaMKII signaling pathways.

Disclosures: **Q. Li:** None. **P. Cui:** None. **X. Li:** None. **F. Li:** None. **F. Gao:** None. **L. Li:** None. **X. Yang:** None. **Z. Wang:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

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Topic: B.03. G-Protein Coupled Receptors

Support: NSFC(No.81322044)

NSFC(No.31370913)

Title: Intercellular GPCR interaction is involved in the neutrophil-neuron contact and nerve recovery

Authors: Y.-W. LIU, *S.- . DAI, T. YANG, L. ZHAO;
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Abstract: The brain is generally considered as immune privilege organ since the integrity of blood-brain-barrier(BBB), impeding the influx of immune cells from blood to the brain. However the BBB breaks down under pathological conditions including traumatic brain injury (TBI), stroke and some degenerative diseases, compelling the infiltration of white blood cells (WBCs) and inducing inflammation of central nerves system(CNS). Neutrophil is recognized as the vanguard during acute phase, but its real effects and detailed mechanisms in regulation of neuron function and recovery are still unclear. In our present study, we found that primary isolated neutrophils could “eat or bite” neurons directly in the coculture model, which was accomplished with down-expression of neuron neurotrophic tyrosine kinase type 2 receptor (NTRK2), leading to the atrophy of axons and dendrites and perishment of neuron. This results were confirmed by live cell imaging system *in vitro* and we described this phenomenon as “neutrophils attack and run”. Further investigations showed that the interaction between two G-protein coupling receptors (GPCRs), metabotropic glutamate receptor 5 (mGluR5) and adenosine 2a receptor (A2AR), were involved in the effects of neutrophils on neuron described above. While being different from the classic GPCR interaction mode to form homo or hetero dimers on the same cell membranes, we found that A2AR on neutrophil contact with mGluR5 on neuron between the cell membranes through their extracellular binding sites. This was testified by A2AR/mGluR5 knockout, Co-IP and fluorescence resonance energy transfer (FRET) technologies. These findings that unveil the regulation upon neuron by intruded circulating neutrophils, may not only be brand-new perspectives about connections between central nervous system and peripheral immune system, but also provide novel vision of nerve recovery. Meanwhile this is the first time to confirm GPCRs between different cell membranes, could interact or bind under specific circumstances, which challenges traditional ligand-receptor signal transduction.

Disclosures: **Y. Liu:** None. **S. Dai:** None. **T. Yang:** None. **L. Zhao:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

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Topic: B.03. G-Protein Coupled Receptors

Support: NIAAA DICBR

NIGMS PRAT Fellowship to K.A.J.

Title: Metabotropic glutamate receptor 2 modulates thalamostriatal neurotransmission

Authors: *K. A. JOHNSON, D. M. LOVINGER;
Lab. for Integrative Neurosci., NIAAA/NIH, Rockville, MD

Abstract: The striatum plays important roles in motor control and action learning. The activity of striatal projection neurons is controlled by inputs from several brain regions, and the strength of these inputs can be modulated by presynaptic G protein-coupled receptors (GPCRs). Most previous efforts to evaluate GPCR-mediated modulation of glutamatergic transmission in the striatum have attributed changes in electrically-evoked transmission to modulation of corticostriatal circuits. However, the contributions of excitatory inputs originating in the thalamus, which represent almost half of excitatory inputs to medium spiny neurons (MSNs), are frequently overlooked. Activation of group II metabotropic glutamate receptors (mGlu2 and mGlu3) is known to produce a strong inhibition of electrically-evoked glutamatergic transmission onto striatal medium spiny neurons (MSNs). To evaluate the input specificity of the mGlu2/3-mediated modulation of striatal glutamatergic transmission, we used a viral strategy to express Channelrhodopsin-2 (ChR2) in neurons projecting to the striatum from the intralaminar nuclei of the thalamus of 5-7 week old male C57Bl/6J mice. 3-6 weeks after virus injection, coronal slices containing the striatum were prepared, and optically-evoked thalamostriatal excitatory postsynaptic currents (oEPSCs) were recorded from MSNs in the dorsolateral striatum. Bath application of the group II mGlu receptor agonist LY379268 (100 nM, 5 min) produced a robust reduction of oEPSC amplitude (peak depression 27.4±6.0% of baseline). Inhibition of oEPSCs by LY379268 was long lasting, persisting for at least 45 minutes after the onset of drug application (oEPSC amplitude 44.5±5.0% of baseline 40-45 minutes after LY379268 application). Experiments in which the group II mGlu-preferring antagonist LY341495 (500 nM, 10 min) was bath applied 10 minutes after LY379268 revealed that the long-term depression induced by LY379268 is fully reversible. To identify which receptor subtype(s) contribute to the inhibition of thalamostriatal transmission, we evaluated the effect of the mGlu2 agonist/mGlu3 antagonist LY395756 (10 μM, 5 min). Interestingly, LY395756 produced a strong, reversible inhibition of oEPSC amplitude (peak depression 22.3±2.5% of baseline), suggesting a major role for mGlu2 in the modulation of thalamostriatal transmission. Ongoing studies will evaluate potential contributions of mGlu3 to the inhibition of excitatory transmission at this synapse. These findings add to our limited knowledge of the mechanisms regulating the thalamostriatal system.

Disclosures: K.A. Johnson: None. **D.M. Lovinger:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.03. G-Protein Coupled Receptors

Support: NARSAD Young Investigator Grant

Title: Modulatory role of Neuregulin 1 signalling on mGluR1 function in DAergic neurons: effects on neurotransmission and synaptic plasticity

Authors: A. LEDONNE[1], A. NOBILI[1], E. C. LATAGLIATA[1], V. CAVALLUCCI[1], E. GUATTEO[1], S. PUGLISI-ALLEGRA[1,2], M. D'AMELIO[1,3], *N. B. MERCURI[1,4]; [1]IRCCS -SANTA LUCIA FOUNDATION, Roma, Italy; [2]Univ. of Rome "La Sapienza", Rome, Italy; [3]Univ. Campus-Biomedico, Rome, Italy; [4]Univ. of Rome “Tor Vergata”, Rome, Italy

Abstract: Neuregulin 1 (NRG1) is a trophic factor involved in neurodevelopment, neurotransmission and synaptic plasticity. Despite the evidence that NRG1 and its receptors, ErbB tyrosine kinases, are expressed in mesencephalic dopaminergic (DAergic) nuclei, and their functional alterations are reported in schizophrenia and Parkinson’s disease, the functional role of NRG1/ErbB signalling in mesencephalic dopaminergic neurons is still elusive. To this regard, recent evidence from our group (Ledonne et al., Mol Psychiatry, 2014) demonstrate that NRG1 modulates the nigrostriatal DAergic system in rodent brain by selectively regulating the functional expression of metabotropic glutamate receptor 1 (mGluR1) in DAergic neurons. We found that endogenous NRG1/ErbB signalling is essential to preserve mGluR1 function in mesencephalic DAergic neurons, by maintaining its surface membrane expression. Consequently, NRG1 tone regulates striatal mGluR1-induced dopamine outflow in *in vivo* conditions. In consideration of this functional interaction between NRG1/ErbB receptors and mGluR1, we questioned whether NRG1 signalling could participate in modulating mGluR1-dependent forms of synaptic plasticity in DAergic neurons. Therefore, using electrophysiological patch-clamp recordings of DAergic neurons in midbrain rodent slices, we investigated the effects of NRG1/ErbB signalling on the mGluR1-dependent long-term depression (LTD) of glutamatergic synaptic transmission in these neurons. Our data support a regulatory role of NRG1/ErbB signalling in the modulation of glutamatergic synaptic strength in DAergic neurons. In consideration of the genetic association of NRG1/ErbB signalling with schizophrenia, and the likely imbalance of glutamatergic and dopaminergic neurotransmission associated with this disease, the discovery of a functional role of NRG1 in tuning the LTD of glutamatergic synaptic transmission in DAergic neurons might provide a framework to comprehend how the NRG1-dependent pathways contributes to the synaptic dysregulations observed in schizophrenia and other neuropsychiatric disorders.

Disclosures: **A. Ledonne:** None. **A. Nobili:** None. **E.C. Latagliata:** None. **V. Cavallucci:** None. **E. Guatteo:** None. **S. Puglisi-Allegra:** None. **M. D'Amelio:** None. **N.B. Mercuri:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.12/A95

Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant R03DC11361

LaBOR RCS Grant LEQSF(2012-15)-RD-A-09

NSF-LA EPSCoR Grant LEQSF-EPS(2014)-PFUND 354

Title: Glutamatergic inhibition of limbic neural circuits

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Abstract: The constituent nuclei of the limbic system are integral components for learning, memory and emotive functions in the brain. Among these many nuclei, the mammillary bodies are essential structures for transmitting information to higher forebrain centers via the mammilothalamocortical pathway. As such, understanding the neuronal processing occurring in the mammillary bodies is essential for deciphering its functional roles. Here, the role of group II metabotropic glutamate receptors (mGluRs) in the mammillary bodies was examined using whole-cell patch clamp recordings from *in vitro* slice preparations in the mouse. We measured whole-cell responses in the presence of pharmacological bath application of group II mGluR agonists and antagonists. In addition, we neuroanatomically examined the expression of mGluR2 in the mammillary bodies and other limbic-related structures. Our results demonstrate a direct role of group II mGluRs for inhibiting neural activity in the mammillary bodies and other limbic-related structures. These data suggest possible mechanisms controlling the activity of limbic circuitry and a molecular factor linking disparate elements of these circuits.

Disclosures: **C.C. Lee:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.13/A96

Topic: B.03. G-Protein Coupled Receptors

Support: Regione Autonoma della Sardegna, LR 7/2007-CRP10810/2012

Title: Evidence that the $GABA_B$ positive allosteric modulator CGP7930 activates MAPK cascade independently of $GABA_B$ receptor

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Abstract: The $GABA_B$ positive allosteric modulator (PAM) CGP7930 potentiates $GABA_B$ receptor signaling in transfected cells (Urwyler et al. Mol. Pharmacol. 60, 963-971, 2001) and rat and human brain (Onali et al., Eur. J. Pharmacol. 471, 77-84, 2003; Olianas et al., Neurochem. Int. 46, 149-158, 2005). A number of behavioral studies have also shown that CGP7930 exerts anxiolytic effects and reduces self-administration of drugs of abuse (Adams and Lawrence, CNS Drug Rev. 13, 308-316, 2007). However, little is known on whether this drug can affect neuronal signaling independently of $GABA_B$ receptor activity. In the present study we report that in human SH-SY5Y neuroblastoma cells, CGP7930 (30 μM) induced a rapid increase of dual phosphorylation (activation) of ERK1/2, which reached a maximum at 15 min and lasted for at least 60 min. CGP7930 also triggered CREB phosphorylation at Ser133 with a similar kinetic profile. Under the same experimental conditions, the $GABA_B$ receptor agonist (-)baclofen (100 μM) failed to affect ERK1/2 phosphorylation, and when combined with CGP7930, it did not elicit any further ERK1/2 stimulation. CGP7930-induced ERK1/2 phosphorylation was not prevented by cell pre-treatment with either the $GABA_B$ receptor antagonists CGP55845A and CGP54626, the $G_{i/o}$-receptor uncoupler pertussis toxin, the $G_{q/11}$ antagonist YM254890, or the tyrosine kinase inhibitor genistein. Conversely, it was completely blocked by the MEK inhibitor PD98059 and significantly attenuated by the protein kinase C inhibitors Go 6983 and bisindolylmaleimide I. CGP7930 (30 μM) was also found to stimulate ERK1/2 phosphorylation in CHO-K1 cells, which do not express $GABA_B$ receptors. However, CGP7930 (1-100 μM) had no effect in HEK293 cells, indicating that ERK1/2 activation was not a generalized cellular response to the PAM. These data indicate that CGP7930 can affect ERK1/2 signaling, which is known to be involved in the control of mood and drug addiction, independently of increased $GABA_B$ receptor activity.

Disclosures: P. Onali: None. **S. Dedoni:** None. **M.C. Olianas:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

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Program#/Poster#: 120.14/A97

Topic: B.03. G-Protein Coupled Receptors

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Vanderbilt University Medical Center/ Howard Hughes Medical Institute Certificate in Molecular Medicine

Postdoctoral Fellowship from Rettsyndrome.org

Title: mGlu7 is critical for hippocampal plasticity and is a potential therapeutic target for the treatment of Rett Syndrome

Authors: *R. KLAR[1], R. G. GOGLIOTTI[2], A. G. WALKER[2], R. ZAMORANO[2], D. W. ENGERS[2], D. GHOSE[3], B. A. GRUETER[4], C. R. HOPKINS[5], C. W. LINDSLEY[5], Z. XIANG[2], P. J. CONN[2], C. M. NISWENDER[2];
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Abstract: Rett Syndrome (RS) is a neurological disorder that is characterized by autism-like symptoms, loss of language skills, seizures, stereotyped behaviors, motor deficits, and cognitive impairments. Most cases of RS result from loss-of-function mutations in the Mecp2 (Methyl CpG Binding Protein 2) gene, which encodes for a protein that binds to methylated CpG islands in DNA and regulates gene transcription. Cell-autonomous knockout (KO) of Mecp2 from neurons recapitulates the cognitive deficits seen in patients with RS and results in impaired synaptic plasticity (induction of long-term potentiation (LTP) and long-term depression (LTD)) at the Schaffer-collateral-CA1 (SC-CA1) synapse in the hippocampus. Metabotropic Glutamate Receptor 7 (mGlu7) is unique from other group III mGlu receptors in that it is located pre-synaptically within the active zones on both glutamatergic and GABAergic terminals, where it

has been shown to regulate neurotransmitter release. Additionally, mGlu7 KO studies have suggested that loss of mGlu7 impairs working memory and fear extinction and results in deficits in short-term plasticity at SC-CA1synapses, similar to synaptic phenotypes seen in Mecp2 KO mice. Our preliminary data show that the GRM7 gene contains an MeCP2 binding site upstream of the transcription start site and that mGlu7 expression is positively regulated by MeCP2. Moreover, loss of Mecp2 in mice results in decreased expression of mGlu7 at the synapse in the hippocampus. Taken together, these data suggest that mGlu7 may be critical for the induction of LTP at SC-CA1 synapses and that potentiation of mGlu7 in Mecp2 KO mice could rescue LTP and improve cognitive impairments. Here we show that mGlu7 is necessary for induction of LTP at SC-CA1 synapses in wild-type mice, and that it facilitates LTP by decreasing GABAergic tone in a frequency-dependent manner. This results in a disinhibition of the synapse and allows for LTP to occur. Additionally, we have shown that application of the group III PAM, VU0422288, is sufficient to restore LTP in Mecp2 KO mice. Furthermore, we have also found that VU0422288 can reverse deficits in the contextual fear conditioning assay. These studies provide evidence for a novel role of mGlu7 in modulating synaptic plasticity in the hippocampus and suggest that activation of mGlu7 is beneficial in improving cognitive deficits in a mouse model of Rett Syndrome.

Disclosures: **R. Klar:** None. **R.G. Gogliotti:** None. **A.G. Walker:** None. **R. Zamorano:** None. **D.W. Engers:** None. **D. Ghose:** None. **B.A. Grueter:** None. **C.R. Hopkins:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bristol Myers Squibb. **C.W. Lindsley:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bristol Myers Squibb, Astra Zeneca, Johnson & Johnson. **Z. Xiang:** None. **P.J. Conn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bristol Myers Squibb, Astra Zeneca, Johnson & Johnson. **C.M. Niswender:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bristol Myers Squibb, Astra Zeneca, Johnson & Johnson.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.15/A98

Topic: B.03. G-Protein Coupled Receptors

Support: R37 NS31373-20

R01 MH62646-16

Vanderbilt Interdisciplinary Graduate Program in Biomedical Sciences

Title: Role of phospholipase D in mGluR-dependent hippocampal and muscarinic-dependent cortical long-term depression

Authors: *S. P. MORAN, M. ALTMAN, A. GHOSHAL, J. T. MAKSYMETZ, J. I. AGUILAR, C. W. LINDSLEY, P. J. CONN;
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Abstract: Activation of metabotropic glutamate receptors (mGluRs) with application of the group I mGluR selective agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) induces long-term depression (LTD) of excitatory synaptic transmission onto hippocampal CA1 neurons. Previously, we have reported selective activation of mGluRs induces a concentration-dependent increase in phospholipase D (PLD) activity. PLD is a lipid signaling enzyme that catalyzes the hydrolysis of phosphatidylcholine into phosphatidic acid and choline. In mammals, the two isoforms PLD1 and PLD2, sharing ~53% sequence identity, have been implicated in critical cell signaling pathways with roles in cancer and central nervous system (CNS) disorders. Using brain slice electrophysiology, we recorded changes in mouse CA1 field excitatory post synaptic potentials (fEPSPs) following DHPG application in the presence of various PLD inhibitors. We report that 10 minute pretreatment of 2μM ML299 (a dual PLD1/PLD2 inhibitor) reduces subsequent 50 μM DHPG induced acute depression in addition to blocking long term depression. Additionally, we demonstrated that selective inhibition of the PLD1 isoform, by 10 minute pretreatment of 370nM VU0359595, recapitulates the blocking effect of ML299 on DHPG induced LTD. However, selective inhibition of the PLD2 isoform by 2μM VU0364739 failed to inhibit either the acute or long term depression. We also report that, other than its role in hippocampal plasticity, PLD can also modulate plasticity in other synapses. Application of ML299 also blocks 50 μM carbachol induced long term depression in the prefrontal cortex (PFC), a form of LTD that requires co-activation of M1 muscarinic and mGlu5 receptors. These data taken together, implicates that dysregulation of PLD function may play a role in CNS disorders in which synaptic plasticity is altered. We are currently performing additional electrophysiology experiments to demonstrate the role of PLD isoforms in induction and expression of LTD and long-term potentiation in the hippocampus and PFC.

Disclosures: S.P. Moran: None. **M. Altman:** None. **A. Ghoshal:** None. **J.T. Maksymetz:** None. **J.I. Aguilar:** None. **C.W. Lindsley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers-Smith, Janssen. **P.J. Conn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers-Smith, Janssen.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

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Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant 5T32MH093366-04

NIH Grant 5R37NS03137321

NIH Grant 5U01MH08796505

Title: Characterization of a novel mGlu2 radioligand

Authors: *D. E. O'BRIEN[1], A. J. CROSS[2], S. WESOLOWSKI[2], C. S. ELMORE[3], P. J. CONN[1];
[1]Pharmacol., Vanderbilt Univ., Nashville, TN; [2]AstraZeneca Neurosci. Innovative Medicines, Cambridge, MA; [3]AstraZeneca Drug Safety & Metabolism, Molndal, Sweden

Abstract: Positive allosteric modulation of the metabotropic glutamate receptor 2 (mGlu2) offers a promising therapeutic target for schizophrenia, pain, addiction, epilepsy, and anxiety disorders. Medicinal chemistry efforts directed at specifically targeting mGlu2 have successfully developed several chemically distinct mGlu2 PAMs that exhibit therapeutic efficacy in preclinical rodent models. In order to better understand the binding modes of these mGlu2 PAMs, we characterized a novel mGlu2 radioligand *in vitro* using recombinant rat mGlu2. We demonstrated that the mGlu2 radioligand possesses suitable characteristics for binding studies. Moreover, we characterized the binding modes of several chemically distinct mGlu2 PAMs in competition binding assays utilizing the mGlu2 radioligand. Taken together, these studies demonstrate the preclinical utility of this mGlu2 radioligand for studying mGlu2 allosteric modulation.

Disclosures: **D.E. O'Brien:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca. **A.J. Cross:** A. Employment/Salary (full or part-time):; AstraZeneca. **S. Wesolowski:** A. Employment/Salary (full or part-time):; AstraZeneca. **C.S. Elmore:** A. Employment/Salary (full or part-time):; AstraZeneca. **P.J. Conn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers Squibb, Jansen Research and Development, LLC.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

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Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant R37 NS31373-20

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Vanderbilt International Scholar Program

Title: Stimulus bias by mGlu5 positive allosteric modulators affects long term potentiation in the hippocampus through involvement of endocannabinoids and GABAergic disinhibition

Authors: *J. T. MAKSYMETZ, A. GHOSHAL, X. LV, J. M. ROOK, C. W. LINDSLEY, Z. XIANG, P. J. CONN;
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Abstract: Previously, we have demonstrated that unique metabotropic glutamate receptor 5 (mGlu5) positive allosteric modulators (PAMs) differentially potentiate NMDAR currents and long-term potentiation (LTP) at the Schaffer collateral-CA1 synapse in the rat hippocampus. The mGlu5 PAM VU0092273 potentiates both NMDAR currents and theta-burst stimulation-induced LTP (TBS-LTP) while VU0409551 does not affect either. It was previously thought that mGlu5 PAMs potentiate TBS-LTP through enhancement of NMDAR currents. However, we now report that the mGlu5 PAM VU-29 potentiates TBS-LTP but has no effect on NMDAR currents. In contrast, another mGlu5 PAM, 5PAM523, potentiates NMDAR currents but does not potentiate TBS-LTP. Thus, these novel mGlu5 PAMs confer distinct forms of stimulus bias to mGlu5 signaling and allow us to show that potentiation of mGlu5 regulation of NMDAR currents is not responsible for the ability of mGlu5 PAMs to enhance hippocampal LTP. Another possible mechanism by which mGlu5 PAMs could enhance hippocampal LTP is through modulation of GABAergic inhibitory transmission onto CA1 pyramidal cells, which is known to play a role in the regulation of LTP in the hippocampus. Previous studies reveal that activation of mGlu5 on hippocampal pyramidal cells induces endocannabinoid production, which then activates CB1 cannabinoid receptors (CB1Rs) on neighboring interneuron presynaptic terminals and causes a long-term depression of inhibitory postsynaptic currents (IPSCs). Interestingly, we found that the CB1R antagonist AM251 completely blocks the ability of the mGlu5 PAM VU0092273 to potentiate threshold TBS-LTP. Furthermore, preliminary whole-cell patch clamp recordings revealed that that application of 1 μM VU0092273 causes a slight reduction in IPSCs in CA1 pyramidal cells that could represent endocannabinoid-mediated disinhibition by GABAergic interneurons. Together, these experiments suggest that mGlu5 PAM potentiation of TBS-LTP is

not dependent on enhancement of NMDAR currents but may be mediated by release of endocannabinoids and a reduction of inhibitory tone. Additional studies will be performed to determine whether VU0092273 reduces IPSCs by a mechanism that involves endocannabinoid signaling to presynaptic GABAergic interneurons and to determine whether this disinhibition is necessary during TBS for the potentiation of LTP. Furthermore, our series of mGlu5 PAMs that display distinct forms of stimulus bias will allow us to determine whether mGlu5 PAMs that do not potentiate induction of TBS-LTP are also without effects on endocannabinoid-mediated disinhibition.

Disclosures: **J.T. Maksymetz:** None. **A. Ghoshal:** None. **X. Lv:** None. **J.M. Rook:** None. **C.W. Lindsley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers Squibb, Janssen Pharmaceuticals. **Z. Xiang:** None. **P.J. Conn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca, Bristol-Myers Squibb, Janssen Pharmaceuticals.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.18/A101

Topic: B.03. G-Protein Coupled Receptors

Support: Pioneer Research Center Program through the National Research Foundation of Korea 2012-0009521

Title: Co-treatment with anthocyanins and vitamin c ameliorates ethanol-induced neurodegeneration via modulation of gaba b receptor signaling in the adult rat brain

Authors: *H. BADSHAH, T.-H. KIM, H.-Y. LEE, T. ALI, M.-O. KIM;
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Abstract: Chronic ethanol exposure is known to cause neuronal damage in both humans and experimental animal models. Ethanol induces neurotoxicity via the generation of reactive oxygen species (ROS), while anthocyanins and ascorbic acid (vitamin C) are free radical scavengers that can be used as neuroprotective agents against ROS. In this study the underlying neuroprotective potential of black soybean anthocyanins and vitamin C was determined. For this purpose, adult rats were exposed to 10% (v/v) ethanol for 8 weeks, followed by co-treatment with anthocyanins (24 mg/kg) and vitamin C (100 mg/kg) during the last 4 weeks. Long term ethanol administration increased the expression of γ-aminobutyric acid B1 receptor ($GABA_{B1}R$) and induced neuronal

apoptosis via alterations to the Bax/Bcl-2 ratio, release of cytochrome C and activation of caspase-3 and caspase-9. Anthocyanins alone and supplementation with vitamin C showed an additive effect in reversing the trend of apoptotic signals induced by ethanol in the cortex and hippocampus. Consequently, anthocyanins also decreased the expression of poly (ADP ribose) polymerase-1 (PARP-1) induced by ethanol and prevented DNA damage. Furthermore, anthocyanins and vitamin C reversed the ethanol-induced expression of $GABA_{B1}R$ and its downstream signaling molecule phospho-cAMP response element binding protein (p-CREB). Moreover, histopathology and immunohistochemistry results showed that anthocyanins and vitamin C significantly reduced ethanol-induced neuronal cell death. Our study revealed a neuroprotective role of anthocyanins and vitamin C via modulation of $GABA_{B1}R$ expression in the adult brain. Hence, we suggest that anthocyanins or co-treatment with anthocyanins and vitamin C may be a new and potentially effective neuroprotective agent for alcohol abuse.

Disclosures: **H. Badshah:** None. **T. Kim:** None. **H. Lee:** None. **T. Ali:** None. **M. Kim:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.19/A102

Topic: B.03. G-Protein Coupled Receptors

Support: SNF

Title: CaMKII-dependent K63-linked ubiquitination of GABAB1 drives lysosomal degradation of GABAB receptors

Authors: *K. ZEMOURA[1], C. TRÜMPLER[2], D. BENKE[2];
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Abstract: GABAB receptors are heterodimeric G protein-coupled receptors, which control neuronal excitability by mediating slow and prolonged inhibition. The magnitude of GABAB receptor-mediated inhibition essentially depends on the amount of receptors in the plasma membrane. One factor that determines receptor availability is regulated protein degradation. GABAB receptors are constitutively internalized and either recycled to the cell surface or degraded in lysosomes. The signal that sorts GABAB receptors to lysosomes is currently unknown. Here we tested whether ubiquitination is the lysosomal sorting signal for GABAB receptors. We found that inhibition of lysosomal activity in cortical neurons increased total and

cell surface GABAB receptors as well as the level of K63-linked ubiquitinated receptors. Mutational inactivation of four putative ubiquitination sites in the GABAB1 subunit significantly diminished K63-linked ubiquitination of GABAB receptors and prevented their lysosomal degradation. Searching for factors that control lysosomal degradation of GABAB receptors revealed that blocking Ca2+/calmodulin-dependent protein kinase II (CaMKII) decreased K63-linked ubiquitination of GABAB receptors and inhibited lysosomal degradation of GABAB receptors by sorting the receptors to the recycling pathway. Finally, triggering lysosomal degradation of GABAB receptors by sustained activation of glutamate receptors, a condition mimicking in brain ischemia, was accompanied with a massive increase of CaMKII-dependent K63-linked ubiquitination of GABAB receptors. Preventing K63-linked ubiquitination by blocking CaMKII, overexpressing ubiquitin mutants or mutant GABAB receptors deficient in GABAB1 K63-linked ubiquitination prevented down-regulation of the receptors. These findings indicate that CaMKII-dependent K63-linked ubiquitination of GABAB1 at multiple sites controls sorting of GABAB receptors to lysosomes for degradation under physiological and pathological condition.

Disclosures: **K. Zemoura:** None. **C. Trümpler:** None. **D. Benke:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.20/A103

Topic: B.03. G-Protein Coupled Receptors

Title: GABAb-receptor neurotransmission changes in the ventral-pallidum are associated to depressive-like behaviors

Authors: *R. E. CONTRERAS[1,2], M. SKIRZEWSKI[3], S. SALMEN[2], L. BETANCOURT[1], L. HERNÁNDEZ[1], P. RADA[1];
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Abstract: Depression affects approximately 121 million people worldwide. It causes more than 800,000 completed suicides by year. Major depressive disorder is an idiopathic syndrome characterized by depressed mood, helplessness, anhedonia, disphoria, altered sleep behavior and appetite and loss of concentration. In a published study we found that GABAergic transmission in the ventral pallidum (VP) seems to play a role in depression-like behaviors. We found an

increased basal GABA extracellular concentration in the VP of rats during the forced swimming test (FST) suggesting an enhancement of GABAergic tone upon depression. However, the specific role for GABAB-R in this brain region during depression remains unknown even though we previously described some GABAB receptor contributions in the VP to this behavior while using specific agonist and antagonist molecules. In this work we demonstrated that intra-VP microinjections of saclofen and then baclofen before day-2 FST significantly exacerbated immobility (477.0 ± 8.07; n=10, $F=3.893$; $p<0.05$) compared to the control group with saline solution (348.9± 31.18; n=10). Then we monitored extracellular GABA when intra-VP saclofen pulses were administered and found a significant increase (480.95±178.43; n=4, $F=10.86$, $Df=1$, $p<0.05$) when compared to the saline control group (77.57±78.68; n=4). Finally, we reported GABAB-R protein and GABAB2 mRNA subunit up-regulation in the VP of depressed animals. Thus, we postulate first the synergic effect of GABAB-R antagonist and agonist in the VP upon pre-synaptic and post-synaptic localization respectively. And second, we report a hypersensitive GABAB-R system that is possibly involved in the pathogenesis and perpetuation in depressive-like behaviors and could be considered as plausible therapeutic target.

Disclosures: R.E. Contreras: None. **M. Skirzewski:** None. **S. Salmen:** None. **L. Betancourt:** None. **L. Hernández:** None. **P. Rada:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.21/A104

Topic: B.03. G-Protein Coupled Receptors

Support: ERC

Avenir

Title: Aversive experiences depress GABAB signaling in the lateral habenula

Authors: *S. LECCA[1,2,3], R. LUJAN[4,5], M. MAMELI[1,2,3];
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Abstract: The lateral habenula (LHb), an ephitalamic structure bridging forebrain and midbrain nuclei, encodes aversive stimuli. Work in primates and rodents indicate that aversive stimuli

phasically increase LHb neurons firing rate (Matsumoto and Hikosaka 2007) and optogenetic activation of LHb terminals onto midbrain neurons induces avoidance behavior in mice (Stamatakis and Stuber 2012; Lammel et al, 2012). Furthermore, in rodents previously exposed to aversive stimuli such as foot-shocks, c-fos expression as well as firing activity increase within the LHb (Brown and Shepard 2013; Li et al, 2011). However, the early cellular adaptations eventually occurring in the LHb after an aversive experience remain to be fully elucidated. In order to test the hypothesis that aversive events can trigger cellular modifications in the LHb underlying changes in neuronal excitability, we took advantage of the inescapable footshock paradigm and ex-vivo patch-clamp recordings in acute slices from mice. To obtain information on the spontaneous output firing of neurons we recorded LHb cells in the cell-attached configurations, one hour after the aversive procedure. We found that, in slices obtained from footshock exposed mice (FsE), neurons presented a significantly higher firing frequency than control mice. We then, sought to understand the molecular mechanisms responsible for the increased firing rate. Performing recordings in whole-cell mode, we find that glutamate and GABAA transmission remained unchanged. In contrast, using whole-cell patch clamp recordings we find that the pharmacological activation of GABAB-Rs by baclofen, elicited a GIRK-mediated outward current, which was reduced in FsE. Employing electron microscopy, we show that FsE leads to a significant reduction in membrane expression of both GABAB1 and GIRK2, with a corresponding increase in their intracellular pool. Interestingly, the reduction of GABAB-GIRK signaling occurred after pain-less aversive experience capable to induce aversive behaviors in mice (odor predator and restraint stress). Finally, we find that in FsE mice GABAB-Rs-mediated inhibition of firing resulted strongly weakened. These data indicate that aversive events drive a rapid and persistent functional reduction of the GABAB-GIRK signal along with a loss of inhibitory control on firing activity of LHb neurons, providing a sensitive cellular mechanism underlying LHb neurons hyperexcitability triggered by aversive events.

Disclosures: **S. Lecca:** None. **R. Lujan:** None. **M. Mameli:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.22/A105

Topic: B.03. G-Protein Coupled Receptors

Support: Swiss National Science Foundation Grants 31003A-152970

National Center of Competence in Research (NCCR) 'Synapsy, Synaptic Bases of Mental Diseases'

Title: GABA-B receptor signaling is regulated by assembly of KCTD proteins with matching G-protein subunits

Authors: T. FRITZIUS[1], R. TURECEK[1], V. BESSEYRIAS[1], M. GASSMANN[1], *B. BETTLER[2];
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Abstract: GABA-B receptors are the G-protein coupled receptors (GPCRs) for GABA, the main inhibitory neurotransmitter in the central nervous system. Upon agonist stimulation, GABA-B receptors activate K+ channels via release of Gβγ heterodimers from the heterotrimeric G protein. GABA-B receptors comprise principle and auxiliary subunits that regulate receptor properties in distinct ways. The principle subunits GABA-B1a, GABA-B1b, and GABA-B2 form fully functional heteromeric GABA-B(1a,2) and GABA-B(1b,2) receptors, while the auxiliary subunits KCTD8, -12, -12b, and -16 influence the kinetics of the receptor response (Schwenk et al., Nature, 2010). Recently, we showed that all KCTD subunits can interact with the Gβγ subunit of the heterotrimeric G protein. Selectively KCTD12 induces a fast desensitization of receptor-mediated Kir3 channel responses by an activity-dependent uncoupling of the Gβγ subunits from the channel (Turecek et al., Neuron, 2014). We will present an in-depth molecular analysis of the Gβγ/KCTD interaction. Specifically, we determined the regions of Gβγ that bind to the KCTDs in a constitutive and activity-dependent manner. Furthermore, we show that KCTD12-induced desensitization of the GABA-B receptor response is not only governed by the presence or absence of KCTD12, but also by the Gβγ subunits available in the cellular environment.

Disclosures: T. Fritzius: None. **R. Turecek:** None. **V. Besseyrias:** None. **M. Gassmann:** None. **B. Bettler:** None.

Poster

120. Metabotropic Glutamate Receptors and GABAB Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 120.23/A106

Topic: B.03. G-Protein Coupled Receptors

Support: NICHD intramural award

Title: GABAB receptor-mediated network function is disrupted by loss of KCTD auxiliary subunits

Authors: *M. T. CRAIG[1], X. YUAN[1], G. A. VARGISH[1], M. GASSMANN[2], B. BETTLER[2], C. J. MCBAIN[1];
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Abstract: Inhibition from interneurons, acting through ionotropic $GABA_A$ receptors, is well established as being essential for generating and maintaining neuronal network rhythms. It is emerging that metabotropic $GABA_B$ receptors also play an important role in regulating network dynamics. Recently, $GABA_B$ receptors were shown to co-assemble with four auxiliary subunits: KCTD8, KCTD12, KCTD12b and KCT16; expression of KCTD12b is restricted to the habenula, but the remainder are expressed throughout the nervous system, with KCTD12 and KCTD16 being widely abundant in most brain regions. The KCTD auxiliary subunits are cytosolic proteins that influence the agonist sensitivity and G-protein signalling of $GABA_B$ receptors, and KCTD12 polymorphisms are associated with bipolar disorders in humans. $GABA_B$ receptors are heterodimers of $GABA_{B1}$ and $GABA_{B2}$ subunits, and $GABA_{B1}$ subunits exist in two isoforms, $GABA_{B1a}$ and $GABA_{B1b}$. Receptors containing $GABA_{B1a}$ are predominantly found in presynaptic locations while those containing $GABA_{B1b}$ are generally found postsynaptically. Previously, we reported that these isoforms had distinct roles in terminating Up states in an *in vitro* mouse model of slow oscillations in the medial entorhinal cortex (mEC), with $GABA_{B1a}$-containing receptors involved in spontaneous termination of Up states while $GABA_{B1b}$-containing receptors were essential for active termination of Up states via afferent stimulation. Using mice in which either KCTD8, KCTD12 or KCT16 had been genetically ablated, we used the mEC model of slow oscillations to determine whether loss of KCTD auxiliary subunits had any consequences for network function. We found that, in wild type mice or those lacking KCTD8 or KCTD16, electrical stimulation in layer 1 of the mEC could halt an ongoing Up state, but that this effect was abolished in mice lacking KCTD12. This demonstrates a functional loss of post-synaptic $GABA_{B1b}$-containing receptors, implying that KCTD12 is necessary for either their function or trafficking. Examining spontaneous termination of Up states suggested that $GABA_{B1a}$-containing receptor-mediated function was impaired, but not lost, in mice lacking KCTD16. These data show that loss of KCTD auxiliary subunits disrupts normal $GABA_B$ receptor-dependent network function, and work is underway to further study these deficits at the electrophysiological, anatomical, and behavioural levels.

Disclosures: **M.T. Craig:** None. **X. Yuan:** None. **G.A. Vargish:** None. **M. Gassmann:** None. **B. Bettler:** None. **C.J. McBain:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.01/A107

Topic: B.04. Ion Channels

Support: School of Communication, Northwestern University

Knowles Hearing Center, Northwestern University

Title: Development of fast and reliable intrinsic excitability in auditory brainstem neurons

Authors: *H. HONG, L. ROLLMAN, J. SANCHEZ;
Communication Sci. and Disorders, Northwestern Univ., Evanston, IL

Abstract: Auditory brainstem neurons can generate extremely fast, reliable and highly synchronized action potentials (APs), a process critical for encoding sound localization and communication cues. This specialization depends on specific potassium and sodium channels found in mature neurons but how these channels shape AP speed and reliability during development are largely unexplored. In this study we characterized the maturation of voltage-gated potassium and sodium channels, and quantified how their currents shape AP properties in the cochlear nucleus magnocellularis (NM) of chickens. Whole-cell patch clamp recordings were obtained from developing chicken embryos before, during, and after hearing onset, corresponding to embryonic (E) days 10-12, E14-16, and E19-21, respectively. Voltage and current steps of varying durations and strengths were injected into the soma of NM neurons. AP properties, along with potassium and sodium currents, were characterized before and during bath application of specific potassium channel blockers. We found that the maturation of both voltage-gated potassium and sodium currents improved AP speed and reliability, in combination with changes in intrinsic membrane properties. With maturation, the time constant of membrane voltage became faster, while input resistance and capacitance decreased. The change in membrane input resistance was due to a significant upregulation in total potassium current. We observed a 2-fold increase in the total amount of potassium current from E10 to E21. Furthermore, we found that high-voltage activated potassium (K^+_{HVA}) channels contribute to the regulation of AP properties during development. When K^+_{HVA} channels were blocked for each age group, APs became significantly slower and less reliable. However, the greatest amount of change in AP properties was most prominent at the youngest ages, suggesting a significant contribution of K^+_{HVA} channels. Indeed, the ratio of K^+_{HVA} current accounted for 86% of the total potassium current for younger neurons; whereas, the ratio dropped to 52% for mature neurons. In addition, sodium currents became larger, faster, and more reliable; there was a 6-fold increase in rise and fall rates with maturation, likely due to changes in the expression of sodium channel subtypes. In conclusion, the refinement of potassium and sodium channels plays an important

role in shaping AP properties in the developing avian NM. Auditory brainstem neuron's ability to fire fast, reliable and synchronized APs is heavily dependent on both potassium and sodium channels and deficits in these channels may underlie aspects of auditory temporal processing disorders.

Disclosures: H. Hong: None. **L. Rollman:** None. **J. Sanchez:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.02/A108

Topic: B.04. Ion Channels

Support: BMBF Grant IonNeurONet 01GM1105A

DAAD Research Grant for Doctoral Candidates

Title: M-current impact on the *in vitro* neuronal network activity during development

Authors: *F. ROSA[1], H. LÖFFLER[1], S. THEISS[2,3], M. DIHNE[4], H. LERCHE[1], S. MALJEVIC[1];
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Abstract: In the last decade, Kv7.2 and Kv7.3 potassium channels underlying the M-current have been linked to epileptic disorders, such as benign neonatal seizures and epileptic encephalopathy. The vast majority of experiments has been performed using single neuron patch clamp recordings or EEG recordings in murine models. Because permanent Kcnq2 knock-out (KO) animals are not viable, little is known about the brain electric activity in the first days after birth in the absence of this subunit. To examine the effect of the M-current on neuronal network activity, we plated E17 hippocampal wildtype (WT), Kcnq2+/- (H) and Kcnq2-/- (KO) cultures on 60-channel microelectrode arrays (MEAs) and recorded the activity on 10, 17 and 24 days *in vitro* (DIV). The network parameters were analyzed before and after application of Kv7.2/7.3 agonist retigabine/ezogabine and the blocker XE991. We assessed standard network activity parameters, including spike and burst frequency, burst duration and number of spikes per burst. The burst parameters refer to the synchronous "population burst" activity observed simultaneously on many electrodes. The characteristic pattern observed at DIV 10 was a regular

firing consisting of short-lasting population bursts, which occurred in all 3 genotypes. However, upon application of 10μM retigabine, mean population burst duration was significantly shorter in WT and H compared to KO networks. Over the following 7 and 14 days, WT and H culture firing patterns massively changed to long-lasting, highly synchronized population bursting, interrupted by long, equally synchronized silent periods. In contrast, KO cultures retained their firing pattern observed on 10 DIV. Application of the XE991 antagonist in a concentration of 20μM altered population burst patterns in WT and H yielding a similar pattern to KO networks. Our results show that the M-current dysfunction in *in vitro* neuronal cultures mostly affects the duration of population bursts. The observed differences between the 3 genotypes and during development corroborate the importance of the M-current for the proper functioning of neuronal networks. Furthermore, this established *in vitro* assay can be used for the substance screening in pharmacological studies targeting the M-channels.

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Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.03/B1

Topic: B.04. Ion Channels

Support: NIH Grant HD067517

Title: Human gain-of-function Slack (KCNT1) K+ channel mutations increase positive cooperativity between individual Slack channels through C-terminal domain interactions

Authors: *J. KRONENGOLD[1], G. E. KIM[1], V. R. GAZULA[1], B. YANG[1], I. QURAISHI[2], H. C. MARTIN[3], G. BARCIA[4], J. TAYLOR[3], L. R. COLLEAUX[4], R. NABBOUT[4], L. K. KACZMAREK[1];
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Abstract: The Na+-activated K+ channel Slack (KCNT1, Slo2.2) is widely expressed in neurons. Slack channels contribute to slow afterhyperpolarizations and regulate firing frequency. We expressed the rat Slack channel in Xenopus oocytes and found two different patterns of channel activity in single channel patch clamp recordings. When few (1-3, low N) channels are

present in the patch, we observe a low channel open probability pattern. In contrast, when 4-5 or more channels are present (high N), a high channel open probability is observed. On-cell recordings of high N patches show coupled gating of the fully open state and all points amplitude histograms show a marked deviation from a binomial distribution expected for independently gating channels. The different patterns of Slack channel activity likely reflect channel-channel interactions that only occur when the channels assemble into clusters, as is the case *in vivo*. Indeed, cortical and hippocampal neurons show Slack channel membrane clusters. Electrophysiological recordings from Slack-expressing auditory brainstem neurons show that the native channels exhibit positive cooperativity. Recent studies have identified KCNT1 point mutations in three early onset epilepsy syndromes; migrating malignant partial seizures in infancy, autosomal dominant nocturnal frontal lobe epilepsy, and Ohtahara syndrome. All three have devastating effects on development and intellectual function. The mutations are found in RCK1 and RCK2 of the large 900aa cytoplasmic C-terminal domain of Slack with one located in the S5 pore forming domain of the channel. Voltage clamp studies of the mutant channels show 3- to 22-fold increases in macroscopic currents with no change in levels of channel protein. Single channel studies show no significant changes in unitary conductance except for the G288S pore mutant which shows a 50% decrease. We found that positive cooperativity is enhanced in all human gain-of-function mutations. Mutations show a significant increase in NPo in multichannel patches relative to the wild type multichannel patches. To explore the allosteric mechanism responsible for channel interactions, we made a C-terminal truncation of Slack to remove RCK2, and found that this eliminated the coupling found in Slack channel clusters. Specifically, there is no difference in open probability in low and high N patches. All points amplitude histograms show that the channel open probability in high N patches follows that predicted from a binomial distribution for independently gating channels. Our findings indicate that cooperative interactions between Slack channels are mediated by the distal cytoplasmic C-terminal domain.

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Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.04/B2

Topic: B.04. Ion Channels

Support: NIH Grant HD067517 (L.K.K.)

Title: The Slack Na+-activated K+ channel contributes to serotonin-sensitive currents of *Aplysia* sensory neurons

Authors: Y. ZHANG[1], A. B. KOHN[2], L. L. MOROZ[2], *L. K. KACZMAREK[1];
[1]Yale Univ. Sch. Med., New Haven, CT; [2]Dept. of Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Modulation of ion channels and neurotransmitter release by serotonin in sensory neurons of *Aplysia* has been widely studied as a model for elementary forms of learning and memory. A primary action of serotonin on these neurons is to activate the cyclic AMP-dependent protein kinase (PKA), which reduces potassium current. This reduced potassium current, in turn, causes membrane depolarization, broadening of action potentials and enhanced neurotransmitter release. In mammalian sensory neurons, it has been established that the Slack sodium-activated potassium channel is a major component of potassium current. Activation of PKA in mammalian sensory neurons increases excitability by reducing potassium current through internalization of Slack subunits (Nuwer et al., 2010). To determine if Slack subunits have a similar role in setting the excitability of *Aplysia* sensory neurons, we have isolated several isoforms of *Aplysia* Slack gene from an *Aplysia* sensory neuron library. *In situ* hybridization experiments show that Slack is expressed at very high levels in mechanosensory neurons in all major ganglia including the abdominal, pleural, cerebral and buccal ganglia. In voltage clamp experiments on isolated pleural mechanosensory neurons, potassium current was reduced on removal of sodium from the external medium. In normal media, application of serotonin reduced overall potassium current, and in current clamp experiments caused depolarization of the resting potential and broadening of action potentials. In contrast, if cells were treated with RNAi against Slack to suppress Slack expression, serotonin failed to reduce potassium current. Slack RNAi-treated cells had more positive resting potentials and broader action potentials than those treated with scrambled RNAi, and application of serotonin produced smaller depolarizations with no broadening of action potentials. Our findings suggest that the Slack sodium-activated potassium channel is highly differentially expressed in *Aplysia* mechanosensory neurons, and contributes in a major way to serotonin-induced changes in excitability.

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Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.05/B3

Topic: B.04. Ion Channels

Support: DFFD grant to PB

Metabolomics NASU grant to PB

Title: Differential Ca^{2+}-dependent signaling of the neuronal Ca^{2+} sensor proteins, Neurocalcin δ and Hippocalcin, in cultured hippocampal neurons

Authors: N. I. KONONENKO[1], J. VIVIANO[3], A. V. DOVGAN[1], V. P. CHERKAS[1], J. ZHANG[3], V. VENKATARAMAN[3], *P. V. BELAN[2];
[1]Lab. of Mol. Biophysics, [2]Bogomoletz Inst. of Physiol., Kiev, Ukraine; [3]Dept. of Cell Biology, Grad. Sch. of Biomed. Sci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ

Abstract: Hippocalcin (HPCA) and Neurocalcin δ (NCALD) are structurally very similar neuronal Ca^{2+} sensor (NCS) proteins controlling neuronal functions in many types of cells and shown to be crucial in regulating slow afterhyperpolarization (sAHP). It has been demonstrated that a time course of sAHP, which controls patterns of neuronal activity, may depend upon Ca^{2+}-induced NCS protein translocation from the cytosol to the plasma membrane. In spite of minor difference in AA sequence between HPCA and NCALD, a primary structure of the latter promotes its Ca^{2+}-dependent dimerization and results in higher affinity for binding Ca^{2+}. Besides, major distinctions in AA sequence between the proteins are located in their N-terminal region responsible for Ca^{2+}-dependent insertion into the plasma membrane. Altogether these differences in structure and biophysical properties suggest different Ca^{2+}-dependent signaling of these proteins during induction of sAHP. To test this hypothesis we studied Ca^{2+}-dependent translocation of HPCA, NCALD and their chimeras in cultured hippocampal neurons. The neurons were co-transfected with HPCA and NCALD (or chimeras) tagged by different fluorescent proteins and stimulated in a voltage-clamp mode to evoke transient increases of intracellular $[Ca^{2+}]_i$ that may produce sAHP. The same spatio-temporal patterns of $[Ca^{2+}]_i$ changes led to different time courses, amplitudes, and locations of HPCA and NCALD translocation. There was substantial (~1s) latency in NCALD translocation as well as its dramatic (~4 times) prolongation compared to HPCA. Ca^{2+} influx was also spatially differentially decoded by HPCA and NCALD translocation in soma and dendrites of cultured hippocampal neurons. Besides, sensitivity of NCALD to Ca^{2+} at low $[Ca^{2+}]_i$ was 4-fold higher compared to HPCA. Initial experiments with chimeras suggested that differences in kinetics of NCALD signaling compared to HPCA is mainly a result of dimerization that stabilizes Ca^{2+}-bound form and increase NCALD binding to the plasma membrane due to its interaction with 2 myristoyl groups. Thus, minor distinctions in AA sequences of HPCA and NCALD does lead to a substantial difference in their Ca^{2+}-dependent signaling via translocation to the plasma membrane and subsequent activation of sAHP. We conclude that differential expression of HPCA and NCALD in CNS neurons may determine the time course of sAHP in particular neurons, thus far controlling their patterns of activity.

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Poster

121. Potassium Channels I

Location: Hall A

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Topic: B.04. Ion Channels

Support: Research Grant Epilepsy Foundation

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Science Foundation Ireland grant 13/IA/1891

Science Foundation Ireland grant 13/SIRG/2014

Science Foundation Ireland grant 13/SIRG/2098

Title: The potassium channel Kv4.2 is regulated by miRNA-induced silencing during seizures

Authors: *C. GROSS[1], X. YAO[2], T. ENGEL[3], L. XING[2], S. W. DANIELSON[2], K. T. THOMAS[2], E. JIMENEZ-MATEOS[3], D. HENSHALL[3], G. BASSELL[2];
[1]Neurol., CCHMC, Cincinnati, OH; [2]Emory Univ., Atlanta, GA; [3]RCSI, Dublin, Ireland

Abstract: Epileptic seizures are bursts of excessive neuronal activity, suggesting that mechanisms controlling brain excitability are compromised. The voltage-gated potassium channel Kv4.2, the predominant mediator of hyperpolarizing A-type currents in the CA1 region of the hippocampus, is a crucial regulator of neuronal excitability. Kv4.2 expression levels are reduced following seizures, but the underlying mechanisms remain unclear. Here, we report that Kv4.2 is translationally repressed by the RNA-induced silencing complex shortly after status epilepticus in mice *in vivo* and after kainic acid treatment of hippocampal neurons *in vitro*. Moreover, we have identified a microRNA that inhibits Kv4.2 protein expression. Antagonizing this microRNA with an antisense oligonucleotide blocks excitotoxicity-induced reduction of Kv4.2 protein in neurons, is neuroprotective, and reduces the severity of kainic acid-evoked status epilepticus in mice. In summary, our results demonstrate that microRNA-mediated

silencing contributes to Kv4.2 suppression during seizures and suggest that Kv4.2-targeting microRNAs might serve as therapeutic targets for epilepsy.

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Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.07/B5

Topic: B.04. Ion Channels

Support: National Natural Science Foundation of China (Grant 31471149)

CAS Hundred Talent Program (S.-Q.C.)

Title: KChIP-like auxiliary subunits of Kv4 channels regulate excitability of muscle cells and control male turning behavior during mating in Caenorhabditis elegans

Authors: *X. CHEN, M.-Y. RUAN, S.-Q. CAI;
Chinese Acad. of Sci., Inst. of Neurosci., Shanghai, China

Abstract: Voltage gated Kv4 channels control the excitability of neurons and cardiac myocytes by conducting rapidly activating-inactivating currents. The function of Kv4 channels is profoundly modulated by K^+ Channel Interacting Proteins (KChIPs) soluble auxiliary subunits. However, the *in vivo* mechanism of the modulation is not fully understood. In this study, we aimed to study the mechanism of KChIPs modulation on Kv4 channels in Caenorhabditis elegans (*C. elegans*). We identified three *C. elegans* KChIP-like (ceKChIP) proteins, NCS-4, NCS-5, and NCS-7. All three ceKChIPs alter electrical characteristics of SHL-1, a *C. elegans* Kv4 channel ortholog, currents by slowing down inactivation kinetics and shifting voltage dependence of activation to more hyperpolarizing potentials. Native SHL-1 current is completely abolished in cultured myocytes of Triple KO worms where all three ceKChIPs genes are deleted. Re-expression of NCS-4 partially restored expression of functional SHL-1 channels, whereas NCS-4(efm), a NCS-4 mutant with impaired Ca^{2+}-binding ability, only enhanced expression of SHL-1 proteins, but failed to transport them from the Golgi apparatus to the cell membrane in body wall muscles of Triple KO worms. Moreover, translational reporter revealed that NCS-4 assembles with SHL-1 K^+ channels in male diagonal muscles. Deletion of either ncs-4 or shl-1

significantly impairs male turning, a behavior controlled by diagonal muscles during mating. The phenotype of ncs-4 null mutant could be rescued by re-expression of NCS-4, but not NCS-4(efm), further emphasizing the importance of Ca^{2+} binding to ceKChIPs in regulating native SHL-1 channel function. Together, these data reveal an evolutionarily conserved mechanism underlying the regulation of Kv4 channels by KChIPs, and unravel critical roles of ceKChIPs in regulating muscle cell excitability and animal behaviors in *C. elegans*.

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Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.08/B6

Topic: B.04. Ion Channels

Title: Intrastriatal administration of retigabine, a pan-Kv7 (KCNQ) channel opener, inhibits post-synaptic neuronal activation

Authors: *H. B. HANSEN[1,3], M. D. MIKKELSEN[3], P. WEIKOP[2], J. D. MIKKELSEN[1,3]; [1]Neurobio. Res. Unit, [2]Neuropsychiatric Lab., Copenhagen Univ. Hosp., Copenhagen, Denmark; [3]NeuroSearch A/S, Ballerup, Denmark

Abstract: Heteromeric Kv7 (KCNQ) channels are voltage-dependent potassium channels composed of four Kv7 subunits being differently expressed in the brain. Striatal neurotransmission is strongly suppressed by systemic administration of various Kv7 channel openers, including the pan-Kv7 channel opener retigabine. This effect presumably involves stimulation of different Kv7 channel isoforms in mesencephalic dopaminergic neurons projecting

to the striatum. However, it remains to be established whether Kv7 channels locally expressed in the striatum may also play an important contributing role in controlling striatal post-synaptic excitability. To further investigate the functional relevance of striatal Kv7 channel expression *in vivo*, we determined the Kv7 subunit mRNA composition in the rat striatum and asked whether retigabine may influence striatal excitability by direct stimulation of striatal Kv7 channel activity. The relative levels of rat striatal Kv7 subunit mRNAs were Kv7.2=Kv7.3=Kv7.5>>Kv7.4. Post-synaptic Kv7 mRNA expression in the rat striatum was suggested by an almost complete ablation of striatal Kv7 mRNA levels following a unilateral intrastriatal application of quinolinic acid. Striatal Kv7 channels were functional, as revealed by a unilateral intrastriatal retigabine application which markedly reduced striatal neuronal c-Fos induction after acute systemic haloperidol administration. Collectively, these data suggests that post-synaptic striatal Kv7 channels play a direct role in modulating striatal excitability.

Disclosures: H.B. Hansen: None. **M.D. Mikkelsen:** None. **P. Weikop:** None. **J.D. Mikkelsen:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.09/B7

Topic: B.04. Ion Channels

Support: Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Title: DPP6 domains responsible for its localization and function

Authors: *L. LIN, L. K. LONG, M. M. HATCH, D. A. HOFFMAN;
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Abstract: Dipeptidyl peptidase-like protein 6 (DPP6) is an auxiliary subunit of the Kv4 family of voltage-gated K^+ channels known to enhance channel surface expression and potently accelerate their kinetics. DPP6 is a single transmembrane protein, which is structurally remarkable for its large extracellular domain. Included in this domain is a cysteine-rich motif, the function of which is unknown. Here we show that this cysteine-rich domain of DPP6 is required for its export from the ER and expression on the cell surface. Disulfide bridges formed at C349/C356 and C465/C468 of the cysteine-rich domain are necessary for the enhancement of Kv4.2 channel surface expression but not its interaction with Kv4.2 subunits. The short

intracellular N-terminal and transmembrane domains of DPP6 associates with and accelerates the recovery from inactivation of Kv4.2, but the entire extracellular domain is necessary to enhance Kv4.2 surface expression and stabilization. Our findings show that the cysteine-rich domain of DPP6 plays an important role in protein folding of DPP6 that is required for transport of DPP6/Kv4.2 complexes out of the ER.

Disclosures: L. Lin: None. **L.K. Long:** None. **M.M. Hatch:** None. **D.A. Hoffman:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.10/B8

Topic: B.04. Ion Channels

Support: R25GM110513

SC1 GM088019

R01 NS065201

G12 MD007583

P031M105050

Title: Novel KCNJ10 SNPs affect Kir4.1 biophysical properties and their modulation by spermine

Authors: *M. MENDEZ[1], Y. KUCHERYAVYKH[1], A. ZAYAS-SANTIAGO[1], W. VELEZ-CARRASCO[1], L. CUBANO[2], S. SKATCHKOV[3], M. EATON*[1];
[1]Biochem., [2]Anat. and Cell Biol., [3]Physiol., Univ. Central Del Caribe, Bayamon, PR

Abstract: KCNJ10-encoded Kir4.1-containing channels generate the major inward rectifying K+ conductance in astrocytes and are essential for buffering excess extracellular [K+]. Mutations in the KCNJ10 gene are causal in certain forms of epilepsy. This gene contains over 120 coding-region single nucleotide polymorphisms (SNPs) of which their molecular effect remains unknown. Here, we investigate the functional consequences of uncharacterized SNP's reported in publically accessible genome data bases (Q212R, L166Q and G83V) that we hypothesize will alter Kir4.1 channel function. Using whole-cell patch clamp of tSA201 cells expressing wild-type (WT) Kir4.1 or its variants, we evaluated the impact of these SNPs on homomeric Kir4.1

and heteromeric Kir4.1/5.1 channel function. Cells expressing WT Kir4.1, Q212R or L166Q channels displayed a hyperpolarized membrane potential, compared with G83V variant. In response to voltage-steps, macroscopic currents from cells expressing WT and Q212R channels displayed no differences, whereas currents from cells expressing L166Q and G83V were reduced. Surprisingly, the current response was rescued when L166Q was co-expressed with Kir5.1. Kir4.1 channels can be modulated by the polyamine spermine (SPM). Using inside-out excised patches we determined SPM sensitivity of homomeric Kir4.1 and heteromeric Kir4.1/5.1 channels and determined V1/2 and offset values by fitting to the Boltzmann function. Both homomeric Q212R and heteromeric Q212R/5.1 displayed greater V1/2 values indicating reduced block by 1μM SPM. At 100μM SPM, the block of Q212R-containing homomeric channels was the same as WT suggesting that the greater driving force of SPM allowed steady state to be achieved. As homomeric L166Q channels show reduced current, we could only assess the effect of SPM on heteromeric L166Q/Kir5.1. Heteromeric L166Q-Kir5.1 channels achieved a higher block than WT at all SPM concentrations tested and most notably with 100 μM SPM. This could be explained if this residue helps to keep SPM in a stable conformation in the deep pore cavity which is supported by the complete block (i.e., offset of 0) seen in these channels. Overall, our data suggest that SNP's of KCNJ10 may affect channel function via different mechanisms.

Disclosures: M. Mendez: None. **Y. Kucheryavykh:** None. **A. Zayas-Santiago:** None. **W. Velez-Carrasco:** None. **L. Cubano:** None. **S. Skatchkov:** None. **M. Eaton*:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.11/B9

Topic: B.04. Ion Channels

Title: The roles of ion channel antibodies on neuron survival

Authors: *N. AYSIT[1], E. TUZUN[2], G. ÖZTÜRK[3];
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Abstract: Neuronal excitability is finely controlled by various membranous channels and associated proteins. In the central nervous system (CNS), the rapidly inactivating voltage-gated potassium channels (VGKC) are the major determinants of dendritic excitability. VGKCs play a critical role in regulating neuronal excitability and synaptic plasticity in the hippocampus.

NMDA receptors and VGKCs are critical for the development of the CNS, generation of rhythms for breathing and locomotion, and the processes underlying learning, memory, and neuroplasticity. Consequently, abnormal expression levels and altered NMDAR function have been implicated in numerous neurological disorders and pathological conditions. NMDAR hypofunction can result in cognitive defects, whereas overstimulation causes excitotoxicity and subsequent neurodegeneration. Subconductance levels have been observed in virtually every type of ion channel, although the number of levels, stability, and abundance vary widely. Sublevels have been well-characterized in potassium channels, which are evolutionarily related to NMDARs. The aim of the study is the contribution of VGKC-complex protein leucine rich glioma inactivated 1 (LGI1) and NMDAR of neuronal survival/death in hippocampal cells. Primary hippocampal cultures were prepared from the brains of newborn Balb-c mice approximately 4 days old. The hippocampus was isolated from the brain of each mice and treated at 4 °C. The cells were suspended in neurobasal medium and were plated at several densities 200 -1600 cells per mm2. IgG was isolated from healthy participants and limbic patients who were NMDAR or LGI1 antibody positive. Different concentrations of isolated IgG were added to the preperation at days 1, 3 and 7 *in vitro*. Imaging was performed on same days by confacal microscobe and dead cells were determined using propidium iodide. In this study t-test was used for analysis and showed that high concentrations of anti-NMDAR and LGI1 IgG cause neuronal and astrocytic death. These result showed that ion channel antibodies do not only cause neuronal dysfunction but also dysregulate neuronal-astrocytic survival.

Disclosures: **N. Aysit:** None. **E. Tuzun:** None. **G. Öztürk:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.12/B10

Topic: B.04. Ion Channels

Title: TWIK-1 /TASK-3 heterodimer contributes to the intrinsic excitability of dentate granule cells in mouse hippocampus

Authors: J. CHOI[1], O. YARISHIKIN[2], E. KIM[3], J.-Y. PARK[3], *E. HWANG[1];
[1]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]The university of Utah, Salt lake city, UT; [3]Korea university, Seoul, Korea, Republic of

Abstract: Two-pore domain K^+ (K2P) channels control resting membrane potential and cellular excitability in neuronalcells. Among the K2P channels, TWIK1 is the first identified member of

K2P channel and highly expressed in brain, but its neuronal role is almost unknown.We recently reported that TWIK-1 contributes to the intrinsic excitability of dentate gyrus granule cells (DGGCs) in mouse hippocampus. However, homodimer function of TWIK-1 is still controversial. Recently, we and Goldstein suggest that TWIK-1 can make heterodimer with TREK-1 in astrocyte and TASK-3 in cerebellum granule cells and act as a functional channel. Talley and his colleagues were already shown that TASK-3 mRNAs were intensively detected in DGGCs as well as cerebellum granule cells, but its protein expression level was not reported.Therefore, we determined TASK-3 expression and the subcellular localization and tested its real- heterodimerization with TWIK-1 using PLA assay in DGGCs. As a result, TASK-3 is highly expressed in DGGCs and can make real-hetrodimers with TWIK-1. In order to verify TWIK-1/TASK-3 heterodimer function in DGGCs, we used TWIK-1 KO mice and TASK-3 specific shRNA containing viral vector. Expectedly, both TWIK-1 KO mice and TASK-3-shRNA infected mice exhibit significantly reduced outwardly rectifying potassium currents and enhanced intrinsic excitability in DGGCs. Interestingly,TASK-3 shRNA infected TWIK-1 KO mice have no additional effect when comparing each condition. Taken together, we suggest that TWIK-1 and TASK-3 mostly make heterodimers in DGGCs and contribute to the intrinsic excitability

Disclosures: **J. Choi:** None. **O. Yarishikin:** None. **E. Kim:** None. **J. Park:** None. **E. Hwang:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.13/B11

Topic: B.04. Ion Channels

Title: Ca^{2+}-dependent modulation of Kv4.2-mediated currents

Authors: *J. J. GUTZMANN, A. M. CHAREST, D. A. HOFFMAN;
NICHD, NIH, Bethesda, MD

Abstract: The sub-threshold activating, A-type potassium current (IA) has long been recognized as a critical component for regulating dendritic excitability and shaping back propagating action potentials. In CA1 pyramidal cells of the hippocampus IA is carried predominantly by Kv4.2 potassium channels, which are associated with a variety of accessory proteins that modulate their trafficking and kinetics. Among these interacting proteins are K-channel interacting proteins

(KChIPs), small cytoplasmic proteins with four calcium binding EF-Hand motifs. IA shows a distinct functional gradient along the apical dendrites of CA1 pyramidal neurons. Synaptic localization and activity dependent trafficking of Kv4.2 indicates an additional, and possibly distinct, role of Kv4.2 in synaptic physiology. The strong and reciprocal relationship between Kv4.2 and the GluN2B subunit of the Calcium conducting N-methyl-D-aspartate (NMDA) receptor opens up the possibility of calcium dependent effects on Kv4.2 function in the synapse. Using a mutated KChIP construct that is unable to bind calcium but still interacts with Kv4.2, we show that changes in intracellular and extracellular calcium concentrations alter Kv4.2 mediated currents in the presence of KChIPs in a heterologous expression system, as well as CA1 pyramidal neurons in acute slices. Specifically, current densities and current decay constants, as well as voltage dependent activation and inactivation states of Kv4.2 depend not only on the presence or absence of KChIPs, but also on the availability of calcium. Since intracellular calcium concentrations are tightly regulated *in vivo*, especially in dendritic spines, and Kv4.2 has a demonstrated intimate relationship with calcium conducting channels as well as calcium binding proteins, understanding the effect of calcium on Kv4.2 mediated currents will be of great value for the understanding of the role that IA plays in shaping postsynaptic currents and dendritic integration in the hippocampus.

Disclosures: J.J. Gutzmann: None. **A.M. Charest:** None. **D.A. Hoffman:** None.

Poster

121. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH Grant NS070261

Barrow Neurological Foundation

Title: Ketogenic diet promotes the upregulation of K_{ATP} channels expression by mediating ketone activity

Authors: H. OH, T. J. METTLER, *D. KIM;
Barrow Neurolog. Institute, St. Joseph's Hosp. & Med. Ctr., Phoenix, AZ

Abstract: Metabolic alteration is being considered as a potential intervention for various neurological disorders, based in part on the functional protection of the high-fat, low-

carbohydrate ketogenic diet (KD) and its metabolic substrates, ketone bodies [KBs; β - hydroxybutyrate (BHB) and acetoacetate (ACA)]. The KD and KBs are involved in producing anticonvulsant properties as well as in broad disease-modifying effects, but the mechanisms underlying their actions have yet to be fully elucidated. Our recent findings have demonstrated that KBs can protect synaptic plasticity against oxidative insults by modulating ATP-sensitive potassium (K_{ATP}) channels (PMID; 25848768). To expand this observation, we asked whether expression profiling of mRNAs and proteins of K_{ATP} channels augments following the administration of the KD or KBs, and if pharmacological blockade and genetic ablation of K_{ATP} channels counteract the neuronal protective effects afforded by KBs. C3HeB/FeJ mice were fed either the KD or a standard diet (SD) from weaning until postnatal day 35 and 60. Hippocampal lysates collected from the KD-fed mice showed a significant increase in the inwardly rectifying K^+ channel subunit Kir6.2 and SUR1, critical components of K_{ATP} channels, as compared to mice fed SD. Consistent with incidence of mild ketosis in KD-fed mice, murine hippocampal HT22 cells exposed to a cocktail of KBs (BHB and ACA, 1 mM each) underwent a higher expression of mRNAs and proteins of Kir6.1, Kir6.2, and SUR1, accompanied with elevation of cell viability against 500 μM H_2O_2 - evoked oxidative insult. The neuroprotective effect was reversed when pharmacological blockers, such as glibenclamide and 5-hydroxydecanoate, or shRNA of K_{ATP} channels were applied with KBs. Further, under lipopolysaccharide (LPS)-induced microglial cell activation, KBs-treated microglial cells (BV2) had a reduction in the release of pro-inflammatory mediators induced by LPS and its effect was linearly correlated with the upregulation of expression profiling of K_{ATP} channels. Kir6.2 immunoreactivity was significantly increased in both HT22 cells and BV2 cells exposed to KBs for 6 hrs. Taken together, our findings suggest that metabolic change by the KD may provide the upregulation of K_{ATP} channels expression through ketone activity to help contribute to its diverse protective actions.

Disclosures: **H. Oh:** None. **T.J. Mettler:** None. **D. Kim:** None.

Poster

121. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH Grant NS078184

Title: The regulation of Slack K(Na) channel trafficking by p38 MAP Kinase

Authors: S. GURURAJ[1], *A. BHATTACHARJEE[2];
[1]Pharmacol. & Toxicology, SUNY at Buffalo, Buffalo, NY; [2]SUNY, Buffalo, Buffalo, NY

Abstract: Nociceptive sensitization is a central component of neuropathic pain, involving an alteration in the membrane properties of the sensory neurons of the Dorsal Root Ganglion (DRG) such that they have a lowered threshold for activation and hence, increased spontaneous activity. This DRG hyperexcitability manifests as the painful physiological symptoms of hyperalgesia and allodynia in patients of neuropathic pain, in whom there is as yet a large unmet need for medical treatment due to a lack of proper understanding of the precise molecular mechanisms involved. The key role of sodium activated potassium (K_{Na}) channels in the maintenance of firing accommodation in DRG neurons makes them important targets to study in the context of DRG excitability, which then deems mechanisms of K_{Na} channel regulation as potential ways to regulate DRG hyperexcitability. In this context, we examined the K_{Na} channel Slack's extensive intracellular C-terminus for possible sites of regulatory protein interactions and found that it contains two putative p38 MAPK phosphorylation sites that are highly conserved across species. While there is no direct link between p38 MAPK and K_{Na} channels, there is evidence for p38 MAPK's role in facilitating neuronal regeneration after nerve crush of the sciatic nerve, indicating that the kinase could be an important player in neuropathy and potentially, in nociceptive sensitization. To answer the question of whether p38 MAPK regulates Slack channels via phophorylation of the C-terminus, we have performed electrophysiology experiments to demonstrate that Slack current is subject to modulation by p38 MAPK, and biochemical experiments to show that Slack is basally phosphorylated by p38 MAPK. Site-directed mutagenesis of the p38 MAPK phosphorylation sites indicates that modulation occurs via phosphorylation at both the sites. Finally, biotinylation assays suggest that the mechanism of modulation of Slack current by p38 MAPK is likely at the membrane via a trafficking mechanism. Together, these results are the first direct demonstration of Slack as a p38 MAPK substrate, and provide evidence for p38 phosphorylation playing a role in Slack channel trafficking and hence, DRG membrane excitability.

Disclosures: S. Gururaj: None. **A. Bhattacharjee:** None.

Poster

121. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH Grant HD067517 (L.K.K.)

HHMI (W.N. and A.H.)

Title: An ALS-associated mutant SOD1 alters sodium-activated potassium currents and neuronal excitability in *Aplysia* neurons

Authors: *Y. ZHANG, W. NI, A. HORWICH, L. L. KACZMAREK;
Yale Univ. Sch. Med., New Haven, CT

Abstract: Mutant human Cu/Zn superoxide dismutase 1 (SOD1) is associated with motor neuron toxicity and death in an inherited form of amyotrophic lateral sclerosis (ALS).The ALS-linked mutations have been associated with a toxic gain-of-function, including the formation of cytotoxic oligomers and aggregates of misfolded SOD1 subunit. The precise forms of the mutant SOD1 that are toxic and the mechanisms by which they produce toxicity and alter neuronal excitability are, however, not clear. To address these questions, we recorded from bag cell neurons of *Aplysia*, a model system to study neuronal excitability. We found that injection of fluorescently-tagged wild-type SOD1YFP or monomeric mutant G85R SOD1YFP had no effect on net ionic currents measured under voltage clamp. In contrast, outward potassium currents were significantly reduced by microinjection of fluorescently-tagged cross-linked dimers of mutant G85R SOD1YFP or by mutant SOD1YFP that was preincubated at 37 degrees. Reduction of potassium current was also seen with G85R SOD1YFP that had been cross-linked to multimeric species of ~300 KDa or to > 300 KDa. In current clamp recordings, microinjection of 300KDa cross- linked G85R SOD1YFP increased excitability by causing depolarization of the resting membrane potential, and decreasing the latency of action potentials triggered by depolarization. The effect of cross-linked 300KDa G85R SOD1YFP on potassium current was reduced by removing sodium from the bath solution, or by knocking-down levels of the Slack sodium-activated potassium channel using RNAi. It was also prevented by pharmacological inhibition of ASK1 (apoptosis signal-regulating kinase 1) or of JNK (c-Jun N-terminal kinase), but not by an inhibitor of P38 mitogen-activated protein kinases. These results suggest that mutant SOD1 aggregates trigger a kinase pathway that regulates levels of sodium-activated potassium current in neurons.

Disclosures: Y. Zhang: None. **W. Ni:** None. **A. Horwich:** None. **L.L. Kaczmarek:** None.

Poster

121. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH R01 HD072056

NIH R01 NS044163

Title: Phosphatidylinositol 4,5-bisphosphate (PIP2) differentially modulates afterhyperpolarizations in oxytocin and vasopressin magnocellular supraoptic neurons

Authors: *M. KIRCHNER, R. FOEHRING, W. E. ARMSTRONG;
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Abstract: Oxytocin- (OT) and vasopressin- (VP) secreting neurohormonal cells play a crucial role in many physiological functions including lactation, parturition (OT) and cardiovascular regulation (VP). The release of both hormones is optimized by a burst-firing pattern of action potentials. Afterhyperpolarizations (AHPs) play a crucial role in shaping bursts and thus are critical for robust hormone release. Three components of the AHP have been described: fast (fAHP), medium (mAHP), and slow (sAHP). Because previous work demonstrated that the lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) enabled the sAHP current in cortical neurons (Villalobos et al., 2011: J Neurosci 31: 18303), we investigated whether PIP_2 served a similar function for OT and VP neurons of the supraoptic nucleus (SON). Using whole cell recording in coronal hypothalamic slices from adult female rats, we demonstrated that wortmannin, which inhibits the rate limiting enzyme of PIP_2 production, PI4Ka, robustly reduced both the sAHP and mAHP of OT neurons with high affinity (EC_{50} = 64 nM). In contrast, VP neurons typically showed no inhibition to wortmannin, even at high concentrations. In VP and OT neurons, the PIP_3 kinase inhibitor LY294,002 (100nM) failed to affect AHPs, suggesting the wortmannin effects were specific to PIP_2 synthesis. We further tested this by introducing a PIP_2 analog (diC_8-PIP_2) into neurons through the patch pipette, which slowed rundown of mAHP and sAHPs over 30+ minutes of recording in both cell types and even enhanced the mAHP and sAHP in VP neurons. Finally, wortmannin failed to inhibit AHPs in the presence of diC_8-PIP_2 in both OT and VP neurons. The results indicate that PIP_2 is necessary for the normal expression of mAHPs and sAHPs in OT neurons, but may nevertheless contribute to the enhancement of AHPs in VP neurons when made available in large quantity.

Disclosures: M. Kirchner: None. **R. Foehring:** None. **W.E. Armstrong:** None.

Poster

121. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NINDS Grant RO1NS062784

Title: The relative contribution of Kir4.1, two-pore domain K+ channels and KCNN2 to the passive conductance of mature hippocampal astrocytes

Authors: *C. M. KIYOSHI, Y. DU, Q. WANG, C. C. ALFORD, B. MA, S. ZHONG, M. ZHOU;
Dept. of Neurosci., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Mature hippocampal astrocytes display a characteristic linear current - voltage (I-V) membrane conductance, or passive conductance. While the K^+ channels are known as the molecular identities underlying the passive conductance, a complete list of these channels remains to be fully identified. The inwardly rectifying Kir4.1 is one of the identified channels, however we have shown that Kir4.1 contributes to less than 50% of the total membrane conductance, leaving the remaining channels to be further uncovered. In an effort to identify these channels, we have previously focused on the two-pore domain K^+ channels, TWIK-1 and TREK-1. However, the functional study from either single-knockouts or double-knockout of these genes resulted in an insignificant alternation in both passive conductances and membrane potentials. We extended the search to genes in other K^+ channel subfamilies that could be responsible for the passive conductance by examining their mRNA expression levels in freshly dissociated hippocampal astrocytes, which includes $K_{ir}5.1$, KCNG4, KCNK10, and KCNN2. We found that the potassium intermediate/small conductance calcium-activated channel, KCNN2, shows the highest mRNA expression among other candidate genes. In addition, KCNN2 shows comparable expression levels to the highest expressing potassium channel, TWIK-1. Interestingly, KCNN2 is a voltage-independent leak K^+ channel that can be dynamically regulated by intracellular Ca^{2+} concentration. Thus KCNN2 is likely a channel contributing significantly to the passive conductance, as well as being able to coordinate intracellular Ca^{2+} signaling with membrane K^+ conductance.

Disclosures: **C.M. Kiyoshi:** None. **Y. Du:** None. **Q. Wang:** None. **C.C. Alford:** None. **B. Ma:** None. **S. Zhong:** None. **M. Zhou:** None.

Poster

121. Potassium Channels I

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Topic: B.04. Ion Channels

Support: BBSRC grant BB/L01565X/1

Title: Kv3 channel subunit localisation and co-expression with phenotypic markers in the murine spinal cord

Authors: *P. MULLEN[1], C. LARGE[2], J. DEUCHARS[1], S. DEUCHARS[1];
[1]Fac. of Biol. Sci., Univ. of Leeds, Leeds, United Kingdom; [2]Autifony Therapeut. Ltd., London, United Kingdom

Abstract: Kv3 voltage-gated potassium ion channels form tetrameric assemblies comprised of alpha subunits Kv3.1-Kv3.4 and are expressed in interneurons throughout the spinal cord (Deuchars et al. 2001). Kv3 subunits decrease with age in the auditory brainstem, a loss thought to underlie age-related hearing deficits (Zettel et al. 2007). We postulate a similar age-related decline in the spinal cord and present here an initial characterisation of the expression of Kv3 subunits in the adult murine spinal cord. 3 wild-type (WT) C57Bl/6 and 3 transgenic GAD67-GFP C57Bl/6 mice (4-6 weeks) were anaesthetised by intraperitoneal injection of sodium pentobarbitone (60mg/kg), perfused with 4% paraformaldehyde (PFA) and dissected to isolate spinal cords and post-fixate in 4% PFA. Spinal cords were sectioned at 40 μm using a vibrating microtome. WT tissue was processed for double labelling immunohistochemistry (IHC) of Kv3 α-subunits and various protein cell markers; parvalbumin and calretinin are calcium binding proteins found in certain interneurons; ChAT (choline acetyl transferase) is expressed in motoneurons and sympathetic preganglionic neurons; and finally vGluT1, vGluT2 and GlyT2 are synaptic transporters for glutamate and glycine, respectively. Transgenic GAD67-GFP tissue was processed for double labelling of Kv3 α-subunits with glutamic acid decarboxylase 67(GAD67). Kv3.1, Kv3.3 and Kv3.4 but not Kv3.2-positive neurons were situated around autonomic and motor regions within the adult murine spinal cord. These neurons were not GABAergic (not containing GAD67) but mostly glycinergic (GlyT2) and/or glutamatergic (vGlut2 but not vGluT1). Kv3.1 and Kv3.4 subunits were expressed in the membranes of ventral horn CHAT-positive putative motoneuron cell bodies. Kv3 subunits were also observed on the membranes of parvalbumin and calretinin positive cell bodies; Kv3.1 with parvalbumin around the intermediolateral horn (IML) and in the ventral horn and both Kv3.3 and Kv3.4 co-expressed with parvalbumin on the membranes of cell bodies in the ventral horn. Kv3.1 was extensively co-expressed with calretinin in cell bodies around the central canal, IML, dorsal horn and ventral horn, the latter thought to represent Renshaw cells. A similar pattern of co-expression was also observed for Kv3.3. We hypothesise that the expression of these ion channels in the murine spinal cord may change through age at a level paralleled by changes in both autonomic and motor outputs. This initial characterisation of Kv3 expression in adult mice will be compared to

that of aged and young mice to elucidate any age-related changes in expression and the functional consequences of these changes.

Disclosures: **P. Mullen:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Autifony Therapeutics Ltd. **C. Large:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autifony Therapeutics Ltd.. **J. Deuchars:** None. **S. Deuchars:** None.

Poster

121. Potassium Channels I

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Topic: B.04. Ion Channels

Support: Fragile X grant: DC01919

Autifony Therapeutics

Title: Novel physiological modulators of Kv3 channels regulate firing rate and temporal accuracy of auditory brainstem neurons in a mouse model of Fragile X syndrome

Authors: *L. EL-HASSAR[1], M. R. BROWN[1], L. SONG[2], C. H. LARGE[3], G. ALVARO[3], L. K. KACZMAREK[1];
[1]Pharmacol. department SHMB 309, [2]Dept. of Surgery, Yale Univ. Sch. of Med., New Haven, CT; [3]Autifony Therapeut. Limited, London, United Kingdom

Abstract: Fragile X syndrome (FXS) is the most common form of inherited intellectual disability. In common with autism, FXS is characterized by hypersensitivity to many types of sensory stimuli, including environmental sounds. Our previous work has shown that mice lacking FMRP (Fragile Mental Retardation Protein), which are a mouse model for Fragile X syndrome, have abnormally elevated levels of Kv3.1-like "high-threshold" potassium currents and significantly decreased levels of Slack Na+-activated K+ currents. Both of these are predicted to increase the firing rate of the postsynaptic neurons and to substantially degrade the accuracy of timing of action potentials in auditory brainstem neurons of the medial nucleus of the trapezoid body (MNTB). Consistent with this, we have found that the firing pattern of MNTB neurons in response to repeated stimulation is severely abnormal in Fragile X mice. We have also found that waves IV of the Auditory Brainstem Response (ABR) recorded *in vivo* are significantly enhanced in Fragile X mice, suggesting that loss of FMRP alters central processing

of auditory signals. Based on these results we are now testing, in Fragile X mice, the physiological effects of two potential therapeutic compounds, AUT1 and AUT2, which modulate the activity of Kv3 family channels in cell lines. To our surprise, we found that the effects of AUT1 on MNTB firing in Fragile X mice are completely opposite to their effects on neurons from wild-type animals. More specifically, while AUT1 decreased firing in MNTB neurons from wild-type animal, it enhanced firing in Fragile X mice. In contrast to AUT1, AUT2 decreased firing in Fragile X mice. Because AUT1 and AUT2 both shift the voltage-dependence of Kv3.1 activation to more negative potentials, but AUT2 is more potent that AUT1, one potential explanation for this difference is that while both compounds increase Kv3.1-like "high-threshold" potassium currents in the Fragile X mice, the greater shift produced by AUT2 increases currents close to the resting potential, raising the threshold for action potential generation. Consistent with this, our preliminary voltage-clamp experiments on MNTB neurons indicate that AUT2 increases K+ current at negative potentials. Ongoing experiments are characterizing the full difference in K+ current responses of wild type and Fragile X mice.

Disclosures: **L. El-Hassar:** None. **M.R. Brown:** None. **L. Song:** None. **C.H. Large:** None. **G. Alvaro:** None. **L.K. Kaczmarek:** None.

Poster

121. Potassium Channels I

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Topic: B.04. Ion Channels

Support: NIH Grant NS-044163

Title: Differential expression of voltage-gated potassium conductances in genetically-defined layer 5 neocortical pyramidal neurons

Authors: *D. GUAN[1], R. C. FOEHRING[2];
[2]Anat. & Neurobio., [1]Univ. Tennessee, Memphis, TN

Abstract: We studied neurons from two BAC transgenic mouse lines that uniquely express EGFP in different subpopulations of layer 5 pyramidal neurons (*etv1* and *glt*) with distinct firing patterns. Our previous study revealed major differences in spike frequency adaptation and firing gain between *etv1* and *glt* cells that were primarily due to differential expression of Ca^{2+}-dependent K^+ conductances (sK and sAHP channels). Here we tested for differential expression of voltage-gated K^+ channels in etv1 and glt neurons. We obtained somatic whole cell recordings

from EGFP-positive neurons in acute brain slices from 2-4 week old mice and then withdrew the pipette to obtain macroscopic outside-out patches whose capacitances were 2 - 4 pf (to overcome space-clamp limitations of the highly dendritic pyramidal cells). Membrane patches were held at -70 mV and 33^{o}C. Outward current was elicited by a 500 ms voltage step to +10 mV. Na^{+} and Ca^{2+} currents were blocked by applying 0.5 μM TTX and 0.2 mM $CdCl_2$ to the external solution. We observed outward currents with both transient and persistent components. Transient components were isolated biophysically and showed slower inactivation in glt patches than in etv1 patches. Persistent currents were measured at 200 ms from the onset of the voltage step. We first recorded current in the control external solution, and then switched to external solution with specific Kv channel blockers to test for the expression of specific Kv channels. Kv1 current was defined by sensitivity to 100 nM Dendrotoxin plus 20 nM Margatoxin. Kv1-mediated current was larger in etv1 patches (18.8% of the current, n = 21) than in glt patches (13.3%, n = 8). Kv2 current was defined by 100 nM Guangxitoxin sensitivity and was significantly larger in glt patches (58.8%, n = 9) than in etv1 patches (30.7%, n = 25). Kv7 current was defined by 2 μM XE991 sensitivity and comprised 7.9% of current in etv1 patches (n = 11) vs. 1.8% in glt patches (n = 6). In additional experiments we perfused the internal solution with Kv2.1 or Kv2.2 antibodies to selectively block Kv2.1 or Kv2.2 channels. Internal perfusion with Kv2.1 antibodies reduced a larger portion of the persistent current in glt patches (55.8%, n = 14) than in etv1 patches (35.5%, n = 11). Internal perfusion with Kv2.2 antibodies inhibited 44.7% of the persistent current in etv1 patches (n = 17) but had no effect in glt patches (n = 7). The greater expression of Kv2.1 conductance in glt cells may facilitate their higher gain and firing rate. Kv2.2 expression in etv1 cells may contribute to lower firing rates and gain. Additional experiments elucidated the biophysical properties and biological roles of these Kv channels in both etv1 and glt cells.

Disclosures: **D. Guan:** None. **R.C. Foehring:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.22/B20

Topic: B.04. Ion Channels

Support: TUBITAK Grant SBAG-110S397

Title: Expression and function of ATP-sensitive potassium channels in the stellate neurons of the mouse ventral cochlear nucleus

Authors: *A. HIM[1], R. BAL[2], G. OZTURK[3], E. ONALAN ETEM[4], N. CENGIZ[5], T. KULOGLU[4], M. TUZCU[4];
[1]Ondokuz Mayis Univ., Samsun, Turkey; [2]Gaziantep Univ., Gaziantep, Turkey; [3]Istanbul Medipol Univ., Istanbul, Turkey; [4]Firat Univ., Elazig, Turkey; [5]Sakarya Univ., Sakarya, Turkey

Abstract: ATP-sensitive potassium (KATP) channels play important roles in many cellular functions by coupling metabolic and electrical activity of the cells and expressed in many types of cells including neurons. Although major voltage-activated ionic channels of stellate cells in the ventral cochlear nucleus are largely characterized previously, the presence of KATP channels in these cells is not known. We aimed to investigate presence, subunit composition and functional role of KATP channels in stellate cells using whole-cell patch clamp and immunohistochemical techniques. Immunohistochemical analyses showed that stellate cell somata were strongly labelled with antibodies against SUR1 and Kir6.2 subunits and moderately labelled with SUR2 antibodies, whereas labelling signals for Kir6.1 subunit were very week. Kir6.2 subunit was strongly co-localized with SUR1 and SUR2 subunits. Whole-cell patch clamp recordings from stellate cells in ventral cochlear nucleus slices revealed that KATP agonists including cromacalim, diazoxide, 3-amino-1,2,4-triazole, NNC 55-0414, NNC 55-0118 and 5-nitro pyridine, and hydrogen peroxide induced membrane hyperpolarization which was accompanied by a decrease in spontaneous firing rate. The hyperpolarizing effects of KATP agonists and hydrogen peroxide were blocked by KATP channel antagonists. Extracellular application of catalase and KATP channel blockers glybenclamid, tolbutamide and 5-hydroxydeconoic acid depolarized stellate cells and increased spontaneous action potential firing rate, suggesting that the channels actively contribute to determine the level of resting membrane potential. We showed that stellate cells of the ventral cochlear nucleus express KATP channels, which are dominantly composed of Kir6.2, SUR1 and SUR2 subunits. Since KATP channels are active at rest and respond oxidative stress they are involved in regulation of the excitability and firing properties of stellate cells, which may be important in processing voice signals in the cochlear nucleus.

Disclosures: A. Him: None. **R. Bal:** None. **G. Ozturk:** None. **E. Onalan Etem:** None. **N. Cengiz:** None. **T. Kuloglu:** None. **M. Tuzcu:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.23/B21

Topic: B.04. Ion Channels

Support: NIH Grant NS-044163

Title: Roles of Kv potassium channels in action potential repolarization in neocortical pyramidal neurons from mouse somatosensory cortex

Authors: D. PATHAK, *R. C. FOEHRING;
Anat. & Neurobio., Univ. Tennessee Hlth. Sci., Memphis, TN

Abstract: In neocortical pyramidal cells (PCs), synaptic inputs are transduced into trains of action potentials (APs). Our understanding of this basic integrative property of PCs is incomplete. Further, the electrical properties of PCs vary between cortical layers and between subtypes of PCs in layer 5. To test for roles of specific Kv channels in AP repolarization, we performed whole cell current-clamp recordings from PCs in layer 2/3 as well as two genetically defined subtypes of layer 5 PCs (etv1 and glt). Previously, we showed that glt cells have narrower APs than either etv1 or layer 2/3 cells and that Ca-dependent K^+ channels did not contribute to AP repolarization in neocortical PCs (Guan et al. 2015. *J Neurophysiol* 113: 2014). APs were elicited with a single 5 ms suprathreshold current injection. We used pharmacological blockers of Kv1 (100 nM α-dendrotoxin: DTX), Kv2 (100 nM guangxitoxin: GTX) or Kv4 (4 mM 4-AP, 150 μM $BaCl_2$, or 200 nM AmmTX3) channels to determine the roles of these voltage gated K^+- channel subtypes in the repolarization phase of the AP. All recordings were obtained in the presence of the synaptic blockers APV (50 μM), DNQX (20 μM) and picrotoxin (100 μM). We concentrate here on AP width (at half amplitude) and rate of repolarization (dV/dt for AP downstroke). Our main findings were (1) DTX significantly broadened the AP and slowed the rate of AP repolarization in layer 2/3 and etv1 PCs but not glt cells, suggesting that Kv1 channels contribute to AP repolarization in layer 2/3 and etv1 cells but not glt cells. (2) GTX had no effects on AP width or repolarization in PCs from layer 2/3, etv1 or glt, indicating no contribution of Kv2 channels to AP repolarization. (3) 150 μM $BaCl_2$ has been reported to block Kv4 channels in dendrites. We found that $BaCl_2$ significantly broadened the AP and slowed repolarization in layer 2/3, etv1 and glt cells. The magnitude of the effects was largest in glt cells. (4) 4 mM 4-AP is frequently used to block Kv4 (A-type) channels. In the presence of 4-AP, APs were broader and repolarization slower in layer 2/3, etv1 and glt cells. Results (3) and (4) are consistent with a major role for Kv4 channels in AP repolarization but both of these agents are relatively unselective. (5) We also tested the peptide blocker AmmTx3, which selectively blocks Kv4 channels associated with DPP10 or DPP6 (Maffie et al. (2013. *J Physiol* 591: 2419). AmmTx3 broadened APs and slowed repolarization in PCs from layer 2/3 as well as etv1 and glt cells. We conclude that AP repolarization mechanisms vary between PC subtypes: Kv4 channels regulate repolarization in glt cells, while the combination of Kv1 channels and Kv4 channels underlie AP repolarization in etv1 and layer 2/3 PCs.

Disclosures: D. Pathak: None. **R.C. Foehring:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.24/B22

Topic: B.04. Ion Channels

Title: Assembly and regulation of functional domains in myelinated axons

Authors: *C. GU;
Biol. Chem. and Pharmacol., Ohio State Univ., Columbus, OH

Abstract: Action potentials propagating along axons play a central role in cell-to-cell communication in the nervous system. Proper action potential firing relies on sequential activation and precise targeting of voltage-gated Na+ (Nav) and K+ (Kv) channels. Nav channels are clustered at the axon initial segment and nodes of Ranvier via ankyrin-G in myelinated axons to support action potential initiation and saltatory conduction, whereas Kv1 channels are clustered in juxtaparanodal domains to prevent repetitive firing. Together with other membrane proteins clustered in nodal or juxtaparanodal domains, they define the function of these key domains in myelinated axons. However, little is known about the mechanisms governing precise delivery of these proteins over long distances and the regulation by myelination and neuronal activity. Here we report that kinesin superfamily 5B (KIF5B) directly binds to ankyrin-G to transport Nav channels into axons, whereas KIF3A transports Kv1.2 channels. Deleting ankyrin-G or interrupting ankyrin-G-KIF5B binding specifically disrupts nodal Nav but not juxtaparanodal Kv1.2 targeting. These results indicate ankyrin-G functions as an adaptor to link Nav channels to KIF5 during axonal transport, before anchoring them to the axon initial segment and nodes of Ranvier. On the other hand, deleting juxtaparanodal proteins only disrupts Kv1 channel targeting, suggesting different assembly mechanisms underlying the axonal domains. Our studies provide new mechanistic insights into the assembly of key functional domains in myelinated axons and elucidate how this process is regulated at the level of axonal transport. These results may in turn contribute to the development of new strategies for treating brain disorders involving disrupted axonal transport and function.

Disclosures: C. Gu: None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.25/B23

Topic: B.04. Ion Channels

Title: Cell depolarization induced by low pHo causes a switch from tonic to burst firing supported by a marked inhibition of BK channels in mouse chromaffin cells

Authors: L. GUARINA[1], D. H. F. VANDAEL[1], *E. CARBONE[2];
[1]Dept. of Drug Sci., [2]Univ. of Turin, Turin, Italy

Abstract: Cell depolarization leads to major changes in spontaneous firing patterns and catecholamine secretion in chromaffin cells. We recently showed that reduced availability of Nav1.3 and Nav1.7 channels induced by steady depolarizations boosts a change from tonic to bursts firing in spontaneously active mouse chromaffin cells (MCCs) (*Vandael et al, J Physiol, 2015*) . As burst firing induces a plateau depolarization that increases ~20-fold the Ca^{2+} flux through Cav1.3 channels, the switch from "tonic" to "bursts" firing appears as a novel command to enhance catecholamine release in a non-neurogenic manner. In the view of identifying the physiological conditions in which chromaffin cell depolarization could induce burst firing, we analyzed the effect of lowering external pH (pHo),which is reported to induce membrane depolarization and catecholamine release by blocking TASK1 channels (*Inoue et al, J Neurochem, 2008*). The mechanism is at the basis of the feedback loop in which adrenaline released from the adrenal medulla triggers the body response to acidosis (*Clausen et al, J Physiol, 1993*). In current-clamp experiments on spontaneously firing MCCs, we first confirmed that lowering pHo from 7.4 to 6.8 the cell depolarized by about 8 mV from their resting potential (V_{rest}). The depolarization was sufficient to cause a net switch from tonic to burst firing of spontaneously active MCCs that was preserved for long periods at a frequency of 1 to 2.5 Hz. Burst firing occurred in most of the cells tested and was characterized by slow plateau depolarizations lasting 100-200 ms on which 3 to 5 action potentials of progressively decreasing amplitude were riding on top. Concerning the firing switch induced by low pHo, we specifically checked whether mild pHo changes from 7.4 to 6.8 besides blocking TASK1 channels affect also Nav, Cav and BK channels that contribute to burst firing generation. We found that Cav channels activation was shifted by about 4 mV toward more positive potentials with little effects on channel conductance, while none of both changes was observed in Nav1.3 and Nav1.7 channels. Regarding the BK channel we found a marked depression of BK currents when activated by either constant or variable pre-conditioning Ca^{2+} loading. The effects were associated to a nearly 60% inhibition of BK channel conductance. In conclusion, the switch from tonic to burst firing in MCCs appears to be induced not only from the expected lowered availability of Nav1.3/Nav1.7 channels that enter a closed-state inactivation during prolonged depolarizations (*Vandael et al, J Physiol, 2015*) but also by a reduced BK channel conductance (*Martinez-Espinosa et al, J Gen Physiol, 2014*).

Disclosures: **L. Guarina:** None. **D.H.F. Vandael:** None. **E. Carbone:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.04. Ion Channels

Support: HSFO 00493

Title: Expression and contributions of the Kir2.1 inward-rectifier K+ channel to proliferation, migration and chemotaxis of microglia in unstimulated and anti-inflammatory states

Authors: ***D. LAM**, L. C. SCHLICHTER;
Genet. and Develop., Krembil Discovery Tower/ Toronto Western Hosp., Toronto, ON, Canada

Abstract: When microglia respond to CNS damage, they can range from pro-inflammatory (classical, M1) to anti-inflammatory, alternative (M2) and acquired deactivation states. It is important to determine how microglial functions are affected by these activation states, and to identify molecules that regulate their behavior. Microglial proliferation and migration are crucial during development and following damage in the adult, and both functions are Ca2+-dependent. In many cell types, the membrane potential and driving force for Ca2+ influx are regulated by inward rectifier K+ channels, including Kir2.1, which is prevalent in microglia. However, it is not known whether Kir2.1 expression and contributions are altered in anti-inflammatory states. We tested the hypothesis that Kir2.1 contributes to Ca2+ entry, proliferation and migration of rat microglia. Kir2.1 (KCNJ2) transcript expression, current amplitude, and proliferation were comparable in unstimulated microglia and following alternative activation (IL-4 stimulated) and acquired deactivation (IL-10 stimulated). To examine functional roles of Kir2.1 in microglia, we first determined that ML133 was more effective than the commonly used blocker, Ba2+; i.e., ML133 was potent (IC50=3.5 μM) and voltage independent. Both blockers slightly increased proliferation in unstimulated or IL-4 (but not IL-10)-stimulated microglia. Stimulation with IL-4 or IL-10 increased migration and ATP-induced chemotaxis, and blocking Kir2.1 greatly reduced both but ML133 was more effective. In all three activation states, blocking Kir2.1 with ML133 dramatically reduced Ca2+ influx through Ca2+-release-activated Ca2+ (CRAC) channels. Thus, Kir2.1 channel activity is necessary for microglial Ca2+ signaling and migration under resting and anti-inflammatory states but the channel weakly inhibits proliferation.

Disclosures: **D. Lam:** None. **L.C. Schlichter:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.27/B25

Topic: B.04. Ion Channels

Support: CIHR

Title: NMDAR-induced plasticity of A-type Kv4 current contributes to long-term potentiation of mossy fiber input in granule cells of posterior cerebellum

Authors: *A. RIZWAN, G. W. ZAMPONI, R. W. TURNER;
Dept. of Neurosci., Univ. of Calgary, Calgary, AB, Canada

Abstract: The mossy fiber (MF) afferents to cerebellum carry information at ultrafast frequencies (~ 1000 Hz) and contact granule cells, a major synaptic relay in cerebellum. In other regions of the brain such high input frequencies can induce long term potentiation (LTP) of synaptic efficacy through a combination of pre- and postsynaptic mechanisms that shape the response to subsequent inputs. We have shown that Kv4 ion channels form a nanodomain complex with Cav3 channels in lobule 9 granule cells, where Cav3 channel-mediated calcium influx selectively promotes a rightward shift in the voltage for inactivation of A-type (IA) current. As a result, the availability of IA is increased in lobule 9 cells, reducing excitability and promoting the tonic rate of firing necessary to respond to vestibular-like sensory stimuli. LTP of synaptic input in hippocampus has been shown to induce a leftward shift in Kv4 voltage for inactivation not unlike that found upon block of the Cav3-Kv4 complex. The current study used *in vitro* slices of rat cerebellum to test the hypothesis that LTP of MF EPSCs modifies the properties of IA in lobule 9 granule cells. We found that LTP of MF input induced by pairing theta burst stimulation with postsynaptic depolarization to -40 mV invoked a select leftward shift of IA voltage for inactivation. Moreover, a late long-lasting slow EPSC (sEPSC) of up to ~ 1 sec was unmasked following LTP of MF input. The LTP-induced shift in IA voltage for inactivation was dependent on calcium influx through NMDA receptors, providing evidence for a novel interaction between NMDAR-mediated calcium influx and Kv4 channels. In contrast, the sEPSC was NMDAR-independent and instead mediated by mGLUR1,5 receptors. Infusion of an antibody against the calcium sensor of the Cav3-Kv4 complex was also sufficient to unmask the sEPSC, indicating a Kv4-mediated baseline suppression of the sEPSC in lobule 9 granule cells. Moreover, the effects of LTP on IA and the sEPSC were observed with or without block of GABAergic inhibition, highlighting the physiological relevance of the results. These data

indicate that there is a novel NMDAR-Kv4 interplay in cerebellar granule cells that functions to regulate postsynaptic excitability and synaptic responses to MF input.

Disclosures: **A. Rizwan:** None. **G.W. Zamponi:** None. **R.W. Turner:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 121.28/B26

Topic: B.04. Ion Channels

Support: the National Program of Basic Research of China (2013CB835100)

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Title: Modulation of Kir4.1 protein and mRNA by activation of mGluR I in rat retinal Müller cells

Authors: ***F. GAO**[1,2], F. LI[2], Y. MIAO[2], L.-D. DONG[2], S.-H. ZHANG[1,2], J. WU[1,2], X.-H. SUN[1,2], Z. WANG[1,2];
[1]Dept. of Ophthalmology at Eye &ENT Hosp., Shanghai, China; [2]Inst. of Brain Sci., Shanghai, China

Abstract: K^+ channels, especially Kir channels, are essential for retinal Müller cells to maintain homeostasis of $[K^+]_o$. Downregulation of Kir currents, which disturbs the characteristic hyperpolarized resting potential of the cells, has been found to contribute to Müller cell gliosis. In the present work, whether and how activation of group I metabotropic glutamate receptor (mGluR I) modulate membrane Kir4.1 protein internalization and Kir4.1 mRNA expression were investigated in purified cultured rat retinal Müller cells by using immunocytochemistry, Western blot and real-time PCR techniques. Our results showed that more than 95% of the cells were Müller cells under our cultured condition. DHPG (10 μM, a selective mGluR I agonist) treatment for different times (0.5 h to 24 h) induced Müller cell gliosis, as evidenced by progressively enhanced glial fibrillary acidic protein (GFAP) expression with a peak at 9 h. Although total Kir4.1 proteins extracted from the DHPG-treated cells kept unchanged, Kir4.1 proteins in the cell membrane compartment were significantly decreased from 0.5 h after DHPG treatment, with a peak at 3 h, which was prior to the change of GFAP in time course. In addition, DHPG (10 and 100 μM) treatment induced a transient decrease in Kir4.1 mRNA expression in the cells. These results suggest that activation of mGluR I may decrease the number of functional Kir4.1

channels in cultured rat retinal Müller cells through modulating Kir4.1 protein and mRNA, thus contributing to Müller cell gliosis.

Disclosures: **F. Gao:** None. **F. Li:** None. **Y. Miao:** None. **L. Dong:** None. **S. Zhang:** None. **J. Wu:** None. **X. Sun:** None. **Z. Wang:** None.

Poster

121. Potassium Channels I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.04. Ion Channels

Support: American Heart Association

National Ataxia Foundation

Chapman SCA Grant

Title: Structural basis for the positive modulation of SK2 channel by Riluzole

Authors: T. N. ALEXANDER, S. A. ALI, S. C. CHIANG, *M. ZHANG;
Dept. of Biomed. and Pharmaceut. Sci., Chapman Univ. Sch. of Pharm., Irvine, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating movement disorder resulting from neurodegeneration of motor neurons in the motor cortex and the brainstem/spinal cord. Evidence indicates that the excitability of ALS patient motor neurons is abnormally elevated. Positive modulation of potassium ion channels has been suggested as a potential therapeutic strategy to reduce the toxic hyperexcitability of ALS motor neurons. Small conductance Ca2+-activated potassium (SK) channels play important roles in regulation of membrane excitability in motor neurons. Riluzole, which positively modulates SK channels, is the only FDA approved drug for ALS, although its therapeutic effects are only modest. One reason that limits the therapeutic effects of riluzole might be its low potency on SK channels. Here we elucidate the binding pocket of riluzole in SK2 channels with crystallography. Site directed mutations in the binding pocket effectively change the potency of riluzole to potentiate SK2 channel. These data suggest that the binding pocket identified by crystallography is indeed the functional binding pocket through which riluzole exerts the positive modulation of channel activity. These studies lay the foundation for structure based drug discovery research with the goal of developing more effective therapeutics for ALS treatment.

Disclosures: **T.N. Alexander:** None. **S.A. Ali:** None. **S.C. Chiang:** None. **M. Zhang:** None.

Poster

122. Monoamine Transporter

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Support: NIH Grant DA035559

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NIH Grant MH064913

Title: Conserved role of the atypical MAP kinase SWIP-13/ERK8 in the regulation of presynaptic dopamine transporters

Authors: *D. BERMINGHAM, J. A. HARDAWAY, S. L. SNIDER, S. M. WHITAKER, R. D. BLAKELY;
Vanderbilt Univ., Nashville, TN

Abstract: The neurotransmitter dopamine (DA) is used across phylogeny, including in the soil-dwelling nematode, *C. elegans*, to modulate a wide range of complex behaviors. The capacity for DA signaling is tightly regulated by the presynaptic DA transporter (DAT/DAT-1), which limits DA availability in space and time. In nematodes, loss of *dat-1* results in a paralysis phenotype that is observed when worms are placed in water, a behavior we have termed Swimming-Induced Paralysis, or Swip. Swip can be reversed by genetic ablation of the TH ortholog *cat-2*, as well as the D2-like receptor *dop-3*, demonstrating a dependence on DA signaling. We identified the mutation vt32 in a forward genetic screen of worms that exhibit DA-dependent Swip, and mapped the molecular lesion to an uncharacterized gene C05D10.2, henceforth called *swip-13*. The *swip-13* gene encodes an ERK-family MAP kinase, orthologous to the mammalian atypical MAP kinase ERK7/8. Functional, GFP-tagged SWIP-13 protein localizes to DA terminals, consistent with a presynaptic contribution to DA signaling. Consistent with these studies, pharmacological and genetic studies of *swip-13* mutants demonstrate the DA-dependence of Swip in these animals and that loss of *swip-13* expression in DA neurons studies accounts for Swip behavior. Using a fluorescence recovery after photobleaching (FRAP) approach to monitor DA neuron synaptic vesicle fusion rates, we found that *swip-13* animals display normal basal

vesicle fusion. However, loss of *swip-13* results in reduced sensitivity to the DAT-1-dependent neurotoxin 6-OHDA, suggesting that a loss of DAT-1 activity may drive Swip. In support of this hypothesis, Swip experiments with *swip-13* and *dat-1* double mutants indicate a lack of additivity, consistent with contributions to swimming behavior in the same pathway. Together, these results are consistent with *swip-13* as a positive regulator of *dat-1*. Excitingly, studies with human ERK8 and human DAT in the human neuroblastoma cell line SH-SY5Y demonstrates a positive functional interaction between these two proteins, with overexpression of ERK8 increasing the uptake capacity of co-transfected DAT. This increase in DA uptake is paralleled by an increase in total and surface DAT protein levels, effects that are lost when DAT is co-transfected with a "kinase-dead" ERK8 mutation. Ongoing studies seek to elucidate the mechanism by which SWIP-13/ERK8 regulates nematode and mammalian DAT expression and function with an eye to whether kinase function can be manipulated for potential therapeutic benefit in disorders linked to perturbed DA signaling.

Disclosures: D. Bermingham: None. **J.A. Hardaway:** None. **S.L. Snider:** None. **S.M. Whitaker:** None. **R.D. Blakely:** None.

Poster

122. Monoamine Transporter

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.05. Transporters

Support: R01DA026947-A1

R01NS071122-A1

S10OD020026

Title: The sigma-1 receptor modulates methamphetamine regulation of dopamine transporter activity

Authors: *D. O. SAMBO[1], M. LIN[2], H. KHOSHBOUEI[2];
[1]Dept. of Neurosci., [2]Univ. of Florida, Gainesville, FL

Abstract: The dopamine transporter (DAT) is a presynaptic membrane protein implicated in multiple physiological and pathological conditions, including movement, reward, neurodegeneration, and drug addiction. DAT functions to translocate dopamine across the plasma membrane and to modulate the excitability of dopaminergic neurons, making it a crucial

regulator of dopamine homeostasis. DAT is also the primary target for methamphetamine (METH), a highly addictive psychostimulant. What is not often recognized, however, is that METH is both a substrate for DAT as well as a ligand for the sigma-1 receptor (σ1R), an intracellular chaperone protein. We have previously shown that the σ1R is a novel protein partner with DAT at the plasma membrane and that both METH and the selective σ1R agonist PRE-084 treatment increases the association of σ1R with DAT. Importantly, we found that σ1R overexpression or σ1R agonist treatment prevented METH-induced, DAT-mediated increases in firing activity in dopaminergic neurons and σ1R agonist also decreased METH-mediated DA efflux, without affecting dopamine uptake or DAT trafficking. Expanding upon these preliminary findings, our current work investigates the potential intracellular mechanisms of σ1R modulation of METH-mediated DAT activity, with a focus on the role of σ1R on METH-mediated, DAT-dependent changes in intracellular calcium homeostasis as well as METH-mediated changes in membrane microdomain distribution of DAT. These findings are important in determining the molecular mechanisms of σ1R modulation of METH-mediated responses and will further examine the potential therapeutic role of σ1R for the treatment of METH addiction.

Disclosures: D.O. Sambo: None. **M. Lin:** None. **H. Khoshbouei:** None.

Poster

122. Monoamine Transporter

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.05. Transporters

Support: R01 DA019676

Title: Impact of S2 disruption on dopamine transporter function

Authors: *J. ZHEN, M. E. REITH;
Dept Psychiatry, New York Univ. Lagone Med. Ctr., New York, NY

Abstract: The structures of the leucine transporter (LeuT) and *drosophila* dopamine transporter (dDAT) show a secondary binding site (designated S_2) for drugs and substrate in the extracellular vestibule towards the membrane exterior in relation to the primary substrate recognition site (S_1). It still is a matter of debate whether the S_2 site plays a role in substrate translocation in human DAT (hDAT) as proposed for LeuT, in which binding of a second substrate molecule to S_2 triggers the release of substrate bound to S_1. The present experiments are aimed at disrupting S_2 by (i) mutating Asp476 to Ala (with D476 in S_2 interacting with DA in the modeling studies of

the Weinstein group and in our own studies), and (ii) mutating Ile159 to Ala (with I159 in S_2 interacting with DA in same models and corresponding to I111 in LeuT mutation of which is known to disrupt S2). D476A hDAT expressed in LLC-pK1 cells displayed a 90% decrease in uptake of 10 nM [^{3}H]DA compared with wild type (WT): the uptake K_m was increased 4-fold, whereas the V_{max} was reduced by an order of magnitude. With the study of binding of [^{3}H]CFT- the cocaine analog- to intact cells, compared with WT, no change was found in the K_d of [^{3}H]CFT and 2-fold reduction was found in the maximal binding sites in D476A. Accordingly, surface expression of D476A DAT, as measured by biotinylation and western blot, was approximately half of that of WT. In turn, D476A hDAT turnover rate was significantly reduced by 7-fold than WT. In LLC-PK_1 I159A-hDAT, [^{3}H]DA uptake was not detectable. Experiments are in progress to enhance expression of I159A hDAT in order to better measure its properties. The results taken together indicate that disruption of S_2 has a more severe impact on transport function of DAT than on cocaine analog binding. These data suggested that S_2 plays a crucial role in DA transport, perhaps by an allosteric interaction between S_2 and S_1. In consonance, the affinity of DA (likely for S_1) as measured by [^{3}H]CFT binding to S_2-disrupted D476A was reduced compared to WT with S_2 intact.

Disclosures: J. Zhen: None. **M.E. Reith:** None.

Poster

122. Monoamine Transporter

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Topic: B.05. Transporters

Support: NIH Grant R01DA02121

Title: Vesicular monoamine transporter-2: Interaction with parkin and mechanism of degradation

Authors: *E. K. STACHOWSKI, T. B. BAUST, G. E. TORRES;
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Abstract: The vesicular monoamine transporter-2 (VMAT2) is essential for monoaminergic homeostasis, as it packages monoamines into synaptic vesicles, readying neurotransmitters for synaptic release. VMAT2 also has a neuroprotective role, given that free monoamines in the cytosol have the potential to auto-oxidize and form dangerous free radicals that can ultimately contribute to oxidative stress and neuronal vulnerability. Despite the importance of VMAT2,

little is known about mechanisms involving VMAT2 degradation. The balance of protein synthesis and degradation is vital in maintaining normal cellular function and deregulation of degradation systems can be detrimental to neurons, as evidenced in disorders such as Parkinson's disease. There are two primary organelles responsible for degradation in mammalian cells: the lysosome and the 26S proteasome. Although synaptic vesicle proteins are thought to be degraded by the lysosome via the endo-lysosomal pathway, little direct evidence exists for this. Furthermore, it remains unclear if these proteins can be differentially regulated or degraded at the vesicle. Utilizing an *in vitro* cellular model we demonstrate here that VMAT2 is degraded primarily through the UPS (ubiquitin-proteasome system), and not by the lysosome. Our data suggests UPS-related degradation of mature VMAT2 is a process independent of endoplasmic-reticulum-associated degradation (ERAD). Specificity for the UPS is primarily determined by E3 ligases, enzymes that tag a specific protein with ubiquitin, destined for the proteasome. We provide evidence that an E3 ligase, parkin, physically and functionally interacts with VMAT2. Since parkin mutations have been associated with autosomal recessive juvenile-onset Parkinsonism, our results suggest that VMAT2 deregulation may be implicated in the pathology of Parkinson's disease.

Disclosures: E.K. Stachowski: None. **T.B. Baust:** None. **G.E. Torres:** None.

Poster

122. Monoamine Transporter

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.05. Transporters

Support: NIH Grant DA027845

NIDA–IRP

Title: Locating the binding site of the benztropine photoaffinity ligand,[125I]GA II 34, on the dopamine transporter

Authors: *M. J. TOMLINSON[1], D. KROUT[1], A.-B. PRAMOD[1], J. R. LEVER[2,3], A. H. NEWMAN[4], L. K. HENRY[1], R. A. VAUGHAN[1];
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Abstract: The dopamine transporter (DAT) is an integral membrane protein responsible for reuptake of dopamine from the synapse. Psychostimulant drugs target the DAT and block reuptake of dopamine thereby affecting dopamine homeostasis. Benztropine, a dopamine reuptake inhibitor, contains a tropane pharmacophore homologous to cocaine, yet has distinct behavioral effects, which has been hypothesized to result from a different mode of interaction with the DAT. Incorporating a photoreactive arylazido group into DAT inhibitors has allowed for covalent attachment of these photoaffinity ligands to the transporter and analysis of inhibitor binding. Our lab has extensively studied the cocaine photoaffinity analogs [^{125}I]MFZ 2-24 and [^{125}I]RTI 82 to characterize their interactions with the DAT. Determining the adduction sites of both [^{125}I]MFZ 2-24 and [^{125}I]RTI 82 and computational ligand docking models identified cocaine coordinates with specific amino acid residues of the DAT. To characterize the binding of benztropine with DAT we labelled the transporter with the irreversible benztropine photoaffinity analog, [^{125}I]*N*-[*n*-butyl-4-(4’’’-azido-3’’’-iodophenyl)]-4’,4’’-difluoro-3α-(diphenylmethoxy)tropane ([^{125}I]GA II 34) and performed trypsin and cyanogen bromide-based peptide mapping. The results narrowed adduction of [^{125}I]GA II 34 to the amino acids Asp-79 and/or Leu-80 in the unwound region of TM1, placing the ligand in close proximity to residues essential for dopamine binding. Computational ligand docking studies, supported by peptide mapping, position [^{125}I]GA II 34 in the high affinity substrate binding site(S1) in a pose similar to MFZ 2-24. To verify this conclusion, the substituted-cysteine accessibility method will be used to determine residues protected by GA II 34 from methanethiosulfonate reagent attack. If the distinct behavioral effects of cocaine and benztropine are due to differential transporter binding properties, our results indicate that these differences are likely to be subtle.

Disclosures: M.J. Tomlinson: None. **D. Krout:** None. **A. Pramod:** None. **J.R. Lever:** None. **A.H. Newman:** None. **L.K. Henry:** None. **R.A. Vaughan:** None.

Poster

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Title: Palmitoylation of the Dopamine Transporter on Cysteine 6

Authors: *D. J. STANISLOWSKI, R. VAUGHAN, J. FOSTER;
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Abstract: The dopamine transporter (DAT) controls synaptic dopamine (DA) levels by reuptake after presynaptic neuronal vesicular release. Several neurological disorders including Parkinson disease, addiction, and ADHD are associated with abnormal DA homeostasis, and DAT dysregulation is hypothesized to contribute to these disorders. DAT function is regulated through posttranslational modifications including S-palmitoylation, the reversible, catalytic addition of palmitate to cysteine via a thioester linkage. S-palmitoylation of DAT opposes PKC-mediated down-regulation and increases DA transport Vmax without altering DAT surface expression. In previous studies we mutated the intracellular Cys residues to alanine, and determined that Cys580 was a major but not sole site of [3H]palmitic acid metabolic labeling. To identify the other site we used the more sensitive acyl-biotinyl exchange (ABE) method to analyze these mutants. The results confirmed the modification of Cys580, but did not detect reduced palmitoylation for other mutated sites (Cys6, Cys135, Cys342, Cys522), suggesting the presence of compensatory modification in response to mutagenesis. We then mutated all but one of the intracellular cysteines to alanine, leaving a single site available for palmitoylation. ABE analysis of these mutants showed significantly elevated palmitoylation of DATs containing Cys6 or Cys580 relative to those containing only Cys135, Cys341, or Cys522, suggesting that Cys6 is the second palmitoylation site. To determine if endogenous palmitoylation is occurring on Cys6, we performed peptide mapping of rat striatal DAT using endoproteinase Asp-N digestion and ABE analysis of N-terminal fragments. Asp-N cleavage of DAT occurs at Asp174, producing a 19 kDa peptide fragment containing Cys6 and Cys135 that can be detected by immunoblotting. ABE analysis of this fragment demonstrated the presence of palmitoylation, indicating that one or both of these residues is palmitoylated. In conjunction with the mutagenesis findings these results strongly support the palmitoylation of DAT at Cys6, suggesting the potential for tethering of the N-terminus to the plasma membrane. The N-terminus of DAT mediates many regulatory functions and is the site for ubiquitylation and phosphorylation. Palmitoylation of Cys6 thus has major implications for transporter structural and functional properties.

Disclosures: **D.J. Stanislowski:** None. **R. Vaughan:** None. **J. Foster:** None.

Poster

122. Monoamine Transporter

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Topic: B.05. Transporters

Support: Intramural Research Program NIMH

Title: Regulation of the Dopamine Transporter by heterotrimeric G-proteins depends on N-terminal phosphorylation

Authors: J. A. BORIS[1], *J. GARCIA-OLIVARES[2], S. G. AMARA[1];
[1]Lab. of Cell. and Mol. Neurobio., Natl. Inst. of Mental Health/NIH, Bethesda, MD; [2]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Dopaminergic neurotransmission has an important role in many complex psychiatric conditions including depression, attention-deficit hyperactivity disorder and drug addiction. Many current therapeutic approaches used in the treatment of these conditions target the dopamine transporter (DAT). A member of the SLC6A family, the DAT clears extracellular dopamine through a sodium-coupled transport mechanism. DAT function is regulated by intracellular mechanisms such as phosphorylation, ubiquitination, and a variety of interactions with other proteins. We recently reported a novel mechanism of regulation of the DAT by heterotrimeric G-proteins. We found that Gβγ subunits bind directly to the C-terminus of the DAT and upon G-protein activation, the release of Gβγ results in a decrease in dopamine (DA) accumulation. In recent studies, we found that the decrease in DA accumulation does not involve a change in uptake, but is instead the result of an increase in DA efflux. Efflux is a complex mechanism that depends on calcium, sodium, and membrane potential and has been linked to the actions of DA-releasing properties of amphetamine. Efflux also involves phosphorylation of residues at the N-terminus, as well as interactions of the C-terminus with Serine/Threonine kinases such as protein kinase C and calmodulin kinase II. To explore whether the DA efflux promoted by the activation of Gβγ subunits also requires phosphorylation of the N-terminus, we used two DAT mutants, hDAT-S/A and hDAT-S/D. These mutants have five N-terminal serines (S2, S4, S7, S12, S13) substituted with alanine (S/A) to eliminate putative phosphorylation sites, or aspartate (S/D), where the negatively charged side chains simulate phosphorylation. We also used pharmacological tools to inhibit kinases and phosphatases in order to explore how the general phosphorylation state of DAT contributes to the regulation of DAT by Gβγ subunits. Our data suggest that the effect on efflux mediated by Gβγ activation depends on the presence of N-terminal serine residues implying that N-terminal phosphorylation is required. Moreover, changes in the global phosphorylation state also modify DAT function. Phosphatase inhibition decreases cytosolic DA, whereas inhibition of Serine/Threonine kinases increases cytosolic DA. Interestingly, the enhanced efflux induced by Gβγ activation is not affected by these manipulations. These results provide a basis for further studies to establish whether substitutions at the N-terminal phosphorylation sites modify the binding of Gβγ to the C-terminus or whether phosphorylation of these residues facilitates a shift in transporter conformation towards an efflux mode

Disclosures: **J.A. Boris:** None. **J. Garcia-Olivares:** None. **S.G. Amara:** None.

Poster

122. Monoamine Transporter

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Title: The molecular mechanism of action of mephedrone

Authors: F. P. MAYER[1], J. S. PARTILLA[2], L. WIMMER[3], A. SEDDIK[4], N. BURCHARDT[1], D. SCHMID[1], S. BULLING[1], M. H. BAUMANN[2], G. F. ECKER[4], M. D. MIHOVILOVIC[3], *H. H. SITTE[1];
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Abstract: 4-methyl-N-metylchathinone (mephedrone, MEPH) is a popular psychostimulant and one of the major representatives of former legal highs, commonly referred to as “bath salt” or “plant food”. MEPH impinges on monoaminergic signaling in the brain in an amphetamine-like fashion and most closely resembles 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”). Thus, MEPH acts at the high-affinity transporters for dopamine (DAT), serotonin (SERT) and norepinephrine (NET) and induces efflux via their cognate transporters. However, the exact interaction of MEPH with monoamine transporters still remains enigmatic and the discrepancy between the long duration of the MEPH induced high and its plasma-half life is unresolved. We sought to investigate the molecular mechanism of action of MEPH and to test its metabolites for psychoactive properties. We investigated the impact of MEPH and its metabolites on DAT, NET and SERT in radiotracer flux experiments using heterologous expression systems as well as rat brain synaptosomes. In addition, we examined the interaction of MEPH with monoamine transporters by use of Foerster resonance energy transfer (FRET) microscopy and electrophysiology. Moreover, we characterized each substance in an *in vivo* approach for their stimulant profiles and generated in silico models of the fit in the binding pockets of DAT, NET and SERT. MEPH was found to increase the proportion of transporters in the inward-facing

conformation and to elicit a depolarizing current. The MEPH-derived metabolites were found to competitively inhibit uptake of radiolabeled substrates via DAT, NET and SERT. Efflux-studies revealed that the examined MEPH metabolites are capable of inducing efflux of preloaded substrates via DAT, NET and SERT. Consistent with *in vitro* findings, the metabolites mimicked the actions of the parent compound *in vivo*. The fact that MEPH is subject to a fast metabolic turnover, yet induces a long lasting high appeared to be inconsistent. Our data unravel the mode of action of MEPH on a molecular level and indicate that the presence of MEPH per se is not the only parameter determining the stimulating effect of this drug.

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Poster

122. Monoamine Transporter

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Topic: B.05. Transporters

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Title: Interplay between the serotonin transporter (SERT) and $5HT_{1A}$ receptors to modulate quantal catecholamine release events in adrenal chromaffin cells

Authors: *R. L. BRINDLEY[1], M. B. BAUER[1], R. D. BLAKELY[2], K. P. M. CURRIE[3]; [1]Dept. of Anesthesiol., [2]Departments of Pharmacol. and Psychiatry, Silvio O. Conte Ctr. for Neurosci. Res., [3]Departments of Anesthesiol. and Pharmacol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: The serotonin transporter (SERT) mediates the reuptake of 5HT at CNS synapses and is a clinically relevant target for some anti-depressants, including the selective serotonin reuptake inhibitors (SSRIs). SERT is also prominently expressed in adrenal chromaffin cells, the neuroendocrine arm of the sympathetic nervous system. In response to stressors, chromaffin cells release catecholamines and neuropeptides that in turn regulate the cardiovascular, endocrine, immune, and nervous systems. However, the effects of 5HT / SERT on chromaffin cell function remain unclear. We show that intracellular 5HT content is >1000 fold lower than the

catecholamines (epinephrine and norepinephrine). One role of SERT is to mediate uptake of this 5HT into chromaffin cells as demonstrated by the ~80% reduction of 5HT in adrenal glands isolated from SERT knockout mice compared to wild-type littermates. The catecholamine content of the same glands was unaltered. To investigate regulation of stimulus-secretion coupling by 5HT / SERT, we used carbon fiber amperometry. Secretion was evoked in chromaffin cells isolated from wild-type or SERT knockout mice by bath perfusion with 30mM KCl for 60s. In control conditions (no extracellular 5HT) there was a significant reduction (~ 35%) in the overall amount of secretion in SERT knockout cells compared to wild-type cells. The number of amperometric spikes (vesicular release events) was not different, but the duration and the charge (i.e. the amount of oxidizable transmitter released) of individual spikes were significantly reduced in SERT knockouts. In SERT knockout cells, acute application of extracellular 5HT (1μM) had an additional effect, significantly inhibiting the number of amperometric spikes. This was prevented by pretreating cells with pertussis toxin (300ng/ml). In wild-type cells, the $5HT_{1A}$ receptor agonist (R)-(+)-8-OH-DPAT (300nM) mimicked the inhibition of number of spikes but 5HT did not unless SERT activity was blocked by acute application of escitalopram (1μM). Under these conditions, 5HT reduced the number of amperometric spikes and this was abolished by the $5HT_{1A}$ receptor antagonist WAY100635 (25nM). Using patch-clamp electrophysiology and fluorescent Ca^{2+} imaging experiments we found no effect of 5HT or escitalopram on baseline intracellular [Ca^{2+}], or Ca^{2+} entry through voltage-gated Ca^{2+} channels. Overall our data identify a complex interaction between SERT and $5HT_{1A}$ receptors to control stimulus-secretion coupling in chromaffin cells. Ongoing work will dissect the underlying mechanisms, and the contribution of adrenal gland 5HT/ SERT to the (patho)physiology of stress and stress-related disorders.

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Poster

122. Monoamine Transporter

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Topic: B.05. Transporters

Title: Interaction of neurokinin signaling and catecholamine transport in amphetamine behavior

Authors: *P. MANNANGATTI, S. RAMAMOORTHY, M. S. BOWERS, L. D. JAYANTHI; Pharmacol. and Toxicology, Virginia Commonwealth Univ., Richmond, VA

Abstract: The psychostimulants cocaine and amphetamine (AMPH) target all three monoamine transporters with AMPH having nearly equal affinity for the catecholamine transporters, the norepinephrine and dopamine transporters (NET & DAT). It is known that both AMPH and activation of the neurokinin-1 receptor (NK1R) can downregulate NET as well as DAT. Interestingly, Substance P, the endogenous NK1R agonist is released in the rat ventral striatum following AMPH, and while Substance P is known to enhance acute stimulatory effects of AMPH, NK1R antagonists decrease psychostimulant locomotor activation. Our previous work demonstrated that Substance P and AMPH mediated NET downregulation is dependent on the transporter T258/S259 trafficking motif (PMC2939970; PMID: 16740633). In addition, NET regulation by NK1R is facilitated by protein-protein interactions that occur in membrane rafts (PMC3789959). These observations indicate that NK1R can modulate the impact of AMPH on catecholamine transporter function. Interestingly, studies examining the behavior of monoamine transporter knockout mice in response to psychostimulants indicate that each amine transporter plays distinct roles in mediating the reinforcing effects of psychostimulants. Here, we extend our studies to in-vivo manipulation of catecholamine transport regulation to test the hypothesis that NK1R-mediated T258/S259-specific downregulation of NET and/or DAT independently or coordinately contributes to AMPH-evoked behaviors. We demonstrate that AMPH administration down-regulated NET in the rat ventral striatum and that the NK1R antagonist aprepitant (emend) prevented these AMPH-mediated effects. Importantly, ventral striatal microinjections of a TAT-peptide targting the T258/S259 motif attenuated AMPH-induced locomotor activation. Our data suggest that catecholamine transport is coupled to NK1R signaling mechanisms and interrupting T258/S259-specific NK1R-mediated NE transport regulation affects AMPH-elicited behavior. The NK1R-mediated T258/S259-dependent catecholamine transport regulation represents a novel molecular mechanism not previously recognized as a potential target of AMPH, and our ongoing studies seek to explore the significance of this molecular mechanism in the rewarding effects of AMPH.

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Poster

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Topic: B.05. Transporters

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Title: Tyrosine 470 and 88 of human dopamine transporter are critical for Tat-mediated allosteric modulation on human dopamine transporter

Authors: *W.-L. SUN[1], A. SUBRAMANIAM[2], P. M. QUIZON[1], C.-G. ZHAN[3], J. ZHU[1]; [1]Dept. of Drug Discovery and Biomed. Sci., Univ. of South Carolina, Columbia, SC; [2]Dept. of Organic Chem., Southern Res. Inst., Birmingham, AL; [3]Mol. Modeling and Biopharmaceutical Ctr. and Dept. of Pharmaceut. Sci., Univ. of Kentucky, Lexington, KY

Abstract: HIV-1 Tat protein and cocaine synergistically disrupt dopamine (DA) transmission by inhibiting human dopamine transporter (hDAT), which exacerbates the progression of HIV-1-associated neurocognitive impairment. Our previous study demonstrated that compared to cocaine alone, Tat slowed cocaine-induced dissociation rate of [^{3}H]WIN35,428 binding and increased IC_{50} value of cocaine inhibiting [^{3}H]DA uptake in wild type (WT) hDAT, indicating that Tat allosterically modulates hDAT. Through the integrated computational modeling and experimental studies, we have identified that mutations of tyrosine470 (Y470H) and tyrosine88 (Y88F) of hDAT, via changing transporter conformational transitions, attenuate Tat-induced inhibition of DAT. Therefore, the present study evaluated the role of Y470H and Y88F in Tat-mediated allosteric modulation of hDAT. SoRI-20041, a novel allosteric modulator of the DAT, significantly increased IC_{50} value for cocaine inhibiting DA uptake by 34% (IC_{50} in nM: cocaine, 323 ± 19; cocaine + SoRI-20041, 443 ± 31) in WT hDAT but did not alter IC_{50} value for cocaine in Y470H (IC_{50} in nM: cocaine, 195 ± 23; cocaine + SoRI-20041, 208 ± 28). Compared to cocaine alone, SoRI-20041 following the addition of cocaine significantly decreased the dissociation rate of [^{3}H]WIN35,428 by 79% (K_{-1}: cocaine, 0.161 ± 0.029; cocaine + SoRI-20041, 0.033 ± 0.008) in WT hDAT, by 42% (K_{-1}: cocaine, 0.169 ± 0.041; cocaine + SoRI-20041, 0.097 ± 0.044) in Y88F and by 18% (K_{-1}: cocaine, 0.076 ± 0.006; cocaine + SoRI-20041, 0.062 ± 0.019) in Y470H, respectively. Collectively, these results demonstrate that tyrosine470 and tyrosine88 are functional recognition residues in hDAT for Tat-induced inhibition of DA uptake and play different roles in Tat-mediated allosteric modulation of DAT. Through known allosteric modulators of DAT, identifying other potential allosteric binding sites in hDAT for Tat is our important ongoing study.

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Poster

122. Monoamine Transporter

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NIH grant 1P30GM103329

Title: Membrane properties of attention-deficit hyperactivity disorder associated dopamine transporter coding variant (A559V) linked with altered palmitoylation and phosphorylation

Authors: *M. J. SHETTY, J. MORRELL, M. HOVLAND, B. GROVE, R. A. VAUGHAN, J. D. FOSTER;
Dept. of Basic Sci., Univ. of North Dakota Sch. of Med. & He, Grand Forks, ND

Abstract: The dopamine transporter (DAT) plays a vital role in maintaining synaptic dopamine (DA) homeostasis in the central nervous system. Many neurological disorders including Parkinson disease, addiction, and attention-deficit hyperactivity disorder (ADHD) are associated with abnormal DA homeostasis, and DAT dysregulation is hypothesized to contribute to these disorders. Recently a DAT coding variant (A559V) has been associated with ADHD that displays anomalous DA efflux and increased distal N-terminal phosphorylation independent of amphetamine (AMPH) stimulation, suggesting alterations in regulatory properties. In previous studies, our laboratory has characterized effects of signaling pathways and abused drugs on DAT palmitoylation, membrane raft localization and site-specific phosphorylation. Here we analyzed the effect of this mutation on these properties using the hDAT A559V form and/or the rat homolog (A558V rDAT), finding multiple alterations in post-translational modifications and regulatory properties. Using a phospho-specific antibody we examined phosphorylation of Thr53, a proline-directed site in the membrane proximal region of the N-terminus that has been linked to altered uptake and efflux kinetics. Our findings show a striking increase in phosphorylation that is equivalent to that induced by AMPH, suggesting a possible mechanistic connection to transmitter efflux. We also found that these mutants induced significant decreases in palmitoylation, the reversible addition of palmitate to a cysteine via a thioester linkage. Palmitoylation is known to influence protein membrane properties including lateral mobility and microdomain targeting. Using fluorescence recovery after photobleaching we found that both A559V hDAT and A558V rDAT showed increased lateral membrane mobility, and using density gradient centrifugation we found that both mutant forms were enriched in membrane rafts compared to the WT proteins, suggesting these properties may contribute to the altered functionality. Ala558/559 is found at the extracellular side of TM12, and DAT is palmitoylated at Cys580/581 at the intracellular side of TM12. The alteration in the palmitoylation properties by this mutation thus suggests the propagation of a structural alteration in the TM12 helix that results in suppression of this modification. We have previously demonstrated the presence of a

reciprocal relationship between DAT palmitoylation and N-terminal phosphorylation, which may be related to the enrichment of the ADHD variants in membrane raft microdomains that may serve as platforms for DAT-mediated DA efflux.

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Poster

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Title: Effects of diet and insulin on DAT activity and expression in rat CPu, NAc and midbrain

Authors: ***K. T. JONES**[1], C. WOODS[2], T. ANTONIO[1], J. ZHEN[1], K. D. CARR[1,3], M. E. A. REITH[1,3];
[1]NYU Dept. of Psychiatry, [2]Ctr. for Neural Sci., New York Univ., New York, NY; [3]Dept. of Biochem. and Mol. Pharmacol., NYU, New York, NY

Abstract: Given that the dopamine transporter (DAT) is regulated by insulin, we wanted to investigate the effects of diet and insulin on DAT expression and function in brain regions important for reward, in particular, the nucleus accumbens (NAc), caudate/putamen (CPu) and midbrain. Freshly processed tissue was studied for dopamine (DA) uptake *in vitro* with rotating disk electrode voltammetry (RDEV), and for binding of the phenyltropane cocaine analog [^{3}H]CFT. We found that the influence of diet differs between NAc, CPu and midbrain. In synaptosomes from NAc, we found no difference in V_{max} and K_m for DA uptake in food restricted (FR) rats compared to *ad libitum* (AL) fed rats but a reduction in V_{max} in obesogenic (OB) rats, in agreement with other reports. [^{3}H]CFT binding to DAT, a reflection of surface DAT, in synaptosomes from NAc showed a reduction in B_{max} with no change in K_d in OB rats while AL and FR rats showed similar values. In contrast, synaptosomes from CPu showed a trend towards a reduction in V_{max}, without a change in K_m, for FR and OB (~30%) animals. The V_{max} effect is likely a result of a decreased surface DAT as indicated by reduced B_{max} of [^{3}H]CFT binding in FR (~24%) and OB (~37%) animals. Direct assessment of surface DAT is

underway in biotinylation studies. Preliminary data collected on somatodendritic DAT in midbrain suggest that diet affects DAT levels as assessed by [^{3}H]CFT binding without changing total DAT protein in synaptoneurosomes. To examine insulin's effect on striatal DAT activity, we monitored DA uptake using classical [^{3}H]DA uptake studies and RDEV. Preliminary voltammetry studies reveal that exogenous addition of insulin (30 nM) enhanced V_{max} in CPu synaptosomes from FR (~70%) rats compared to AL (~50%) fed rats but had less of an effect in synaptosomes from OB rats (~35%). This phenomenon was observed in the NAc to a lesser degree. Additionally, classical [^{3}H]DA uptake studies from AL rats confirmed the voltammetry data and indicated that insulin's effect on V_{max} in both NAc and CPu (~30%) was blocked by an insulin antibody (InsAb), whereas InsAb alone or IgG was without effect. This data along with the existing literature supports a role for diet in affecting DAT in CPu, NAc and midbrain while insulin can increase DAT-mediated DA uptake in both CPu and NAc.

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Poster

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F31NS084739

Title: Enhanced vesicular monoamine transporter level modifies synaptic L-DOPA-derived dopamine handling

Authors: *K. M. LOHR, G. W. MILLER;
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Abstract: The vesicular monoamine transporter 2 (VMAT2) is responsible for the packaging of monoamine neurotransmitters into vesicles for their subsequent release following an action potential. We have previously shown that elevated VMAT2 levels in mice (VMAT2-HI mice) increases vesicular storage capacity for dopamine, vesicle size, and stimulated dopamine release, suggesting that vesicular transporter expression is able to dictate dopaminergic output. Since VMAT2 overexpression augments dopamine handling in this way, we wanted to examine the

effects of a flooded dopamine system. To address this question, we applied L-DOPA to a striatal slice and, following a wash, recorded the stimulated dopamine release in the presence of exogenously stored dopamine using fast-scan cyclic voltammetry. We find that both wildtype and VMAT2-HI mice have significantly increased peak dopamine release following L-DOPA administration, with VMAT2-HI mice still showing an enhancement in dopamine release in the presence of drug. However, when L-DOPA is applied, the plasma membrane dopamine transporter (DAT)-mediated uptake of extracellular dopamine is significantly faster in the VMAT2-HI mice as measured by the rate constant tau ($p<0.05$). The level of the DAT is unchanged between the genotypes, suggesting a change in the function of the DAT. Taken together, these results suggest that vesicular filling is capable of modifying synaptic handling of exogenous dopamine.

Disclosures: K.M. Lohr: None. **G.W. Miller:** None.

Poster

122. Monoamine Transporter

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Dept. of Veterans Affairs I01BX002106 (TJP)

Intramural Research Program of the National Institute of Mental Health (SGA, SMU)

Title: Amphetamine increases NMDA-GluN2B synaptic currents in substantia nigra dopamine neurons

Authors: *S. L. INGRAM[1], M.-H. LI[1], T. J. PHILLIPS[2], S. G. AMARA[3], S. M. UNDERHILL[3];
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Abstract: The psychostimulant amphetamine and it's derivatives have significant abuse potential, but the cellular mechanisms underlying amphetamine addiction are not understood.

Here we show that amphetamine increases evoked NMDA-mediated synaptic currents (EPSCs) at -70 mV in 0 Mg2+ extracellular solution by 35% (control: -134 ± 20 pA, amphetamine: -202 ± 36 pA; paired t-test, $t(5) = 3.456$, $p < 0.05$). AMPA/NMDA ratios measured at +40 mV in normal extracellular solution were decreased by approximately 30% (control: 2.6 ± 0.24; amphetamine: 1.9 ± 0.12; paired t-test, $t(6) = 3.872$, $p < 0.01$). The potentiation of NMDA EPSCs is dependent on the dopamine transporter (DAT) but not on activation of dopamine receptors. The DAT inhibitor GBR12909 (1 μM) was superfused prior to amphetamine and completely blocked potentiation of NMDA EPSCs by amphetamine (GBR12909: -184 ± 18 pA compared to -200 ± 20 pA in GBR12909 + amphetamine). NMDA-GluN2B subunit inhibitors, ifenprodil (1 - 10 μM) and Ro 25-6981 (1 μM), inhibited the effects of amphetamine without affecting basal evoked NMDA currents indicating that different NMDA receptors are activated in the presence of amphetamine. A selective peptide inhibitor of amphetamine-dependent trafficking of the neuronal excitatory amino acid transporter (EAAT3 blocked the ability of amphetamine to potentiate NMDA EPSCs (scrambled peptide: 58 ± 19 % increase compared to EAAT3 peptide: -19 ± 8%, $t(9) = 4.437$, $p < 0.05$). In addition, the amphetamine-mediated potentiation was absent in mice lacking EAAT3. These data provide evidence that EAAT3 internalization increases extracellular glutamate concentrations and activates GluN2B-containing NMDA receptors. Experiments with the use-dependent NMDA blocker, MK-801, indicate that potentiated NMDA receptors are localized to the plasma membrane and are not inserted de novo. Finally, inhibition of NMDA-GluN2B receptors with Ro 04-5595 blocked methamphetamine (2 mg/kg, IP)-induced locomotor stimulation in a dose-dependent manner indicating that these receptors are important in the behavioral effects of amphetamines. These results reveal an important interaction between dopamine and glutamatergic signaling in midbrain dopamine neurons that is dependent on amphetamine modulation of neurotransmitter transporters.

Disclosures: **S.L. Ingram:** None. **M. Li:** None. **T.J. Phillips:** None. **S.G. Amara:** None. **S.M. Underhill:** None.

Poster

122. Monoamine Transporter

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Support: R01DA026947-A1

R01NS071122-A1

S10OD020026

Title: Prolonged METH exposure suppresses BK channel activity in the dopamine neurons

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Abstract: Previous studies have shown acute methamphetamine (METH) rapidly increases the excitability of dopamine neurons. However, the effect of longer METH exposure on the intrinsic firing activity of dopamine neurons is unknown. In cultured midbrain dopamine neurons, we found METH initially (1-2 min, 10 microM) increases the rate of spontaneous spike activity of dopamine neurons followed by a progressive reduction of the spontaneous spike activity (10-15 min, 10 microM). Longer METH exposure slows action potential repolarization and decreases the amplitude of afterhyperpolarization (AHP). These results suggest methamphetamine potentially affects the activity of large-conductance Ca2+-activated K+ (BK) channels in these neurons. We found both METH and blockade of BK channels increase the spike half-width and decrease the amplitude of afterhyperpolarization (AHP). Therefore, we tested the hypothesis that prolonged METH exposure inhibits the activity of BK channels to modulate the excitability of dopamine neurons. Using excised patch single-channel recordings, we identified the BK channels by their voltage-dependence and responsiveness to pharmacological manipulations such as using a specific channel blocker paxilline (10 microM) or channel opener NS1619 (10 microM). While METH exposure suppressed the amplitude of BK channel-mediated unitary currents, the BK channel opener, NS1619, attenuated the effects of METH on the AP half-width, AHP and the rate of spontaneous spike activity. Western blot and immunostaining analysis revealed the α1 / β4 subunits of BK channel are expressed in the midbrain dopamine neurons. The literature suggests the α1 subunit of BK channel increases the activity of the channel, whereas the β4 subunit suppresses the activity. While METH exposure decreased α1 subunit level, it enhanced β4 subunits level at the plasma membrane of these neurons. Taken together, our results support a fundamental role for BK channels' fine-tuning of the intrinsic firing activity of dopamine neurons and METH regulation of neuronal activity. Our ongoing experiments examine the mechanism of METH regulation of α1 / β4 subunits of BK channel in the dopamine neurons.

Disclosures: **M. Lin:** None. **D. Sambo:** None. **H. Khoshbouei:** None.

Poster

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Title: Dopamine transporter expression, transport capacity, and membrane lateral mobility is regulated by palmitoylation

Authors: *D. E. RASTEDT, J. D. FOSTER, R. A. VAUGHAN;
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Abstract: The dopamine transporter (DAT) mediates the reuptake of synaptic dopamine and thus regulates the spatio-temporal dynamics of dopaminergic neurotransmission. Complex control of DAT is exerted by various regulatory processes including posttranslational modifications. We previously found that DAT is palmitoylated, via the addition of a 16-carbon palmitic acid moiety to Cys 580, and that depalmitoylation led to reductions in Vmax and transporter levels. Palmitoylation is a reversible process catalyzed by palmitoyl acyltransferases (PATs) (also called DHHC enzymes based on the conserved active site sequence Asp-His-His-Cys), and palmitoyl-protein thioesterases (PPTs). Recent studies identified a family of 23 PAT enzymes in the human genome with distinct tissue distributions (e.g. neuronal vs non-neuronal), and with some associated with DA diseases such as schizophrenia. To identify PATs that can catalyze DAT palmitoylation and alter function, we co-expressed a specific subset of DHHC enzymes individually with DAT and assessed DAT palmitoylation, expression, surface levels, DA transport capacity, and lateral mobility. Palmitoylation assessed by acyl-biotin exchange revealed that the neuronal PATs, DHHC2, DHHC3, DHHC8, DHHC15, and DHHC17 increased DAT palmitoylation, while several others had no effect. We observed a correlation between increased DAT palmitoylation and increased total DAT levels, consistent with a role for palmitoylation in opposing DAT degradation. Increased DAT palmitoylation also led to enhanced DA uptake with no effect on surface levels, suggesting palmitoylation increases transport capacity via an alteration of DAT transport kinetics. Finally, increased DAT palmitoylation increased the time for recovery after photobleaching, suggesting that increased palmitoylation decreases the membrane lateral mobility of the transporter. Palmitoylation of DAT thus plays a role in both short- and long-term regulation of DAT, and may represent a point of DA reuptake dysregulation in dopaminergic disorders.

Disclosures: D.E. Rastedt: None. **J.D. Foster:** None. **R.A. Vaughan:** None.

Poster

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Support: R01DA038058

Title: Rare autism associated variants implicate syntaxin 1 (STX1 R26Q) phosphorylation and the dopamine transporter (hDAT R51W) in dopamine neurotransmission and behaviors

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Abstract: Syntaxin 1 (STX1) is a presynaptic plasma membrane protein that coordinates synaptic vesicle fusion. STX1 also regulates the function of neurotransmitter transporters, including the dopamine (DA) transporter (DAT). The DAT is a membrane protein that controls DA homeostasis through the high-affinity re-uptake of synaptically released DA. We adopt newly developed animal models and state-of-the-art biophysical techniques to determine the contribution of the identified gene variants to impairments in DA neurotransmission observed in autism spectrum disorder (ASD). Here, we characterize two independent autism-associated variants in the genes that encode STX1 and the DAT. We demonstrate that each variant dramatically alters DAT function. We identify molecular mechanisms that converge to inhibit reverse transport of DA and DA-associated behaviors. These mechanisms involve decreased phosphorylation of STX1 at Ser14 mediated by casein kinase 2 as well as a reduction in STX1/DAT interaction. These findings point to STX1/DAT interactions and STX1 phosphorylation as key regulators of DA homeostasis. We determine the molecular identity and the impact of these variants with the intent of defining DA dysfunction and associated behaviors as possible complications of ASD.

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Poster

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Support: NIH Grant R01 DA038598

Title: G beta-gamma subunits of heterotrimeric G-proteins interact with the dopamine transporter to evoke DA efflux

Authors: ***T. B. BAUST**[1], J. GARCIA-OLIVARES[4], K. SALERNO[1], E. STACHOWSKI[1], E. THIELS[1,2,3], S. G. AMARA[4], G. E. TORRES[1,5];
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[5]Pharmacol. and Therapeutics, Col. of Med., Univ. of Florida, Gainesville, FL

Abstract: The dopamine transporter (DAT) regulates extracellular dopamine (DA) levels and signaling via uptake and efflux. Addictive psychostimulants such as amphetamine increase extracellular DA levels in motivational and reward brain areas by targeting DAT. Examining the basic mechanism(s) that affect DAT efflux is critical for understanding both fundamental aspects of DA regulation and for clinical intervention in DA-related brain disorders associated with the therapeutic use and abuse of psychostimulants. Several studies have revealed a plethora of protein-protein interactions influencing DAT distribution and activity; suggesting that the fine-tuning of dopamine homeostasis occurs via an elaborate interplay of multiple mechanisms. We recently reported that beta-gamma (βγ) subunits of G proteins bind directly to the C-terminus (residues 582-620) of DAT to down-regulate uptake activity. Here, we report that the novel DAT/ Gβγ interaction also promotes DA efflux through DAT. Specifically, activation of Gβγ subunits using mSIRK increased DA efflux through DAT using both heterologous cells and primary neurons in culture. This effect was blocked in the presence of gallein, a Gβγ inhibitor. Likewise, a TAT-peptide containing the Gβγ interacting domain of DAT blocked the ability of mSIRK to induce DA efflux, suggesting the effect of Gβγ on efflux is a result of a direct interaction with the transporter. Based on these data, we hypothesized that Gβγ may also be

involved in the actions of amphetamine. In similar efflux experiments, amphetamine induced a dose-dependent increase in DA efflux in both heterologous cells and primary neurons in culture. More importantly, amphetamine-induced efflux was blunted in the presence of either gallein or the TAT-peptide containing the DAT interacting domain. Collectively, our data suggest that the direct interaction of Gβγ subunits with DAT has an important role in both physiological and amphetamine-induce DA efflux. Additional studies are examining the role of the DAT/ Gβγ interaction on amphetamine-evoked behaviors *in vivo*.

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Poster

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NIH grant F32DA034408

Title: Brain region-specific trafficking of the dopamine transporter

Authors: ***A. SORKIN**[1], E. BLOCK[1], J. J. BALCITA-PEDICINO[2], J. NUTTLE[2], S. SESACK[2]; [1]Cell Biol., [2]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The dopamine (DA) transporter (DAT) controls dopaminergic neurotrasmission by removing extracellular DA. While DAT activity is proposed to be regulated by its traffic to and from the cell surface, the membrane trafficking system involved in the endocytic cycling of DAT in the intact mammalian brain has not been characterized. Hence, we performed immunostaining and quantitative analysis of the subcellular and regional distribution of DAT using the transgenic knock-in mouse expressing hemagglutinin (HA) epitope tagged DAT (HA-DAT), and by employing a combination of electron microscopy and a novel method for immunofluorescence labeling of HA-DAT in acute sagittal brain slices. Both approaches demonstrated that in midbrain somatodendritic regions, HA-DAT was present in the plasma membrane, endoplasmic reticulum, and Golgi complex, with a small fraction in early and recycling endosomes, but not in late endosomes and lysosomes. In the striatum, as well as in axonal tracts between the midbrain and striatum, HA-DAT was detected predominantly in the plasma membrane, and quantitative

analysis revealed increased DAT density in striatal compared to midbrain plasma membranes. Endosomes were strikingly rare and lysosomes were absent in striatal axons, where there was little intracellular HA-DAT. Acute administration of amphetamine *in vivo* or to slices *ex vivo* did not result in detectable changes in the patterns of DAT distribution. Altogether, these data provide evidence for regional differences in DAT plasma membrane targeting and retention, and suggest a surprisingly low level of endocytic trafficking of DAT in the striatum along with limited DAT endocytic activity in somatodendritic areas.

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Poster

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Title: HIV-1 Tat protein augments methamphetamine-induced impairment of dopamine transporter activity

Authors: ***R. STEGER**[1], K. SAHA[1], D. SAMBO[1], T. ALONGE[1], B. BUTLER[1], C. APPADOO[1], J. GOODWIN[2], S. BUCH[3], H. KHOSHBOUEI[1];
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Abstract: HIV-1 contributes to cognitive and behavioral decline in infected individuals, in part, through the production of HIV-1 transactivator of transcription (Tat) protein. While antiretroviral agents reduce viral load, they do not eliminate the production of Tat and thus are ineffective against neuropathologies caused by HIV-1. Specifically, the Tat protein has been shown to play a critical role in perturbations of the dopamine neurotransmission in the brain. In addition, there also exists a high comorbidity between HIV-1 infection and methamphetamine (METH) abuse. Both the Tat protein and METH exert their effects through the dopamine transporter (DAT). DAT is a major regulator of extracellular dopamine and thus dopamine neurotransmission in the

brain and it is implicated in neurological and neuropsychiatric disorders such as Parkinson's disease and drug addiction. Recent reports suggest the Tat protein enhances METH-induced impairment of dopamine transmission, albeit with a less understood mechanism. Under control conditions, METH increases the DAT-mediated inward current, increases the spontaneous firing activity of dopaminergic neurons and increases DAT-mediated DA efflux. However, we found that intracellular Tat-101(200ng/ml) protein attenuated basal and METH (10μM) induced DAT-dependent DA efflux, in both bath solution or delivered directly into the dopamine neurons via the patch electrode. Furthermore, we found intercellular Tat-101 significantly decreases METH-induced inward current, supporting the hypothesis that Tat-101 might affect ionic permeability of DAT regulating dopamine neurotransmission. Consistent with previous reports we found methamphetamine or Tat-101 alone decreases dopamine uptake, while pretreatment of DAT cells with 100 nM Tat for 15 minutes synergistically decreases dopamine uptake. Previously we have shown DA efflux via DAT is regulated by intracellular calcium and its associated kinases including Calcium calmodulin-dependent protein kinase II (CaMKII) and Protein Kinase C (PKC). Currently we are examining the hypothesis that Tat-101 regulates DAT activity by regulating intracellular calcium homeostasis and signaling molecules such as PKC and CaMKII. These preliminary findings provide mechanistic insight involved in the combined deleterious effects of HIV-1 Tat and methamphetamine in the brain with the ultimate goal of testing novel therapeutics.

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Poster

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Title: Role of interaction between the Dopamine Transporter and G protein βγ subunits in amphetamine-induced hyperlocomotion

Authors: ***J. C. MAUNA**[1], M. R. MARKS[1], N. ARIAS[1], T. B. BAUST[1], S. HARRIS[1], G. E. TORRES[1,2], E. THIELS[1,3,4];

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Abstract: Dopamine (DA) plays a crucial role in several brain functions, including locomotion and motivated behavior. Consequently, DA deregulation contributes to the development of a number of brain disorders such as ADHD, Parkinson's disease, and drug addiction. The primary mechanism used by neurons to maintain extracellular levels of DA is reuptake of the transmitter through the Dopamine Transporter (DAT). DAT is a symporter protein that uses the sodium gradient to pump DA back into the presynaptic terminal. It was shown that amphetamine reverses DAT directionality, causing a massive efflux of DA into the extracellular space. The molecular mechanism by which DAT works in reverse is poorly understood. Recently, our laboratory identified a novel interaction between the intracellular carboxy terminal region of DAT and G protein βγ subunits. Experiments in cells in culture demonstrate that activation of Gβγ subunits induces DA efflux similar to that observed with amphetamine. Here, we examined the effect of pharmacological manipulation of Gβγ subunits on amphetamine-induced locomotor activity and on DAT/Gβγ interaction *in vivo*. Adult rats received bilateral infusions into the nucleus accumbens (NAc) of either the Gβγ activator mSIRK, scrambled mSIRK peptide (control), the Gβγ inhibitor gallein, or vehicle solution (control) before being injected i.p. with either amphetamine (3mg/kg), cocaine (20 mg/kg), or physiological saline (control). Activity in an open-field chamber was monitored before and after drug treatment. We found that neither the Gβγ activator nor the Gβγ inhibitor alone (saline-injected controls) had an effect on locomotor activity compared to the respective control groups. In sharp contrast, the Gβγ activator markedly enhanced amphetamine-induced hyperlocomotion, whereas the Gβγ inhibitor significantly attenuated it. Different from the Gβγ activator's effect on amphetamine-induced hyperlocomotion, the activator had no effect on cocaine-induced hyperlocomotion. Because cocaine blocks DAT whereas amphetamine reverses DAT directionality, these data suggest that amphetamine's actions on DAT are mediated through Gβγ interaction with DAT. To test this idea directly, we treated rats as described above and harvested striatal tissue for co-immunoprecipitation of DAT and Gβγ subunits. We found that amphetamine increases the interaction between DAT and Gβγ and that prior intra-NAc infusion of the Gβγ inhibitor attenuates this effect. Taken together, our results identify Gβγ subunits as a novel mechanism in the regulation of amphetamine actions *in vivo*.

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Poster

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Support: NIH Award MH094527 (RDB)

Title: State-dependent protein interactions with the presynaptic serotonin transporter

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Abstract: Disruptions in serotonin (5-hydroxytryptamine, 5-HT) signaling have been implicated in multiple neurological disorders, including depression, autism and schizophrenia. The antidepressant-sensitive 5-HT transporter (SERT, SLC6A4) affords powerful control over 5-HT signaling via rapid clearance of 5-HT from the extracellular space. Evidence suggests that SERT responds to intrinsic and extrinsic factors to modulate 5-HT uptake activity, regulating 5-HT availability. For example, systemic administration of lipopolysaccharide (LPS) in rodents shifts the transporter to a high-affinity state (SERT*), in a p38 MAPK dependent manner, promoting efficient clearance at low 5-HT concentrations. Recently, our lab identified a gain-of-function, autism-associate coding variant, SERT Ala56, and provided evidence that its conformations and insensitivity to PKG/p38 MAPK signaling are such as to suggest constitutive occupancy of the SERT* state. Interestingly, stimulation of kinase-linked pathways that alter SERT activity appears to also modulate SERT interacting proteins (SIPs). We hypothesize that SERT exists in multiple activity states, defined and mediated by distinct SIP complexes. Clearly defining the protein complexes of the different SERT conformational states will be useful in identifying possible drug targets to modulate SERT activity. However, identifying these macromolecular complexes is technically challenging as biochemical preparations of native SERT likely feature multiple such complexes that are difficult to follow independently. We developed a SERT Ala56 knock-in mouse model, providing access to an enriched pool of SERT* and a platform for the identification of proteins that differentially interact with the SERT* state *in vivo*. Using candidate and proteomic-based approaches, we are assessing differences between WT and SERT Ala56 SIPs, as well as whether these changes identified are paralleled by SIP changes obtained when comparing saline vs. LPS injected animals. Co-immunoprecipitation analysis reveals that previously reported SIPs, such as protein phosphatase 2A and syntaxin 1A, differentially associate with SERT Ala56 compared to wild-type SERT (WT). Ongoing studies seek to validate and characterize novel SIP interactions with the SERT* state and to relate these interactions to SERT trafficking/activation mechanisms *in vivo*.

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Poster

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Novo Nordisk Foundation, Grant 10959

Title: The betaine-GABA transporter (BGT1; slc6a12) may play its main roles in the liver rather than in the brain by help preserving choline levels in hepatocytes

Authors: *Y. ZHOU[1], K. CORBIN[2], Y. XIANG[3], W. ESKILD[3], S. ZEISEL[2], N. DANBOLT[1]; [1]Inst. of Basic Med. Science, Univ. of Oslo, Oslo, Norway; [2]Dept. of Nutr., UNC Nutr. Res. Inst., Kannapolis, NC; [3]Dept. of Biosci., Inst. of Mol. Biosci., Oslo, Norway

Abstract: The betaine/GABA transporter (BGT1; slc6a12) has received attention both as an osmolyte transporter in the kidney and as a potential controller of transmitter GABA in the brain. The expression of BGT1 in the brain, however, is low and is mostly limited to the leptomeninges suggesting a minor role in inactivation of GABA (Epilepsy Res 95:70-81). Most of the BGT1 protein turns out to be in the liver (Am J Physiol Renal Physiol 302:F316-28). Hepatocytes contain large amounts of betaine as well as betaine homocysteine S-methyltransferase (BHMT). This enzyme allows betaine to be used as a methyl donor. Hepatocytes can acquire betaine either by oxidizing choline to betaine, or by absorbing betaine (by means of transporters) from the portal blood. These observations make it important to determine (a) the precise localizations of BGT1 in the liver, (b) the contribution of BGT1 to the total betaine uptake, and (c) the importance of betaine uptake for hepatocyte betaine content and health. Here we have isolated cells from mouse liver (from both wildtype mice and BGT1-deficient mice). The cells were examined for BGT1 content (Western blots and immunocytochemistry) and for betaine uptake activity. BGT1 was detected only in hepatocytes and not in the other cell types found in the liver (Kuppfer cells, stellate cells and endothelial cells). The betaine uptake activity was significantly impaired in BGT1-deficient hepatocytes while GABA and choline uptake activities were unaffected in agreement with the notion that these compounds are primarily transported by other mechanisms. Further choline metabolites analysis showed that the livers from mice lacking

BGT1 had reduced concentrations of choline (78 ± 2 %; $p < 0.03$). **Conclusions**: BGT1 expression is limited to hepatocytes under normal physiological conditions. Deletion of BGT1 reduces the capacity of betaine transport by hepatocytes and affects the liver choline content possibly by increasing choline consumption.

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Poster

122. Monoamine Transporter

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Topic: B.05. Transporters

Title: Differential effects of maternal and fetal genetic disruption of the serotonin transporter on placental and fetal brain biogenic amine function and thalamocortical axon pathway formation

Authors: *J. C. VELASQUEZ[1], E. HUGHES[2], K. FISH[2], E. EMERY[2], A. BONNIN[1], S. HEROD[2];
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Abstract: Serotonin (5-HT) is a monoamine neurotransmitter that is fundamental to brain development, and low circulating levels of 5-HT are associated with clinical psychopathologies including anxiety and depression. Interestingly, the placenta is a significant source of 5-HT to the fetus early in fetal development. It remains unclear whether hyposerotonemia in mothers with such pathologies might affect fetal brain development, either directly or through alteration of placental function. B6.129(Cg)-*Slc6a4*^{tm1Kpl}*/J* serotonin transporter knockout (SERT-KO) mice were used as a model of genetically-induced hyposerotonemia. Mice were bred heterozygous (HET) by HET to generate wildtype (WT), HET, and knockout (KO) experimental genotypes. Females of each genotype were bred with HET males, and fetal and placental tissue was harvested at four different timepoints during embryonic development (E12.5, E14.5, E16.5, E18.5). High performance liquid chromatography (HPLC) was used to measure concentrations of 5-HT and 5-HIAA, the main metabolite of 5-HT, present in fetal forebrains, hindbrains, and placentas across all maternal and fetal genotypic combinations. Results reveal an age-dependent influence of maternal and fetal genotypes on these parameters. At E14.5, a significant decrease of 5-HT concentration was observed in the KO placenta; a change driven by the maternal

genotype, independent of fetal genotype. The impact on fetal brain 5-HT was minimal, consistent with a placental (fetal origin) source of 5-HT to the fetus. In contrast, at E18.5 fetal brain 5-HT levels appeared to be driven by the genotype of the fetus, and not the mother, reflecting an impact of SERT function on the endogenous (fetal dorsal raphe) source of 5-HT. At both ages, absence of SERT function in either the mother or the fetus led to a decrease in 5-HIAA in the fetal brain, suggesting that 1) maternal blood 5-HT drives fetal brain levels of 5-HIAA, and 2) absence of SERT in the fetus affects fetal 5-HT metabolism. In addition, immunohistochemical analyses of fetal brains were performed by co-staining for 5-HT and Netrin-G1a (NetG1), a marker of developing thalamocortical axons (TCAs). Initial analyses of fluorescent imaging of WT and KO fetal brains at E14.5 from the same HET mother showed no differences in the 5-HT neuronal localization. However, NetG1 staining in the KO revealed decreased fasciculation of TCAs in the midbrain region as compared to WT. These results suggest that even small alterations of 5-HT metabolism in the forebrain can alter the early phase of TCAs pathfinding and/or TCAs expression of NetG1.

Disclosures: J.C. Velasquez: None. **E. Hughes:** None. **K. Fish:** None. **E. Emery:** None. **A. Bonnin:** None. **S. Herod:** None.

Poster

122. Monoamine Transporter

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 122.26/B53

Topic: B.05. Transporters

Support: NSF 0922085

Title: Organic cation transporter 3 (OCT3) and plasma membrane monoamine transporter (PMAT) are increased in parietal cortex following chronic administration of citalopram

Authors: *J. C. MOLINARO[1], J. S. TALBOOM[1,2,3], M. ORCHINIK[1];
[1]Arizona State Univ., Tempe, AZ; [2]Banner Sun Hlth. Res. Inst., Sun City, AZ; [3]Arizona Alzheimer's Consortium, Phoenix, AZ

Abstract: The activity of extracellular monoamine neurotransmitters in the brain can be regulated not only by the uptake of presynaptic transporters that have high affinity for a single neurotransmitter, but also by lower affinity “polyspecific” transporters which can take up several different neurotransmitters. Among these polyspecific neurotransmitters are the organic cation transporter 3 (OCT3) and the plasma membrane monoamine transporter (PMAT), both of which

appear to be widespread in the human and rodent brain. OCT3 and PMAT are each capable of transporting serotonin, and OCT3 has been reported to be increased in the brains of mice lacking the serotonin transporter. We hypothesized that these transporters might compensate for increased serotonin levels produced by selective serotonin reuptake inhibitor (SSRI) antidepressant drugs and might thereby mitigate the efficacy of SSRIs. To test this hypothesis, we investigated if chronic SSRI administration causes changes in OCT3 and PMAT protein levels in the hippocampus and parietal cortex as these brain regions have been implicated in the behavioral changes induced by SSRI drugs. Young adult male Sprague-Dawley® rats were administered either the SSRI citalopram, or vehicle, for 28 days via subcutaneous Alzet® mini-osmotic pumps at a dosage of 10/mg/kg/day. This dose has been shown to result in plasma serum levels of citalopram comparable to that of human serum levels during citalopram pharmacotherapy and corresponds to the typical length of pharmacotherapy required for the development of therapeutic effects. Western blots showed that OCT3 and PMAT protein levels were both significantly increased in the parietal cortex of rats given citalopram compared to controls, although OCT3 and PMAT levels were unchanged in the hippocampus. Increases in these transporters could attenuate the behavioral effects of SSRIs. On the other hand, since OCT3 is inhibited by physiological levels of corticosteroids (CORT), this might represent a mechanism through which SSRIs alter neuronal-sensitivity to CORT or affect the stress response. Future investigations will test if there is an interaction between SSRIs, polyspecific transporters in the cortex, and behavior.

Disclosures: **J.C. Molinaro:** None. **J.S. Talboom:** None. **M. Orchinik:** None.

Poster

123. Other Transporters

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 123.01/B54

Topic: B.05. Transporters

Support: UAMS COM pilot grant program

Arkansas Science and Technology Authority

Title: Transgenic mouse model to selectively identify alpha$_3$ Na,K-ATPase expressing cells in the nervous system

Authors: ***M. DOBRETSOV**[1], D. D. PIERCE[2], K. E. LIGHT[3], N. T. KOCKARA[4], M. KOZHEMYAKIN[5], P. A. WIGHT[4];

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Abstract: The α_3 Na^+,K^+-ATPase (α_3 NKA) is one of four known α isoforms of the mammalian transporter. A deficiency in the α_3 NKA associates with severe impairment of movement control, and in some cases, seizure disorder. Understanding the pathogenesis of these disorders is limited by incomplete knowledge of the expression of the various isoforms in brain tissue, as well as the challenges in exploring the functional alterations of these isoforms in electrophysiological studies. To address this problem, a 1,758-bp fragment of the mouse α_3 subunit gene (*Atp1a3*) for Na^+,K^+-ATPase, which encompasses the promoter and 5′-untranslated region, was fused to a promoterless ZsGreen1 reporter gene, and α_3 NKA-ZsGreen1 transgenic mice were generated in the C57BL/6 genetic background. Founder mice were bred to C57BL/6 wild-type (WT) mice, and three transgenic lines were established. Expression of ZsGreen1 does not interfere with expression of the endogenous α_3 subunit of NKA as evidenced by normal growth, phenotype and breeding capacity of α_3 NKA-ZsGreen1 mice in our colonies. Transverse and sagittal sections of whole brain from five adult transgenic mice were analyzed. Consistent with published results on α_3 NKA distribution, the display of ZsGreen1-labeled neurons varied considerably (non-uniform), with highest density observed in hypothalamic, midbrain, pontine, brain stem, deep cerebellar and select thalamic nuclei. Intensively labeled neurons were also present in the cerebellar cortex, neocortex, and hippocampus. ZsGreen1-labeling was not observed in glial cells or white matter-enriched brain regions. In electrophysiological experiments, discharges of ZsGreen1-labeled hippocampal interneurons were sensitive to 1 μM ouabain (a concentration too small to inhibit rodent α_1 NKA), while non-fluorescent interneurons did not show this sensitivity. Thus, the α_3 NKA-ZsGreen1 transgenic mice model constitutes a novel and versatile tool to elucidate the properties, regulation, and functional significance of α_3 NKA in its native environment. Identification of functional α_3 NKA-expressing neurons *in situ* simplifies electrophysiological and biophysical studies addressing the discharge properties of these neurons, as well as provides a means to study the biophysical properties of the transporter and understand its spatiotemporal regulation under normal and pathological conditions. [Supported by UAMS COM pilot grant program and by Arkansas Science and Technology Authority]

Disclosures: M. Dobretsov: None. **D.D. Pierce:** None. **K.E. Light:** None. **N.T. Kockara:** None. **M. Kozhemyakin:** None. **P.A. Wight:** None.

Poster

123. Other Transporters

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Program#/Poster#: 123.02/B55

Topic: B.05. Transporters

Support: Norwegian Research Council Grant 240844

Novo Nordisk Fonden Grant 10959

NIH NINDS

Title: Generation and characterization of BAC transgenic GABA transporter 2 (GAT2; slc6a13) tdTomato reporter mice

Authors: *N. C. DANBOLT[1], Y. ZHOU[1], J. XU[2], B. S. KHAKH[2];
[1]Univ. of Oslo, Oslo, Norway; [2]Physiology, David Geffen Sch. of Med., UCLA, Los Angeles, CA

Abstract: Background: GABA transporters 1 and 3 (GAT1 and GAT 3) are known for their functions in uptake of the neurotransmitter GABA in the central nervous system, but less is known about the function of GAT2. We have recently reported that GAT2 acts as a taurine transporter in both peripheral organs and in the brain (Journal of Biological Chemistry 287, 35733-46). We reasoned that generation of GAT2 reporter mice would advance the study of GAT2 expression and distribution as well as permit detailed study of the cells where it is expressed. GAT2-tdTomato reporter mice were generated by bacterial artificial chromosome (BAC) mediated transgenesis. Since BACs are about 200 kb, they should retain the transcriptional regulatory elements that regulate GAT2 expression and thus GAT2-tdTomato reporter mice should faithfully report GAT2 endogenous expression by virtue of the orange/red fluorescence of tdTomato. Here, we test this by comparing the distribution of tdTomato in GAT2-tdTomato reporter mice with the distribution of GAT2 immunoreactivity (using GAT2 knockout mice as negative controls). The GAT2-tdTomato transgenic mice were viable and fertile. Neither abnormal behavior nor abnormal brain amino acid composition was observed. In adult mice, we found tdTomato expression in hepatocytes and proximal tubules as well as in the leptomeninges and some cerebral blood vessels. **Conclusions**: The GAT2-tdTomato mice faithfully report the expression patterns of GAT2. These mice will be useful in studies of GAT2 in liver, kidney and leptomeninges.

Disclosures: **N.C. Danbolt:** None. **Y. Zhou:** None. **J. Xu:** None. **B.S. Khakh:** None.

Poster

123. Other Transporters

Location: Hall A

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Program#/Poster#: 123.03/B56

Topic: B.05. Transporters

Support: CONACYT-CB-2009-01 Grant #130194 for Ureña-Guerrero, M.E.

Title: GABAA receptor activation in newborn male rats modifies the developmental profile of NKCC1, KCC2 and alpha1 and gamma2 GABAA receptor subunits in entorhinal cortex

Authors: *J. MURGUIA CASTILLO[1], M. E. UREÑA-GUERRERO[1], S. A. OROZCO-SUAREZ[2], C. BEAS-ZÁRATE[1], A. I. FERIA-VELASCO[1];
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Abstract: GABA is considered as the main inhibitory neurotransmitter in adulthood, but it can induce neuronal depolarization and excitation during early development of the brain. Both, GABA-mediated inhibition and excitation depend on the GABAA receptor (GABAA-R) activation, which mainly promotes facilitated diffusion of chloride (Cl-). NKCC1 acts as Cl- importer and is highly expressed when GABA induces neuronal depolarization, whereas KCC2 operates as Cl- exporter and it is expressed in neurons to reach the establishment of the GABA-mediated inhibition. *In vitro* studies have been suggested that in early developmental stages, the GABA-mediated neuronal excitation increases the KCC2 expression and improves the neuronal inhibitory response to the GABA application. In this work, an *in vivo* GABAA-R activation was induced in newborn male Wistar rats through the subcutaneous (s.c.) administration of muscimol (1 mg/kg of body weight) at postnatal day (PD) 0. After the treatment, the expression levels of NKCC1, KCC2, and alpha1 and gamma2 GABAA-R subunits were estimated in entorhinal cortex at PD1,3,5,7,9,11,13,15,21,30,60, 90 and 120 using western-blotting assays. The muscimol treatment modified the developmental profile of all proteins studied. Briefly, the expression peak of NKCC1 protein observed at PD11 in the control group was not observed after muscimol treatment. However, after the treatment, the expression of NKCC1 increased gradually to reach lightly higher levels than control group from PD30 to PD120. In respect to KCC2 protein, muscimol treatment increased significantly its expression at PD9-120, reaching its maximal level at PD15 in four fold higher respect to control group and before the expression peak observed at PD30 in control group. Similarly, the expression peak for alpha1 GABAA-R subunit and the maximal expression level for gamma2 GABAA-R subunit, both observed at PD21 in the control group, were observed before in the muscimol treated group at PD13. Results suggest that the early activation of GABAA-R with muscimol modifies the developmental profile expression of NKCC1, KCC2 and alpha1 and gamma2 GABAA-R subunits in male entorhinal cortex, in a way where GABA-mediated inhibitory signaling could be improved in magnitude and in its time for establishment, which should be better characterized.

Disclosures: **J. Murguia Castillo:** None. **M.E. Ureña-Guerrero:** None. **S.A. Orozco-Suarez:** None. **C. Beas-Zárate:** None. **A.I. Feria-Velasco:** None.

Poster

123. Other Transporters

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 123.04/B57

Topic: B.05. Transporters

Support: NHMRC Project Grant 1082570

NHMRC CDF 1048784

Title: An allosteric mechanism for lipid inhibition of glycine transporter 2

Authors: ***R. J. VANDENBERG**[1], S. MOSTYN[2], J. E. CARLAND[2], R. M. RYAN[2];
[2]Pharmacol., [1]Univ. Sydney, Sydney, Australia

Abstract: The glycine transporter, GlyT2, is the target for a number of drugs that can alleviate neuropathic and inflammatory pain. We have identified a series of lipids that inhibit GlyT2 and also show promise as leads for the development of a novel class of analgesics. The three lipids that show highest affinity and efficacy are N-Arachidonyl-Glycine (NAGly), N-Oleoyl-Glycine (NOGly) and Oleoyl-L-Carnitine (OLCarn). All three lipids are non-competitive inhibitors and are unlikely to bind at the substrate binding site. We have investigated the mechanism of inhibition by first constructing a homology model of GlyT2 based on the crystal structures of the related bacterial amino acid transporter, LeuT, and the *drosophila* melanogaster dopamine transporter, dDAT. We then used site-directed mutagenesis to selectively disrupt the inhibitory actions of the lipids, whilst retaining glycine transport function. Mutations in four distinct regions of the protein influence lipid potency and efficacy, extracellular loop 4 (EL4), transmembrane domain 7 (TM7), TM8 and the substrate binding site . EL4 undergoes considerable conformational changes during the transport cycle, and the I545L mutation at the apex of this loop disrupts inhibition by all three lipids. This site is unlikely to form a lipid binding site, but it may influence the conformational changes of the protein. We reasoned that the transmembrane domains flanking EL4, TM7 and TM8, may also influence the conformational changes required for lipid inhibition. The L569F mutation on the membrane-exposed surface of TM8 reduced the potency of OLCarn and NOGly, but had no effect on NAGly. This suggests that TM8 may influence lipid tail interactions. The F515Y mutation in TM7 caused an increase in both potency and efficacy of all three lipids. Finally, we investigated

how disruptions in the substrate binding site may alter lipid activity. The S479G mutation relaxes the substrate selectivity to also allow sarcosine to be transported, and also reduces the potency of all three lipids. No other mutations in the substrate binding sitealtered lipid inhibition. We propose a model where the lipids bind to the external membrane-exposed surface of the transporter, which then alters the conformational changes in EL4 and disrupts substrate interactions, causing a reduced rate of transport.

Disclosures: R.J. Vandenberg: None. **S. Mostyn:** None. **J.E. Carland:** None. **R.M. Ryan:** None.

Poster

123. Other Transporters

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 123.05/B58

Topic: B.05. Transporters

Title: Regulation of cation-chloride cotransporters by N-methyl-D-aspartate receptors

Authors: *J. CHEVRIER, V. MAHADEVAN, M. WOODIN;
Cell & Systems Biol., Univ. of Toronto, Toronto, ON, Canada

Abstract: The strength of gamma-aminobutyric acid (GABA)-mediated inhibitory synaptic transmission is determined by the electrochemical gradient of chloride across the plasma membrane. Within the electroneutral family of transmembrane cation-chloride cotransporters (CCCs), NKCC1 and KCC2 are the primary transporters responsible for regulating chloride transport in the central nervous system. In immature neurons, NKCC1 expression predominates and mediates chloride influx, resulting in high intracellular chloride ([Cl-]i) and depolarizing GABA potentials. In mature neurons, the chloride-extruding transporter KCC2 is upregulated and maintains low [Cl-]i, which is required for hyperpolarizing GABAergic inhibition. Recently, excitatory kainate-type glutamate receptors (KARs) were observed to post-translationally regulate KCC2 expression and function by physical interaction. In the present study, we used biochemical techniques to investigate the regulation of KCC2 and NKCC1 by another class of glutamate receptors: N-methyl-D-aspartate receptors (NMDARs). Previously, the Ca2+-dependent protease calpain was found to cleave KCC2 and attenuate its function upon pathophysiological NMDAR activation. *In vivo* and in heterologous expression systems, our immunoprecipitation assays revealed that NMDAR subunits interact with KCC2 and NKCC1 in a developmentally-dependent manner. These interactions between excitatory ionotropic glutamate receptors and inhibitory cation-chloride cotransporters suggest the existence of a

functional glutamate-dependent complex capable of regulating local neuronal chloride homeostasis.

Disclosures: J. Chevrier: None. **V. Mahadevan:** None. **M. Woodin:** None.

Poster

123. Other Transporters

Location: Hall A

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Topic: B.05. Transporters

Support: LUNDBECK FOUNDATION GRANT R118-A11564

Title: Pharmacological characterization of a BGT1-selective compound with a biphasic inhibition profile and anticonvulsive properties in mice

Authors: *M. E. LIE[1], J. SKOVGAARD-PETERSEN[1], S. B. VOGENSEN[1], R. P. CLAUSEN[1], B. FRØLUND[1], A. SCHOUSBOE[1], S. WHITE[2], P. WELLENDORPH[1];
[1]Dept. of Drug Design and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark; [2]Dept. of Pharmacol. and Toxicology, Univ. of Utah, Utah, UT

Abstract: Seizures caused by hyper-excitable neurons can be alleviated by enhanced inhibitory neurotransmission via GABAergic signalling. The inhibitor N-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-hydroxy-4-(methylamino)-4,5,6,7-tetrahydrobenzo[d]isoxazol-3-ol (EF1502) targeting GAT1 and BGT1, exerts a synergistic anticonvulsant effect in several mouse seizure models when tested in combination with GAT1-selective compounds. BGT1-selective compounds that can enter the brain are limited, however, a newly developed compound in our laboratories, (1*R*,2*S*)-2-((4,4-bis(3-Methylthiophen-2-yl)but-3-en-1-yl)(methyl)amino)cyclohexanecarboxylic acid (SBV-114), seems to fulfil these criteria. A pharmacological characterization of SBV-114 was performed using the [^{3}H]GABA uptake assay in recombinant HEK293 cells stably expressing mouse GABA transporters (GAT1-3 or BGT1), tsA cells transiently expressing human BGT1 and MDCK cells endogenously expressing BGT1. The anticonvulsant activity and motor impairment of SBV-114 were examined using the audiogenic seizure-susceptible Frings mouse, and the scPTZ-induced seizure threshold test, maximal electroshock seizure (MES) test and Rotarod test in the CF-1 mouse. SBV-114 inhibited mouse BGT1 with a biphasic inhibition profile (27% and 60% uptake-inhibition at the low (4.0μM) and high (411.3μM) IC_{50}, respectively). SBV-114 displayed negligible inhibitory activity at GAT1-3 (IC_{50}>1,000μM). The biphasic inhibition profile was also observed at the

human BGT1 and in MDCK cells. The profile was neither due to kinetic differences between SBV-114 and GABA (no profile change after 10 minute pre-incubation) nor to differences in expression levels of BGT1 across cell types. Interestingly, the calcium chelator BAPTA-AM converted the biphasic profile into a monophasic inhibition profile, indicating that the biphasic behaviour of SBV-114 is Ca^{2+}-dependent. When administered systemically, SBV-114 displayed anticonvulsive effects in the audiogenic seizure (50 and 120mg/kg) and MES test (ED_{50}=139mg/kg), but was unable to block seizures induced by scPTZ(150mg/kg). SBV-114 (150mg/kg) did not cause any motor impairment at doses tested. We report that SBV-114, a novel GABA-analogue, is a BGT1-selective compound with a unique biphasic inhibition profile at recombinant transporters and anticonvulsant properties in mouse seizure models.

Disclosures: M.E. Lie: None. **J. Skovgaard-Petersen:** None. **S.B. Vogensen:** None. **R.P. Clausen:** None. **B. Frølund:** None. **A. Schousboe:** None. **S. White:** None. **P. Wellendorph:** None.

Poster

123. Other Transporters

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 123.07/B60

Topic: B.05. Transporters

Support: NIH NS078220

NIH NS092545

Title: Suprachiasmatic nucleus chloride cotransporter (nkcc1) regulation of behavioral circadian rhythms

Authors: *J. K. MCNEILL, IV[1], J. C. WALTON[2], H. E. ALBERS[2];
[2]Neurosci. Inst., [1]Georgia State Univ., Atlanta, GA

Abstract: γ-aminobutyric acid (GABA) and ionotropic GABAA receptors are expressed throughout the suprachiasmatic nucleus (SCN); the location of the master circadian pacemaker. Evidence from our lab shows that acute activation of GABAA receptors (GABAARs) inhibits the phase shifting effects of photic cues and promotes the phase shifting effects of non-photic cues. More recently, we have shown that the sustained activation of GABAARs can mimic the phase delaying effects of light and that the sustained inhibition of GABAARs can inhibit the phase delaying effects of light. Although GABA is most commonly described as the primary

inhibitory neurotransmitter in the CNS, *in vitro* studies suggest the presence of excitatory GABAA responses in the adult SCN. The ratio of inhibitory to excitatory responses to GABAAR binding depends on chloride ion gradients as maintained by chloride co-transporters. Importantly, excitatory GABA responses in the adult SCN disappear upon inhibition of the inward chloride co-transporter, Na+/K+/Cl- co-transporter 1 (NKCC1). We have previously shown that the sustained inhibition of NKCC1 enhances phase delays to light. The following experiments aimed to further characterize the role of chloride co-transporter activity in behavioral entrainment. Adult male Syrian hamsters were housed in constant dark conditions until the formation of stable free-running activity rhythms. Hamsters received injections of bumetanide (a NKCC1 inhibitor) or vehicle into the SCN region followed by phase delaying (CT 13.5) or advancing (CT 19) light pulses. Phase shifts were calculated using regression lines fitted to activity onsets before and after test days. Acute NKCC1 inhibition did not alter the magnitude of light-signaled phase delays. However, NKCC1 inhibition in the late subjective night decreased the magnitude of photic phase advances and when injected alone led to substantial phase delays. These data suggest that endogenous excitatory SCN responses to GABA in the late subjective night may advance the behavioral rhythm.

Disclosures: **J.K. McNeill:** None. **J.C. Walton:** None. **H.E. Albers:** None.

Poster

123. Other Transporters

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 123.08/B61

Topic: B.05. Transporters

Title: Functional consequences of a loss-of-function mutation in the SLC39A8 (ZIP8) gene in NMDA receptor signaling

Authors: ***M. L. WEBER**[1,2], P. O'DONNELL[1,2], T. A. LANZ[1,2], V. REINHART[1,2], D. L. BUHL[1,2], D. BERTRAND[4], C. R. SCHUBERT[1,3];
[1]Pfizer Worldwide Res. & Dev, Cambridge, MA; [2]Neurosci. & Pain Res. Unit, [3]Pharmatherapeutics Clin. Res., Pfizer, Cambridge, MA; [4]HiQscreen, Geneva, Switzerland

Abstract: The trace element zinc (Zn2+) is found in most all tissues throughout the body, including the CNS, and is known to modulate a variety of ligand- and voltage-gated ion channels such as NMDA, GABAA, calcium, and potassium channels. In the CNS, Zn2+ concentration is highly regulated in cells and at the synapse by a number of transporters including ZnT and ZIP transporters. Zn2+ may be best known for its potent (low nM) inhibition of NMDA receptors,

specifically allosteric modulation of GluN2A subunit-containing receptors. Genome wide association studies on schizophrenia patients have recently implicated a mutation (A391T) in the SLC39A8 gene, which encodes the ZIP8 Zn2+ transporter. Our data show that this mutation, which resides in a coding region, results in a partial loss-of-function in the Zn2+ transport (or see SFN poster - Schubert & Bertrand). To elucidate the role of ZIP8 in NMDA-mediate currents at the synapse, we created SLC39A8 shRNA to selectively knock down ZIP8-mediated Zn2+ transport in primary rat cortical co-cultures and measured NMDA-mediated current density in pyramidal neurons using whole-cell patch clamp. Following ~75% ZIP8 mRNA knockdown measured by RT-PCR, we observed an apparent paradoxical increase in NMDA current density. In another set of studies measuring spontaneous synaptic activity (sEPSCs) in rat cortical neurons, we observed a decrease in NMDAR-mediated sEPSC amplitude after ZIP8 shRNA knockdown, indicating that loss of the ZIP8 transporter at cortical synapses has a negative effect on glutamatergic signaling. We hypothesize that this mutation is a loss-of-function in ZIP8-mediated transport, impairing Zn2+ uptake and contributing to NMDA receptor hypofunction observed in schizophrenia.

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Poster

123. Other Transporters

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Topic: B.05. Transporters

Support: Croatian Science Foundation Grant 09.01/414

Jane and Aatos Erkko Foundation

ERA-NET NEURON II CIPRESS (Academy of Finland)

Title: Developmental expression patterns of KCC2 and functionally-associated molecules in the human brain

Authors: M. PUSKARJOV[1], G. SEDMAK[2], N. JOVANOV-MILOŠEVIC[2], M. ULAMEC[3], B. KRUŠLIN[3], *K. KAILA[1], M. JUDAŠ[2];
[1]Univ. Helsinki, SF-00014 Helsinki, Finland; [2]Univ. of Zagreb Sch. of Medicine, Croatian Inst.

for Brain Res., Zagreb, Croatia; [3]Univ. of Zagreb Sch. of Medicine, Clin. Hosp. Ctr. Sisters of Mercy, Zagreb, Croatia

Abstract: Work on rodents has shown that steep up-regulation of KCC2, a neuron-specific Cl-extruder of the SLC12 cation-chloride cotransporter (CCC) family, commences in supraspinal structures at around birth, leading to the establishment of hyperpolarizing GABAergic responses. KCC2 is also a structural protein necessary for spinogenesis in cortical principal cells. Here, we describe the spatiotemporal expression profiles of the entire SLC12 gene family, using microarrays, in the human cerebrum, thalamus and cerebellum. Translation of KCC2 was validated by immunohistochemistry. KCC2 mRNA was observed already at the 10th postconceptional week (PCW) in the amygdala, cerebellum and thalamus. KCC2-immunoreactive neurons were abundant in the neocortical subplate at 18 PCW. Commencing at 19-24 PCW, most subplate and cortical plate neurons became KCC2-positive by 25 PCW. The mRNA expression profiles of Na-K ATPase α and β isoforms as well as TrkB were consistent with what is known from studies on rodents about their interactions with KCC2. Thus, in the human brain, expression of KCC2 and its functionally-associated proteins begins in the early fetal period. Our work facilitates translation of results on CCC functions from animal studies to the human and, more specifically, refutes the view that the poor efficacy of anticonvulsants in the term human neonate is attributable to a lack of KCC2.

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Poster

123. Other Transporters

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 123.10/B63

Topic: B.05. Transporters

Title: Deciphering ZIP8 activity using an electrophysiology reporter assay

Authors: *C. R. SCHUBERT[1], T. D. HELTON[3], J. A. ALLEN[2], J. R. WENDLAND[2], P. O'DONNELL[2], D. BERTRAND[4];
[1]PTx Clin. Res., [2]Pfizer, Cambridge, MA; [3]Brown Univ., Providence, RI; [4]HiQScreen, Geneva, Switzerland

Abstract: Zinc (Zn^{2+}) is the second most important metal ion in the body, and its tight regulation is a key element in the functioning of the central nervous system. Best known for its intracellular

modulation of many biochemical events, including the regulation of transcription or modulation of enzymes, Zn^{2+} also plays an important role in the modulation of ion channels such as N-methyl-D-aspartic acid receptors (NMDA), gamma-aminobutyric ($GABA_A$) ligand-gated receptors, or voltage-gated calcium channels (VGCC). To date, Zn^{2+} activity has been monitored mainly using radioactive or fluorescent measurements, both methods with intrinsic limitations. The observation that activity of the voltage-gated calcium channel $Ca_v1.2$ is exquisitely modulated by intracellular Zn^{2+}, however, offers novel possibilities to investigate Zn^{2+} activity using an electrophysiological reporter assay. Here, we investigate at the single cell level properties of the zinc transporter *SLC39A8* (ZIP8) and its naturally occurring mutant A391T that was found to be associated with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Monitoring ZIP8 activity using $Ca_v1.2$ as a reporter assay revealed that increase in the submicromolar range of Zn^{2+} in the extracellular fluid is sufficient to trigger a rapid inhibition of calcium channel activity. These findings indicate that augmentation of the intracellular Zn^{2+} occurs with a time constant in the seconds range and reveals both the efficiency of ZIP8 activity and its sensitivity to Zn^{2+}. In addition, these data also demonstrate the functional relevance of intracellular free Zn^{2+} on ion channel activity. Measurements conducted at the naturally occurring ZIP8 mutant A391T confirmed that this mutation alters Zn^{2+} transport that could, at least in part, be contributing to the origin of neuropsychiatric disorders. Offering several advantages, the use of a voltage-gated ion channel as reporter assay for the intracellular Zn^{2+} concentration allows for the first time to investigate the role of this divalent cation in the vicinity of the plasma membrane. Placed in perspective of intracellular recordings conducted *in vitro* and *in vivo*, this work illustrates the determinant role of this often neglected metal ion and its possible contribution in the manifestation of neuropsychiatric and neurological diseases.

Disclosures: **C.R. Schubert:** A. Employment/Salary (full or part-time):; Pfizer, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc.. **T.D. Helton:** None. **J.A. Allen:** A. Employment/Salary (full or part-time):; Pfizer, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc.. **J.R. Wendland:** None. **P. O'Donnell:** A. Employment/Salary (full or part-time):; Pfizer, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer, Inc.. **D. Bertrand:** None.

Poster

123. Other Transporters

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 123.11/B64

Topic: B.05. Transporters

Title: SorLA trafficking in physiological barriers

Authors: ***M. S. NIELSEN**[1], S. C. KLINGER[1], A. HØJLAND[1], P. MADSEN[1], J. BONIFACINO[2];
[1]Univ. of AARHUS, Aarhus, Denmark; [2]NIH, Washington, DC

Abstract: Cells throughout the body are polarized in order to achieve and support the specialized functions of the organs. Epithelial and endothelial cells are essential in maintaining the barriers separating different parts of the body, like the brain endothelial cells in the blood-brain barrier, epithelial cells in plexus choroideus and epithelial cells in the intestine. Cells like astrocytes and neurons are polarized having end-feet, axons and dendrites. To generate and maintain polarized structures, cells express a subset of specialized cytosolic adaptor proteins that facilitates asymmetric trafficking of receptors and their cargo. The adaptor proteins bind to motifs in the cytosolic domain of the receptors, thereby guiding them to their appropriate polarized cellular destination The Vps10p domain receptor SorLA, is a type I sorting receptor that harbors several functional adaptor binding motifs in its short cytosolic domain. Using MDCK cells as an epithelial model and primary hippocampal neurons, we have demonstrated that SorLA displays a polarized localization in these cell types. In a tight epithelial MDCK cell barrier, SorLA is localized on the basolateral membrane from where it is sorted to apically-localized endosomes. Using antibodies, we have furthermore demonstrated that SorLA mediates transcytosis across the MDCK barrier. In neurons, SorLA is localized throughout the dendrites but not found the axons. Using yeast 2-hybrid screens and expression of cytosolic mutated SorLA constructs, we are now mapping the sites and some of the adaptor proteins involved in polarized localization and sorting. Although there are some redundancies in the signals, preliminary results points to an extended acidic cluster in SorLA as the main actor in the observed polarized sorting. In addition, we find that the adaptor complex AP-1 is involved in the asymmetric sorting. Although there is evidence that epithelial and endothelial cells as well as neurons use a similar cytosolic sorting machinery to orchestrate polarized organizations, there are also some differences. We have recently found that SorLA is expressed in primary brain endothelial cells and we are currently investigating whether SorLA shows a similar polarized trafficking in these cells.

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Poster

124. Presynaptic Structure and Neurotransmitter Release II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 124.01/B65

Topic: B.06. Neurotransmitter Release

Title: Calcium dynamics in boutons of layer V pyramidal neurons in the rat somatosensory cortex

Authors: *L. TRAN[1], C. STRICKER[1,2];
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Abstract: In response to stimulation, a transient rise in intracellular [Ca^{2+}] causes transmitter release from presynaptic terminals. As presynaptic Ca^{2+} dynamics can affect the efficacy, reliability, and plasticity of synaptic connections, it is essential to understand Ca^{2+} homeostasis in nerve terminals. **Objective:** Ca^{2+} dynamics associated with action potentials (APs) was investigated in boutons of layer V pyramidal neurons in the rat somatosensory cortex. **Methods:** Brain slices were obtained from male Wistar rats (P15-20). Layer V pyramidal cells were filled through the patch pipette with Alexa Fluor 568 (50 μM) and various concentrations of Oregon Green 488 BAPTA-1 (OGB-1, K_D = 205 nM; 40, 80, 120, 240 μM) or OGB-6F (K_D = 3 μM; 50, 100, 200 μM). Imaging was done at 35 ± 1 °C, using a laser-scanning confocal microscope equipped with a 40x 1.0 NA objective. For each bouton, three increases in [Ca^{2+}] were measured: one evoked by an AP and two by AP trains of different frequencies (OGB-1: 10-40 APs at 10-40 Hz, OGB-6F: 60-80 APs at 80-100 Hz). APs were evoked with 2 ms somatic current injections. [Ca^{2+}] increases caused by AP trains were used to determine the maximal fluorescence at saturating [Ca^{2+}]. All calculations of [Ca^{2+}] from fluorescence intensity were done according to a published method (1). **Results:** Analysis of Ca^{2+} transients measured from fluorescence of OGB-1 and OGB-6F under steady-state conditions (> 90 min after break-in) shows similar results (p = 0.1-1.0); thus, data with these two dyes are pooled (94 boutons, 34 cells). The free volume-averaged [Ca^{2+}] is 43 ± 7 nM at rest and rises by 820 ± 320 nM immediately after an AP. The ratio of endogenous buffer-bound Ca^{2+} to free Ca^{2+} measured under steady-state conditions is 66 ± 27, similar to that measured during OGB-1 loading (< 60 min after break-in; 73 ± 19, 9 boutons, 9 cells). This suggests that the majority of endogenous buffers in these boutons are fixed, while mobile buffers contribute minimally. In addition, the total [Ca^{2+}] increase immediately after an AP is calculated to be 55 ± 31 μM, corresponding to influx through 20 ± 10 voltage-gated Ca^{2+} channels. The decay time constant of Ca^{2+} transients evoked by an AP is 37 ± 15 ms, indicating an extrusion rate of 1800 ± 1000 s^{-1}. These values for Ca^{2+} increases, endogenous Ca^{2+} binding ratio, and clearance kinetics are similar to those in boutons of layer II/III pyramidal neurons (2). **Conclusion:** There exists a large variation in presynaptic Ca^{2+} dynamics. This may reflect target cell-specific differences in transmitter release properties. 1. M. Maravall, Z.F. Mainen, B.L. Sabatini, K. Svoboda, *Biophys. J.* **78**, 2655 (2000). 2. H.J. Koester and B. Sakmann, *J. Physiol.* **529**, 625 (2000).

Disclosures: **L. Tran:** None. **C. Stricker:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 124.02/B66

Topic: B.06. Neurotransmitter Release

Support: NIH Grant NS075506

Title: Acute inhibition of the ubiquitin signaling system induces changes in the synaptic ubiquitome and alters synaptic transmission in mammalian neurons

Authors: *A. CAPUTO[1], K. M. MYERS[1], A. A. VASHISHT[2], Y. WU[3], J. WOHLSCHLEGEL[2], F. E. SCHWEIZER[1];
[1]Neurobio., [2]Biol. Chem., [3]Dept. of Anesthesiol., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: The ubiquitin signaling system (USS) regulates several cellular processes including synaptic development and physiology. Available data indicate that proteasomal degradation of synaptic proteins can profoundly impact synaptic function. Much less is known about the non-degradative roles of protein ubiquitination at the synapse. Here we show that in rat primary cortical neurons inhibition of *de novo* ubiquitination or inhibition of deubiquitination acutely and rapidly affect synaptic activity. In particular, we found that both inhibitions strongly augment the frequency of minis and drastically diminish the amplitude of evoked responses. To identify the molecular mechanisms involved, we used a quantitative proteomic approach in synaptoneurosomes derived from primary neurons. We thus defined a set of synaptic proteins that change their ubiquitination levels following acute USS inhibition. In addition, we validated some of the hits of our proteomic analysis and provide evidence that ubiquitination modulates synaptic transmission by affecting the levels of SNARE complexes, the microtubule cytoskeleton and the activity of CaMKII. Our results highlight new functions of protein ubiquitination at synapses and pave the way for a better understanding of the complex role of this post-translational modification in governing neuronal health and disease.

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Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Research to Prevent Blindness Senior Scientific Investigator Award (WBT)

Title: G-protein beta-gamma subunits regulate synaptic vesicle exocytosis by cone photoreceptors

Authors: *M. VAN HOOK[1], Z. ZURAWSKI[2], H. E. HAMM[2], W. B. THORESON[1]; [1]Ophthalmology & Visual Sci., Univ. of Nebraska Med. Ctr., Omaha, NE; [2]Dept. of Pharmacol., Vanderbilt Univ. Med. Sch., Nashville, TN

Abstract: The earliest stages of visual processing in the vertebrate retina are under dynamic control by a variety of G-protein coupled receptors (GPCRs) that regulate gap junctional coupling, voltage-dependent currents, and synaptic transmission. As at other synapses, G-protein α subunit (Gα)-dependent signaling pathways act on voltage-gated Ca2+ currents (ICa) to regulate neurotransmitter release by rod and cone photoreceptors. Synaptic output can also be influenced by the G-protein βγ complex (Gβγ), which modulates ICa and K+ currents and can directly interact with the vesicle fusion machinery (SNARE complex). Because it is unknown whether or how Gβγ influences photoreceptor exocytosis, we tested for direct effects of Gβγ on synaptic transmission by cone photoreceptors in the vertebrate retina. Experiments were performed using whole-cell voltage-clamp recordings in vertical slices of retinas from tiger salamanders (Ambystoma tigrinum). We measured synaptic transmission using paired recordings of cones and post-synaptic horizontal cells and capacitance measurements from cones. Gβ1γ1 subunits (100 nM - 2 μM) were introduced directly into the cone via the whole-cell patch pipette. For control recordings, we boiled the Gβ1γ1 stock solution for >20 minutes before diluting to the working concentration. ICa was recorded with a P/8 leak subtraction protocol. Spontaneous glutamate transporter current amplitude and kinetics were measured to test for effects of Gβ1γ1 on quantal content and vesicle fusion mode. In paired whole-cell recordings, dialyzing cones with Gβ1γ1 caused a gradual, dose-dependent reduction (IC50 = 80 nM) in the cone-driven

responses recorded from post-synaptic horizontal cells. Consistent with an inhibition of vesicle fusion rather than vesicle glutamate content or a switch in fusion mode, Gβ1γ1 also caused a reduction in exocytosis measured with whole-cell capacitance recordings. Gβ1γ1 had no effect on quantal glutamate transporter current amplitude or kinetics, but did cause a slight reduction in spontaneous event frequency. The reduction in exocytosis was not the result of changed ICa properties, as Gβ1γ1 altered neither the amplitude nor voltage-dependence of cone ICa. The use of voltage-clamp recordings precluded potential effects of Gβγ on K+ currents from altering the presynaptic voltage-response. Thus, Gβγ subunits appear to inhibit exocytosis by cone photoreceptors by a mechanism downstream of ICa. This suggests that Gβγ subunits in photoreceptors can directly inhibit synaptic transmission by acting on the vesicle fusion machinery.

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Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Topic: B.06. Neurotransmitter Release

Support: NIH Grant EY10291

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NIH Grant T32

Title: Modulation of synaptic transmission by Gbetagamma: specificity of Gbeta and Ggamma to inhibitory adrenergic A2a receptor and SNARE

Authors: ***Y. YIM**[1], K. BETKE[1], H. MCDONALD[2], R. GILSBACH[3], Y. CHEN[4], K. HYDE[1], Q. WANG[4], L. HEIN[3], K. SCHEY[2], H. HAMM[1];
[1]Pharmacol., [2]Biochem., Vanderbilt Univ., Nashville, TN; [3]Univ. of Freiburg, Freiburg, Germany; [4]Univ. of Alabama at Birmingham Sch. of Med., Birmingham, AL

Abstract: The modulation of neurotransmitter exocytosis by activated Gi/o-type G-protein coupled receptors (GPCRs), such as the α_{2a} adrenergic receptor (α_{2a}-ARs), is a universal regulatory mechanism used both to avoid overstimulation and to influence circuitry. Upon the activation of α_{2a}-ARs, Gβγ are released to directly interact with soluble N-ethylmaleimide-

sensitive factor attachment protein receptor (SNARE) to inhibit neurotransmitter release. There are 5 Gβs and 12 Gγs, and it is not known whether specific Gβγs are activated by a given GPCR. Presynaptic α_{2a}-ARs in both adrenergic (auto α_{2a}-ARs) and non-adrenergic neurons (hetero α_{2a}-ARs) work in a similar manner to inhibit neurotransmitter release and have various physiological functions such as anesthetic sparing and working memory enhancement. It has been shown that synaptic regulatory mechanisms can go awry in various neurological disorders such as anxiety and working memory deficits. In this project, we are investigating whether autoreceptors in sympathetic neurons use the same Gβγ subunits as heteroreceptors in other neuronal types. We use several mice models including transgenic Flag-α_{2a}-ARs, knock-in HA-α_{2a}-ARs, and wild-type mice. The Gβγ specificity is determined by co-immunoprecipitating Gβγ with α_{2a}-ARs and SNARE from synaptosomes, followed by mass spectrometry analysis. We hypothesize that specific Gβγ subunits interact with activated auto- and hetero α_{2a}-ARs and inhibit exocytosis by interacting with SNARE. We have identified Gβ1, Gβ2, Gβ4, Gβ5, Gγ2, Gγ3, Gγ4, Gγ7, Gγ12, and Gγ13 which interact with α_{2a}-ARs and SNARE. Out of these G protein, Gβ2, Gγ4, and Gγ13 preferentially interact with activated auto α_{2a}-ARs. We found no difference in Gβγ specificity to SNARE following α_{2a}-ARs activation. Further understanding Gβγ specificity may offer new insights into the normal functioning of the brain, as well as better understanding of disease progression. The Gβγ-SNARE interaction may be a new therapeutic target to modulate exocytosis in neural disorders in combination with drugs targeted to Gi/o-type GPCRs.

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Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Support: GGNB Excellence Stipend

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Title: A usage-dependent mechanism measures synaptic vesicle age and prevents the use of old vesicles in synaptic transmission

Authors: *S. TRUCKENBRODT[1], S. O. RIZZOLI[2], A. VIPLAV[2], E. F. FORNASIERO[2], A. DENKER[2];
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Abstract: Old organelles can become a hazard for cellular function, due to the accumulation of damaged molecules. Mechanisms that measure organelle age, and prevent old organelles from participating in cellular reactions, are therefore necessary. The prevaling assumption is that organelles are funtionally employed in cells until degradation via damage response mechanisms. We have identified an additional mechanism, which measures the usage of synaptic vesicles and functionally inactivates them long before degradation. Using cultured hippocampal neurons, we found that newly synthesized vesicles are preferentially employed in neurotransmitter release. They recycle only ~270 times, over ~24 hours. During recycling they become contaminated with a molecule from the plasma membrane, which interferes with a vital component of the synaptic vesicle release machinery. This process renders the old vesicles less competent to release than their newly synthesised counterparts. The inactivation can be accelerated by increasing the activity of neurons, suggesting that the cell uses this mechanism to directly measure the number of times a vesicle has been used, rather than its age. The old vesicles are eventually targeted for degradation, but their functional inactivation precedes degradation by several days. We conclude that the contamination is a timing mechanism needed to ensure that old vesicles are not used in neurotransmitter release. Synaptic transmission is presumably too sensitive to tolerate accumulation of damage on synaptic vesicles until they can be recognised by classical damage response mechanisms, necessitating this additional functional inactivation.

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Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Program#/Poster#: 124.06/B70

Topic: B.06. Neurotransmitter Release

Support: CIHR

CRC

Title: Intersectin1 is required for developmental enhancement of Ca^{2+}-dependent replenishment of the readily-releasable synaptic vesicles

Authors: *Y. YANG, A. SENGAR, J. AITOUBAH, S. EGAN, M. SALTER, L.-Y. WANG; The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Intersectin1 (Itsn1) is an evolutionally conserved scaffold protein engaged in recruiting dynamin and other accessory proteins for clathrin-mediated endocytosis, and hence a key molecular substrate for the coupling between exocytosis and endocytosis. However, it remains unknown if Itsn1 is required for development of synaptic functions in mammalian central synapses. To address this issue, we examined synaptic properties of the developing calyx of Held synapse in the auditory brainstem from wild-type (WT) and Itsn1 knockout (KO) mice at different postnatal (P) stages (immature P8-10 vs mature P16-20). Immunohistochemical analyses showed that Itsn1 proteins co-localized with a presynaptic marker vGlut1 in WT synapses for both age groups but completely absent in Itsn1 KO synapses. By stimulating presynaptic axons, we found that deletion of Itsn1 had little effect on basal excitatory synaptic transmission or short-term depression (STD) as a result of depletion of synaptic vesicles (SVs) from the readily-releasable pool (RRP) in immature and mature synapses. In contrast, the fast Ca2+-dependent component of recovery from STD was selectively attenuated in mature Itsn1 KO synapses but remained unaltered in immature Itsn1 KO synapses. Surprisingly, blockade of the interactive partner of Itsn1, dynamin, with specific inhibitors did not slow down the recovery time course at the mature WT synapses. These results indicate that the fast Ca2+-dependent replenishment of SVs in the RRP to the depleted sites is mediated by distinct mechanisms at different developmental stages, being strongly enhanced by Itsn1-dependent and dynamin-independent signaling during synapse maturation.

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Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Topic: B.06. Neurotransmitter Release

Support: Neurocure Excellencecluster

Title: The role of synaptotagmin-1 C2B domain in synaptic vesicle docking in hippocampal neuron

Authors: *S. CHANG, T. TRIMBUCH, C. ROSENMUND;
Neurocure, Charité - Universitätsmedizin Berlin, Berlin, Germany

Abstract: Synaptotagmin-1 (Syt-1) is a Ca2+ sensor for neurotransmitter release. Whether it plays a role in vesicle docking remains unclear. While studies examining the ultrastructure of presynaptic terminal in Syt-1 knock-out (KO) mice using conventional chemical fixation show unaltered docked vesicle number (Geppert et al, 1999), those using the high-pressure freezing (HPF) for fixation show a 39%-50% reduction in docked vesicle number (Liu et al, 2009; Imig et al 2014). Using HPF technique, we indeed found that the docked vesicle number was reduced by 53% in Syt-1 knockout mass culture neurons, while the total number of vesicles within 100 nm of the active zone was not significantly altered. Furthermore, we utilized mutations in three regions of the C2B domain of Syt-1 that severely impair vesicle exocytosis by 70%-95% (Li et al 2006; Xue et al 2008; Nishiki et al, 2004) to investigate whether there is a link between exocytosis and vesicle docking. We expressed the mutant proteins in autapic/mass cultured Syt-1 KO hippocampal neuron, and surprisingly found that mutating either the residues at polybasic stretch (K325, K327) or at the bottom of the C2B domain (R398, R399) led to a reduction of docked vesicle by 49% to 63% compared to wild-type synapses. In contrast, while mutating residues for Ca2+ binding in the top loop (D309, D363, D365) did not affect vesicle docking. However, none of the mutants displayed a difference in the primed vesicle number as assayed by sucrose-mediated response in autapic culture. This argues against the notion that functional priming of vesicles from the electrophysiology results is the same process as morphology docking in the EM images. Subsequently, we investigated whether Ca2+ entry influences vesicle docking by using flash and freeze technique to resolve changes in vesicle-plasma membrane distance upon action potential triggering. Surprisingly, we found an increased number of vesicles in the proximity of active zone after Ca2+ influx on the timescale of milliseconds, suggesting that the Ca2+ induced phospholipid binding of C2B may be capable of compensating for the loss of membrane tethering before Ca2+ influx. These results indicate 1) Syt-1 regulates synaptic docking via its side and bottom loops of C2B domain, 2) vesicle priming and docking are not necessary linked and 3) Ca2+ triggering may induce vesicle docking via its Ca2+ dependent phospholipid binding ability of top loop of C2B domain.

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Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Support: NRSA NS080605 to KG

NIH Grant #NS44421 to PS

Karolinska Institute Fellowship to MI

Title: The role of SNAP-25 in presynaptic mechanisms of long-term plasticity of vesicular release at glutamatergic synapses in the hippocampus

Authors: *K. R. GOPAUL[1], M. IRFAN[1,2], X.-L. ZHANG[1], C. BARK[2], P. K. STANTON[1]; [1]Cell Biol. and Anat., New York Med. Col., Valhalla, NY; [2]Dept. of Mol. Med. and Surgery, Rolf Luft Ctr. for Diabetes Res. & Endocrinol., Karolinska Inst., Stockholm, Sweden

Abstract: Two glutamate receptors, N-methyl-D-aspartate receptors (NMDAR) and group II metabotropic glutamate receptors (mGluRII), can independently induce long-term depression (LTD) of presynaptic transmitter release at hippocampal synapses. Recent work from our laboratory indicates that presynaptic LTD involves the interaction of a G protein with one of the SNARE proteins, synaptosomal-associated protein 25 (SNAP-25). LTD, but not long-term potentiation (LTP), is significantly impaired when the 9 amino acid c-terminal region of SNAP-25 is cleaved, suggesting that a differential interaction with SNAP-25 is required for the coordination of these distinct forms of long-term, activity-dependent presynaptic plasticity. Elevation of cyclic guanosine monophosphate (cGMP) by NMDAR-mediated generation of the intercellular messenger nitric oxide, when paired with Gα liberated by activation of G protein-coupled receptors such as mGluRIIs, is sufficient to induce LTD. Given that Gα and Gβγ are co-released by activation of G protein-coupled receptors, we hypothesized that elevated [cGMP], inhibition of PKA by Gαi, and binding of Gβγ to the C-terminus of SNAP-25, are all in a common final pathway of NMDAR and mGluRII induction of presynaptic LTD of vesicular glutamate release. We tested this hypothesis by monitoring changes in vesicular release from rodent hippocampal Schaffer collateral synapses with the fluorescent membrane dye, FM1-43, using two-photon laser scanning microscopy. In SNAP-25 transgenic mice that express only an immature form of SNAP-25 (SNAP-25a) and not the mature form (SNAP-25b), we found that paired-pulse facilitation and presynaptic transmitter release from the sucrose-loaded readily-releasable vesicle pool were reduced compared to wildtype littermate control mice. SNAP-25b deficient mice also showed impairments in both LTD and LTP of synaptic strength. We are examining the relative expression of NMDAR and mGluRII-dependent forms of LTD in these mice, and how blocking components of each pathway alters the expression of LTD of vesicular

release. Our findings suggest that synaptic plasticity that plays an important part in learning and memory is functionally modulated by isoforms of the SNARE protein SNAP-25, and that both LTP and LTD can be influenced by this protein.

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Poster

124. Presynaptic Structure and Neurotransmitter Release II

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MOST

DOE 2011-program

Title: Mechanisms of calcium independent but voltage dependent secretion (CiVDS) in somata of mammalian primary sensory neurons

Authors: *Z. ZHOU[1], Z. CHAI[1], X. ZHANG[1], R. HUANG[1], Y. WANG[1], R. ZHOU[1], Q. WU[1], M. HU[1], X. WU[1], H. LIU[2], T. LIU[1], Y. WANG[1], W. GUO[1], H. ZHENG[1], W. XIONG[1], C. ZHANG[1], J.-P. DING[2], C. WANG[1];
[1]Inst. Mol. Med, Peking Univ., Beijing, China; [2]Inst. of Biophysics and Biochemistry, Sch. of Life Sciences, Huazhong Univ. of Sci. and Technol., Beijing, China

Abstract: The somata of primary sensory neurons, including dorsal root ganglion (DRG) neurons, release neurotransmitters and neuropeptides. Following physiological action potentials, in addition to Ca^{2+}-dependent secretion, we have discovered and studied Ca^{2+}-independent but voltage-dependent secretion (CiVDS) in somata of freshly isolated DRG neurons (Zhang et al, 2002, 2004; Zheng et al, 2009, Liu et al, 2011). Major open question of CiVDS is molecular mechanism, including 3 components: fusion pore machinery (FP), voltage sensor (VS) and the FP-VS linker (LK). Here we report, by using exocytosis assays of patch-clamp recording of membrane capacitance, and single vesicle imaging (EM and TIRF), (1) FP is jointly contributed by 2 components of FP-complex SNARE, SNAP25 and syntaxin; (2) VS is jointly contributed by 2 voltage-gated Ca channels (VGCCs), Cav2.2 (N-type VGCC) and Cav1.3 (L-type VGCC); (3) LK is the “synprint”, Cav-S2-S3 intracellular loop718-963aa (Catterall, 1999); (4) following

automatic knockdown of CiVDS by 3d-culture of DRG neurons, CiVDS is rescued by overexpressing any component of FP (SNAPE25or syntaxin) or VS (Cav2.2 or Cav1.3); (5) CiVDS is inhibited by blockers against FP (SNAP25 or sytaxin), Cav(2.2 or 1.3) and FL (synprint-truncated); (6) finally, CiVDS is blocked by Cav2.2-RNAi-KD in DRG pre-transfected *in vivo*. *Supported by grants from NSFC, MOST and DOE 2011-program*

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Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Topic: B.06. Neurotransmitter Release

Support: NIH Grant 1R01NS082759

Title: Facilitation of vesicle endocytosis by rho kinase at immature calyx of held synapses

Authors: H. YUE, L. LI, A. SATHYAMURTHY, L. MEI, *J. XU;
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Abstract: A kinase closely related to cellular cytoskeleton, Rho kinase (ROCK) affects synapse development and neuronal functions. In the calyx of Held synapses after maturation, vesicle endocytosis can be accelerated by retrograde activation of ROCK by nitric oxide released from postsynaptic cells, which involves presynaptic cyclic GMP-dependent protein kinase (PKG) and phosphatidylinositol-4,5-bisphophate (PIP2). Because this pathway of retrograde activation is suggested to function only in mature calyces, a role of ROCK in endocytosis at immature synapses remains to be determined. This possible regulation of synaptic endocytosis was now explored at the calyces from postnatal 7-11 days old rat pups by performing the whole-cell membrane capacitance measurement to monitor endocytosis. In our tests, pharmacological inhibition of ROCK activity led to slower endocytosis. Elevation of intracellular PIP2 or block of NMDA receptor did not influence effects of ROCK inhibition, excluding the involvement of PKG and PIP2. In addition, the slow calcium chelator, EGTA, did not generate synergistic effect when combined with ROCK inhibitors. These observations suggest that ROCK can regulate endocytosis in immature synapses by a potentially calcium-dependent and PKG-independent

pathway. It is possible that ROCK participates in regulation of endocytosis in both immature and mature synapses, but using different molecular mechanisms for its activation.

Disclosures: **H. Yue:** None. **L. Li:** None. **A. Sathyamurthy:** None. **L. Mei:** None. **J. Xu:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Topic: B.06. Neurotransmitter Release

Support: DFG grant SFB889

NIH Grant EY017836

Title: Unique role of complexin in shaping the biphasic response at a mammalian ribbon synapses in the rod pathway

Authors: ***L. S. MORTENSEN**[1], J. KE[2], K. REIM[1], C. IMIG[1], B. H. COOPER[1], E. KALOGERAKI[3], S. LÖWEL[3], N. BROSE[1], J. H. SINGER[2], J. RHEE[1];
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Abstract: At the rod bipolar cell (RBP) to amacrine AII cell ribbon synapse in the inner retina, contrast and luminance is encoded in the transient and sustained components of the response to sustained input. While it is well described that the partial depletion of the readily releasable pool (RRP) underlies this biphasic response, the molecular players involved in regulating vesicle release in RBP to AII synapses have not been investigated in depth. Complexins (Cplx) are synaptic proteins that exist in four isoforms in mammals. All isoforms bind to and stabilize SNARE complexes, and their deletion leads to disturbances in vesicle release. Cplx-3 is the only isoform found at RBP to AII cell ribbon synapses. We performed paired recordings from synaptically coupled RBP and AII cells in acute retinal slices from Cplx-3 genetic deletion mutants and wild type controls. While synchronous release was strongly reduced in Cplx-3 deletion mutants, we found that asynchronous, sustained and delayed release was unaltered. Studies regarding the clamping function of Cplx-1/2 in conventional synapses have caused some controversy, however, we do not report any sort of clamping function upon loss of Cplx-3.

Although the readily releasable pool (RRP) was significantly reduced, we still observed coordinated multivesicular release, which was not depressed by partial depletion of the RRP. This finding is in contrast to previous studies linking multivesicular release to a high initial release probability. Electron tomographic analysis suggests that Cplx-3 is not involved in tethering/docking of vesicles at the presynaptic active zone. By simulating physiological stimuli electrophysiologically and by behavioural analysis we show the importance of Cplx-3 in generating the biphasic response needed to faithfully encode contrast in the inner retina. Our data indicate that the release machinery driving synchronized and sustained/delayed release rely of different components, with complexin being significant only for the transient phase of release, and that the molecular configuration of the release machinery is crucial for signal processing in the retina.

Disclosures: **L.S. Mortensen:** None. **J. Ke:** None. **K. Reim:** None. **C. Imig:** None. **B.H. Cooper:** None. **E. Kalogeraki:** None. **S. Löwel:** None. **N. Brose:** None. **J.H. Singer:** None. **J. Rhee:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Title: Complexin increases the coupling of presynaptic calcium influx and quantal release

Authors: E. A. QUIROZ[1], *R. A. JORQUERA[1,2];
[1]Neurosci., Univ. Central Del Caribe, Sch. of Med., Bayamon, PR; [2]Physiol. and Biophysic Program, Univ. de Chile, Sch. of Medicine, Inst. of Biomed. Sci., Santiago, Chile

Abstract: At nerve terminals, the coupling between the Ca^{2+} influx and the quanta ready for release is critical for synaptic communication. However, the molecular mechanism by which the coupling occurs remains under investigation. Complexin binds the SNARE complex necessary for vesicle docking and fusion, preventing the spontaneous vesicle fusion and increasing a pool of quanta ready for release. In addition, Complexin speeds the timing for quantal release and may affect coupling. Nevertheless, Complexin function is still under debate. Here, using the *Drosophila* NMJ we investigate the role of Complexin coupling the Ca^{2+} influx and quantal release at complexin null ($cpx^{-/-}$). Kinetic analysis of glutamatergic EPSCs in the presence of

Cd^{2+}, Ca^{2+} channel inhibitor PLTXIIa, Sr^{2+} or intracellular Ca^{2+} buffers EGTA and BAPTA were explored at different frequency of nerve stimulation. Cd^{2+} and PLTXIIa display different effects in control and *cpx*$^{-/-}$ at the EC50 and EC75 values indicating a role of complexin in the sensitivity of Ca^{2+} influx in the release process. Sr^{2+} promotes asynchronous release in a higher extent in *cpx*$^{-/-}$ vs control, suggesting an increased pool or sensitivity of asynchronous quanta. Intracellular Ca^{2+} buffering with EGTA has a minor effect in control but suppresses the asynchronous release in *cpx*$^{-/-}$ without affecting the spontaneous release, confirming the role of complexin in coupling. As is expected, in null and control animals, BAPTA slows the EPSCs kinetics and decreases the spontaneous release in *cpx*$^{-/-}$. Our data is consistent with a modulatory role of complexin in the Ca^{2+} sensitivity of the release machinery. In addition, complexin increases coupling, probably by clamping the SNARE complex at the release site.

Disclosures: E.A. Quiroz: None. **R.A. Jorquera:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Title: Local nanometer organization and the function of PI(4,5)P2 during vesicle exocytosis

Authors: *C. JI[1], Y. ZHANG[2], T. XU[2], X. LOU[1];
[1]Univ. of Wisconsin-Madison, Madison, WI; [2]Natl. Lab. of Biomacromolecules, Inst. of Biophysics, Chinese Acad. of Sci., Beijing, China

Abstract: PI(4,5)P2 has been identified as an independent plasma membrane determinant inositol lipid that is essential for multiple cellular functions. However, their nanometer scale organization in native cell plasma membrane (PM) remains controversial and the exact function of PI(4,5)P2 in differential dynamic stages of exocytosis remains elusive. Using single-molecular super resolution imaging, photo-activatable PH-domain probes, and optogenetic tools, we

explored the structure and function of PI(4,5)P2 in different molecular interactions during vesicle trafficking. We examined different imaging approaches and variable conditions for PI(4,5)P2 nanoscale distribution, and we found relatively homogeneous distribution of PI(4,5)P2 in the large areas of PM. Moreover, using optogenetic tools, we successfully manipulated PI(4,5)P2 levels in live cells, we are aiming to dissect its interactions with different effectors proteins in exocytosis. Our preliminary data suggest PI(4,5)P2 regulated exocytosis at multiple steps, including docking, priming, and final membrane fusion via different mechanisms.

Disclosures: **C. Ji:** None. **Y. Zhang:** None. **T. Xu:** None. **X. Lou:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Whitehall Foundation

Title: Spontaneous and evoked recycling pools of synaptic vesicles in cultured striatal neurons of a DYT1 dystonia mouse model

Authors: *N. C. HARATA, S. IWABUCHI;
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Abstract: Body movement is severely affected in the brain disorder dystonia. Hallmarks of this disease are involuntary skeletal muscle contractions and abnormal postures. In spite of the fact that dystonia is the third most common movement disorder in humans, treatment options remain limited. In part this is because the mechanisms whereby dystonia affects brain functions remain unclear. One of the pathophysiological features of this disease is thought to be abnormal synaptic transmission, e.g. in the striatum, a brain region that plays an important role in motor control. We

previously reported that in DYT1 dystonia - the most common form of inherited dystonia - synaptic vesicle recycling is affected in the nerve terminals of cultured mouse striatal neurons. Using live-cell imaging of the vesicle-recycling indicator FM1-43 dye, we had demonstrated the presence of two vesicle pools, which were mobilized during spontaneous or evoked synaptic activities (spontaneous and evoked recycling pools, respectively). We further found that the size of spontaneous recycling pool was increased in mutant neurons that bear a TOR1A mutation that is present in human patients (i.e. in a heterozygous ΔE-torsinA knock-in mouse). We have extended the previous study, evaluating the changes in the evoked recycling pool and also the transfer of vesicles between the two pools. We found that in the mutant neurons, the size of the evoked recycling pool is slightly reduced relative to that in wild-type counterparts. Moreover, vesicles that were loaded with FM dye during a synaptic activity of one type later unloaded the dye during an activity of the other type, indicating that vesicles can be transferred from one pool to another. The amount of this transfer from the evoked to the spontaneous pools was higher than in wild-type neurons, and was lower in the opposite direction. These results suggest that the mutant neurons tended to rely more on the spontaneous than the evoked recycling pools. Overall, our findings demonstrate a high level of complexity in the influence of the dystonia-associated mutation on the presynaptic factors.

Disclosures: **N.C. Harata:** None. **S. Iwabuchi:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

Location: Hall A

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Program#/Poster#: 124.15/B79

Topic: B.06. Neurotransmitter Release

Title: Measuring glutamate transients in the cleft of individual Schaffer collateral synapses

Authors: ***C. D. DÜRST**, J. S. WIEGERT, C. SCHULZE, T. G. OERTNER;
Ctr. For Mol. Neurobio. Hamburg (ZMNH),, Hamburg, Germany

Abstract: Whether central synapses can release only a single vesicle, or multiple vesicles per action potential, or even sub-quantal amounts of glutamate, is still a matter of debate. It is possible to detect glutamate release indirectly by electrophysiological recordings of postsynaptic AMPA receptor mediated currents (EPSCs). It is not straightforward, however, to distinguish between multisynaptic connections and multivesicular release from a single synaptic site. In addition, due to cooperative glutamate binding, saturation, and desensitization of AMPA receptors, EPSC amplitudes are not linearly related to cleft glutamate concentrations. The

complex nature of postsynaptic responses makes it difficult to investigate the mode of transmitter release at central synapses. To directly monitor cleft glutamate during synaptic transmission, we took advantage of the genetically encoded membrane-bound glutamate sensor iGluSnFR that we expressed in CA3 pyramidal neurons through single cell electroporation. Fast two-photon imaging of single boutons in CA1 during propagation of single action potentials allowed us to optically measure glutamate release from individual release sites with high spatial and temporal precision. We show that our method is sensitive enough to detect the release of individual transmitter vesicles at a single presynaptic terminal. Release events could be mapped to an area smaller than the resolution limit of the microscope. By altering external calcium concentrations, we found that increased release probability was accompanied by increased glutamate concentrations in the synaptic cleft. This increase in glutamate concentration followed binomial statistics and most likely arose from the simultaneous release of several vesicles at a single release site. Multivesicular release was also observed at near-physiological calcium concentrations, suggesting that synaptic transmission at Schaffer collateral synapses is not limited to a single quantum per action potential and release site.

Disclosures: **C.D. Dürst:** None. **J.S. Wiegert:** None. **C. Schulze:** None. **T.G. Oertner:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Program#/Poster#: 124.16/B80

Topic: B.06. Neurotransmitter Release

Title: Synaptotagmin-1 and synaptotagmin-7 differ in their stimulus and Ca^{2+}-dependence of activation

Authors: ***T. RAO**[1], A. R. PELEMAN[1], D. R. GIOVANNUCCI[2], A. ANANTHARAM[1]; [1]Biol. Sci., Wayne State Univ., Detroit, MI; [2]Raymond & Beverly Sackler Lab. for Neuroendocrine Tumor Res., The Univ. of Toledo, Toledo, OH

Abstract: The secretory response of adrenal chromaffin cells varies in accordance with the stimulus applied. Mild stimulation favors limited release of granule cargo through a narrow fusion pore while strong stimulation leads to greater release through wide pore expansion. The trigger for release in these cells is a rise in intracellular Ca2+ through voltage-gated channels. The level of intracellular Ca2+ accumulation varies directly with the strength of depolarization. Ca2+ triggered exocytosis in chromaffin cells also requires the protein synaptotagmin (Syt) as a sensor. The two isoforms expressed in these cells - synaptotagmin-1 (Syt-1) and synaptotagmin-

7 (Syt-7) - have distinct Ca2+ affinities, are usually sorted to separate granules, and favor fundamentally different modes of exocytosis. Therefore, we wanted to determine whether changes in stimulation intensity drive release by selectively, or differentially, activating granules with Syt-1 or Syt-7. To address this question, we depolarized cells with varying levels of extracellular KCl. We found that while 56 mM KCl equally drove fusion of Syt-1 and Syt-7 granules, 10 and 25 mM KCl were far more effective at driving fusion of Syt-7 granules. To directly investigate Ca2+-dependence of Syt isoform fusion, we perfused permeabilized cells with Ca2+ buffered to different concentrations with EGTA. Both Syt granule populations fused effectively at highest concentrations of Ca2+ (30 μM and 100 μM), although there were small differences in their overall rate constants for activation. A greater fraction of docked Syt-7 granules fuse with significantly faster kinetics at a lower Ca2+ concentration (10 μM). Our results demonstrate chromaffin cells exploit Syt isoform diversity to modulate the secretory response. A significant consequence of synaptotagmin isoform segregation is that individual granules are endowed with unique Ca2+-sensitivities, allowing rapid and local control of the fusion mode based on local Ca2+ levels.

Disclosures: T. Rao: None. **A.R. Peleman:** None. **D.R. Giovannucci:** None. **A. Anantharam:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Topic: B.06. Neurotransmitter Release

Title: A chimeric approach to studying the Syntaxin Habc domain uncovers roles in exocytosis and Syntaxin trafficking

Authors: *L. A. PARRA, M. PALFREYMAN, E. JORGENSEN;
Univ. of Utah, Salt Lake City, UT

Abstract: Synaptic vesicle fusion is mediated by the concerted action of Munc18 and SNARE proteins. Munc18 has multiple binding modes with syntaxin. Key to understanding this interaction is the highly conserved three-helix bundle (known as the Habc domain) that is present on the SNARE protein syntaxin. This domain is the major interaction interface between Munc18 and the SNARE proteins. To test the role of the Habc domain and Munc18 proteins *in vivo*, we are analyzing different Habc domain variants using the nematode *C. elegans*. Specifically, we are exchanging the Habc domain of the worm with the respective Habc domains of other species.

Using this chimeric approach we hope to identify key components that will help to mechanistically characterize the interaction between the SNAREs and the Munc18 proteins. Previous evidence suggests the interaction of Munc18 and the SNAREs serves a role in trafficking and in exocytosis. Our evidence shows that replacing the worm Habc with the choanoflagellate Monosiga brevicollis (MoBr) Habc does not disrupt trafficking whereas the yeast Saccharomyces cerevisiae (SaCe) Habc domain does result in partial trafficking defects. Despite the correct localization, the choanoflagellate Habc chimera animals are still uncoordinated and have dramatic decrease in synaptic vesicle fusion -thus, demonstrating the important role for the Habc domain downstream of trafficking. Interestingly, the roles downstream of trafficking might result from specific interactions between the Habc domain and Munc18. Specifically, the inviability of yeast Habc chimeras can be rescued by expression of Sec1p, the yeast homolog of Munc18. In this combination the viability is restored independent of an observable restoration of wild-type syntaxin localization. I am currently performing further chimeric analyses to understand the physiological implication of syntaxin Habc domain interaction with Munc18. These experiments will parse the roles of Munc18 in the trafficking of syntaxin and the exocytosis of synaptic vesicles.

Disclosures: **L.A. Parra:** None. **M. Palfreyman:** None. **E. Jorgensen:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Support: NIH Grant MH084874

Title: Quantitation and simulation of presynaptic evoked calcium signals

Authors: *E. Y. HAMID[1], E. CHURCH[2], S. ALFORD[2];
[1]NIH, Bethesda, MD; [2]Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Presynaptic Ca^{2+} entry evokes neurotransmitter release, compensatory endocytosis, and short-term plasticity. The true spatiotemporal profile of Ca^{2+} entry and diffusion at synaptic molecular targets is difficult to determine, because imaging has limited resolution. To overcome these limitations, we used Ca^{2+} dyes as buffers to compute presynaptic Ca^{2+} signals and constructed models of diffusion and Ca^{2+} binding. Action potential-evoked Ca^{2+} responses in CA1 pyramidal cell varicosities were quantified using Ca^{2+} dyes as buffers. Peak transient

amplitudes (789 ± 39 nM) and decay times (119 ± 10ms), which showed little variation when measured with low affinity Fluo-5F, occurred within 2 ms of stimulation. A higher affinity Ca^{2+} dye (Fluo-4) was used to buffer Ca^{2+} transients. We determined endogenous Ca^{2+} buffering capacities, single action potential evoked free [Ca^{2+}] and total molar Ca^{2+} entering terminals. These results provided the basis of a Monte Carlo (MCell) simulation of Ca^{2+} entry, buffering and dispersal. Data were well fit using a model of Calbindin with two high and two moderate-rate binding sites (40 μM Calbindin). The model, while reproducing experimentally obtained data, demonstrates that Ca^{2+} enters varicosities with non-uniform distribution and diffused to fill the structure within 8 ms. During stimulus trains, free Ca^{2+} throughout the terminal showed super-linear summation from one period of entry to the next. At nm domains around primed vesicles Ca^{2+} peaks remained constant with repetitive stimulation as diffusion dominated. A kinetic model of synaptotagmin Ca^{2+} binding indicates that even with high (10 μM) total varicosity Ca^{2+} concentrations, synaptogmin must be with tens of nm of a channel but can be activated at greater distances with repetitive stimulation.

Disclosures: **E.Y. Hamid:** None. **E. Church:** None. **S. Alford:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Topic: B.06. Neurotransmitter Release

Title: Calcium-dependent activator protein for secretion function in murine dorsal root ganglion neurons

Authors: ***A. H. SHAIB**[1], M. KLOSE[2], R. MOHRMANN[2], J. RETTIG[2], U. BECHERER[2]; [1]Universty of Saarland, Physiol. Inst., Homburg, Germany; [2]Univ. of Saarland, Homburg, Germany

Abstract: Dorsal root ganglion (DRG) neurons transmit sensory information to the central nervous system through glutamatergic synaptic transmission that can be modulated by neuropeptides. They are contained in large dense core vesicles (LDCVs) and can be as diverse as calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), substance P, etc. LDCVs are exocytosed in response to specific stimulus at the cell somata and in afferent terminals along with synaptic vesicles (SVs). The release machinery of these LDCVs is poorly understood. Thus, we investigate the role of both isoforms of the priming the factor Calcium-dependent Activator Protein for Secretion (CAPS1 and CAPS2). Using PCR and immunocytochemistry, we verified

that both CAPS isoforms are expressed. With total internal fluorescence reflection microscopy, we visualize in real time the release of LDCVs that are labeled through Lenti virus driven expression of NPY-Venus. We found that the amount of secreting neurons was raised by 20% upon CAPS 1 or CAPS 2b overexpression and that the number of secreted LDCV per responding neuron was more than twice as high in comparison to WT control. CAPS1 and 2 double Knockout showed significant reduction in the number of secreted vesicles and 20% decrease in the amount of secreting cells was observed. Since the density of LDCVs at the plasma membrane was not affected by CAPS expression level, our result suggest that CAPS can be a priming factor for LDCVs in DRG-neurons. We also examine the role of CAPS in SV priming. For this we have established a DRG-dorsal horn neurons co-culture to allow synapse formation. Additionally, we designed a Lenti virus encoding for vGlut tagged to the pH sensitive fluorescent protein mNectarine to specifically label SVs and visualize their exocytosis. With these tools we are investigating whether CAPS plays a differential role in priming of LDCV vs. SV.

Disclosures: **A.H. Shaib:** None. **M. Klose:** None. **R. Mohrmann:** None. **J. Rettig:** None. **U. Becherer:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Title: The role of vti1a- and VAMP7-driven spontaneous vesicle fusion in synaptic plasticity

Authors: *D. C. CRAWFORD, D. M. O. RAMIREZ, B. TRAUTERMAN, L. M. MONTEGGIA, E. T. KAVALALI;
UT Southwestern Med. Ctr., Dallas, TX

Abstract: Spontaneous neurotransmission is a ubiquitous feature of neuronal synapses and occurs when synaptic vesicles fuse with the plasma membrane irrespective of the presence of action potentials. It is becoming increasingly apparent that this form of neurotransmission plays important roles in synaptic development and plasticity, for example in synaptic homeostasis. Prior studies probing the physiological functions of spontaneous neurotransmission, however, have been hampered by an inability to selectively manipulate vesicles destined for spontaneous fusion. Recently two vesicular soluble NSF attachment protein receptors (v-SNAREs), vps10p tail interactor 1a (vti1a) and vesicle-associated membrane protein 7 (VAMP7/TI-VAMP), were identified as specific facilitators of spontaneous vesicle fusion. To determine how spontaneous neurotransmission modulates synaptic function *in vitro* and *in vivo*, we used lentiviral and AAV vectors to deliver shRNAs against both of these proteins to rodent hippocampal neurons (hereafter referred to as double knockdown, or DKD). DKD in cultured rat hippocampal neurons significantly reduced the frequency of miniature AMPA receptor-mediated excitatory postsynaptic currents but increased their amplitudes, and these effects were rescued by co-expression of shRNA-resistant vti1a. Additionally presynaptic terminals from DKD cultures that were treated with tetrodotoxin took up significantly less antibody against the luminal domain of synaptotagmin 1. Spontaneous network activity, which requires action potential propagation in neurons, and inhibitory miniature postsynaptic current amplitudes were, however, unchanged after DKD. These results suggest that excitatory spontaneous neurotransmission specifically driven by these v-SNAREs is important for synaptic homeostasis but does not grossly alter action potential-driven activity. In brain slices from mice stereotaxically injected with an AAV driving DKD, axons from infected neurons exhibited reduced spontaneous uptake of antibody against the luminal domain of synaptotagmin 1 in a manner similar to DKD in cultured neurons. Using field potential recordings of Schaffer collateral synapses after CA3-specific injections of the DKD AAV, we observed that paired-pulse ratios and long-term potentiation were unaltered by DKD, suggesting that at least some short-term and long-term forms of synaptic plasticity were intact. Together our data suggest that vti1a and VAMP7 specifically modulate spontaneous vesicle fusion events *in vitro* and *in vivo*, and we are currently investigating how these proteins alter synaptic plasticity and animal behavior.

Disclosures: **D.C. Crawford:** None. **D.M.O. Ramirez:** None. **B. Trauterman:** None. **L.M. Monteggia:** None. **E.T. Kavalali:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Title: Quantitative analysis of vesicle recycling at the calyx of Held synapse

Authors: Q. ZHU, *X. QIU, J. SUN;
Inst. of Biophysics, Chinese Acad. of Sci., Beijing, China

Abstract: Vesicle recycling is pivotal for maintaining reliable synaptic signaling, but its basic properties remain poorly understood. Here, we developed an approach to quantitatively analyze the kinetics of vesicle recycling with exquisite signal and temporal resolution at the calyx of Held synapse. The combination of this novel electrophysiological approach with electron microscopy revealed that ~80% of vesicles (~270,000 out of ~330,000) in the nerve terminal are involved in recycling. Under sustained stimulation, recycled vesicles start to be reused in tens of seconds when ~47% of the preserved vesicles in the recycling pool (RP) are depleted. The heterogeneity of vesicle recycling as well as two kinetic components of RP depletion revealed the existence of a replenishable pool of vesicles prior to the priming stage and led to a realistic kinetic model that assesses the size of the sub-pools of the RP. Thus, our study quantified the kinetics of vesicle recycling and kinetically dissected the whole vesicle pool in the calyceal terminal into the readily-releasable pool (~0.6%), the readily-priming pool (~46%), the pre-mature pool (~33%) and the resting pool (~20%).

Disclosures: **Q. Zhu:** None. **X. Qiu:** None. **J. Sun:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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European Union (EU) FP7-308943

Title: Synapse-specific determinants of single-vesicle recycling kinetics in central presynaptic terminals

Authors: *M. WAGNER, K. STARAS;
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Abstract: In small central synaptic terminals efficient stimulus-driven fusion and recycling of neurotransmitter-filled synaptic vesicles is critical for the maintenance of synaptic performance. In recent years considerable effort has been invested in understanding these processes, facilitated by advances in imaging techniques and development of improved fluorescent reporters of synaptic function. It is widely accepted that synapses exhibit striking heterogeneous activity across populations but the factors that underlie this variability are poorly understood. Here we exploited high-sensitivity imaging approaches to examine whether expression of synaptic profiles was predictable at the level of individual release events and the rules that underlie this. Specifically, we imaged synaptophysin-2x-pHluorin (sypHy2x)-expressing synapses in dissociated rat hippocampal neurons. Using minimal stimulation protocols, we collected response profiles and generated frequency distribution plots. These were highly quantized, allowing us to sub-divide profiles into quantal and multi-quantal events using simple custom-written algorithms. Regarding the timecourse of endocytosis, we found that individual traces were highly variable across the whole population of synapses. Nonetheless, the expression of different profiles was not random but instead determined by factors related to the identity of individual terminals. This relationship was true for single vesicle retrievals but became less robust in the case of multiple fusion events. By inducing a form of homeostatic plasticity we were able to manipulate these parameters and change vesicle kinetics in predictable ways, suggesting that they are highly linked. Our findings offer new insights into the rules that determine single vesicle retrieval events in individual synapses.

Disclosures: **M. Wagner:** None. **K. Staras:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

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Support: MEXT KAKENHI

Title: CAPS1 stabilizes docking state of SVs in hippocampal CA3-CA1 synapses

Authors: *Y. SHINODA[1], C. ISHII[1], Y. FUKAZAWA[2], T. SADAKATA[3], T. IWASATO[4], S. ITOHARA[5], T. FURUICHI[1];
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Abstract: Calcium-dependent activator protein for secretion 1 (CAPS1) is a cytosolic protein, which associates with dense-core vesicle secretion in endocrine cells, however, their neuronal function is still largely unknown because of Caps1 knock-out (KO) results in prenatal death. Here we show that CAPS1 stabilizes the docking state of synaptic vesicle (SV) to presynaptic active zone using forebrain specific Caps1 conditional KO (cKO) mice. The synaptic transmission is strongly reduced and paired-pulse facilitation shows significant alteration in Caps1 cKO. Morphological analysis shows accumulation of SVs in presynapse without any other morphological changing. Interestingly, even though SV accumulation is occurred, the percentage of presynaptic button contained docked vesicle is markedly reduced in Caps1 cKO. These data suggest that CAPS1 stabilizes SV docking state to enhance SV release.

Disclosures: Y. Shinoda: None. **C. Ishii:** None. **Y. Fukazawa:** None. **T. Sadakata:** None. **T. Iwasato:** None. **S. Itohara:** None. **T. Furuichi:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 124.24/B88

Topic: B.06. Neurotransmitter Release

Support: NIH R01DK093953

Title: Dynamin-1 is required for short-term synaptic plasticity and transmission fidelity independent of vesicle re-availability at a mature central synapse

Authors: *S. MAHAPATRA, F. FAN, X. LOU;
Dept of Neurosci., Univ. of Wisconsin, Madison, Madison, WI

Abstract: Endocytosis is critical to generate new synaptic vesicles (SVs) during sustained synaptic transmission and to promote efficient clearance of vesicle components from release sites (1). The latter may become a rate-limiting step in response to high frequency action potential (AP) firing (2), when vesicle fusion demand at the active zone is strongly enhanced. Dynamin is

a large GTPase that plays a key role in membrane fission by pinching off the SVs from plasma membrane (3, 4). Perturbation of dynamin function, which delays the reformation of new SVs, may hinder the release site clearance and rapidly affect transmission in a much fast time scale. However, still after more than two decades of work on dynamin, such role of dynamin remains elusive. Calyx of Held is a fast relay synapse in the auditory brainstem that fires very high frequency APs with high fidelity. Using patch-clamp recording from mature Calyx of Held synapses (P16-20) from the tissue-specific dynamin-1 knockout (Dyn-1f/f Krox20Cre+ or KO) and their littermate controls (Dyn-1f/f), we examined *in vivo* function of dynamin 1 in short-term synaptic plasticity. We found that the basal transmission in KO mice was largely intact. However, strikingly, KO synapses exhibited a significant reduction of short-term synaptic depression (STD) and loss of transmission fidelity during high frequency stimulations. The STD reduction did not depend on the commonly known mechanisms for STD at central synapses, such as postsynaptic receptor desensitization/saturation, presynaptic calcium channel modulation, or vesicle recovery from depletion. But disruption of actin polymerization by Latrunculin B blocked such effect and brought the STD in KO synapses to the same levels as control. This data suggest actin plays a critical role at downstream of vesicle fusion, such as enhanced site clearance presumably through the up-regulated bulk endocytosis as demonstrated by EM tomography in dynamin-1 KO synapses (5, 6). Moreover, transmission failure in KO was not due to vesicle depletion but a presynaptic AP failure. These data demonstrated that dynamin is required to preserve the normal synaptic plasticity and transmission fidelity in a native circuit. This study uncovers a distinct role of dynamin-1 to regulate neurotransmission through an ultrafast feedback directly from endocytosis, in addition to its classical function in membrane fission. References: 1. Kononenko et al. (2015). Neuron 85:484 2. Neher E. (2010). F.S.Neurosci 2:144 3. Lou et al. (2012). PNAS 109:E515 4. Ferguson et al. (2007). Science 316:570 5. Hayashi et al. (2007). PNAS 316:570 6. Wu et al. (2014). Elife 3:e01621

Disclosures: **S. Mahapatra:** None. **F. Fan:** None. **X. Lou:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 124.25/B89

Topic: B.06. Neurotransmitter Release

Support: NIH Grant MH066198

Title: The role of synaptotagmin 1 in single synaptic vesicle endocytosis

Authors: *Y. LI, B. TRAUTERMAN, E. T. KAVALALI;
Neurosci., UT Southwestern, Dallas, TX

Abstract: Synaptic vesicle recycling is essential for maintaining normal synaptic function. The coupling of exo- and endocytosis allows for continued rapid synaptic transmission; however, the molecular mechanisms of this process are not well understood. A key candidate for the regulation of vesicle trafficking is synaptotagmin 1 (syt1) which has been established as the calcium sensor for fast synchronous neurotransmitter release. Biochemical experiments show that syt1 interacts with a variety of endocytosis associated proteins including clathrin adaptor protein 2 (AP-2). Live fluorescence imaging experiments show slowed endocytosis after strong stimulations in syt1 knockout neurons. However, the impaired exocytosis in these models is a major confounding variable. To uncover the specific role of syt1 in endocytosis, I am using single-vesicle imaging to isolate unitary exo- and endocytosis events and analyzing the properties of activity-driven synaptic vesicle trafficking. By knocking down syt1 and expressing a pHluorin-tagged vesicular probe I am able to examine the role of syt1 in single vesicle endocytosis. These experiments demonstrate minimal regulation of single vesicle endocytosis after syt1 knockdown, although there is still slowed endocytosis after larger stimulations as previously observed. Earlier studies from our lab have shown a slowing of single-vesicle endocytosis with increasing calcium however this effect is greatly diminished in the syt1 knockdown (KD) background, suggesting syt1 may function as a calcium sensor for single-vesicle endocytosis.

Disclosures: Y. Li: None. **B. Trauterman:** None. **E.T. Kavalali:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 124.26/B90

Topic: B.06. Neurotransmitter Release

Title: Evidence for PKA dependent regulation of tomosyn

Authors: *S. J. ZINN[1], D. FEATHERSTONE[2], J. RICHMOND[2];
[2]Biol. Sci., [1]Univ. of Illinois, Chicago, Chicago, IL

Abstract: Tomosyn, a syntaxin binding protein, has been postulated to negatively regulate synaptic vesicle fusion by forming nonfusogenic complexes with the plasma membrane SNAREs, syntaxin and SNAP-25, thereby inhibiting SNARE complex assembly. Using RNAi

knockdown we recently demonstrated that *Drosophila* tomosyn not only inhibits synaptic transmission but also disrupts PKA-dependent aversive olfactory learning in flies1. Biochemical evidence indicates that vertebrate tomosyn is a direct PKA-target. Phosphorylation reduces the ability of tomosyn to inhibit fusogenic SNARE complex assembly by lowering its syntaxin binding affinity2. The possibility that tomosyn is a potentially important PKA-target *in vivo* is supported by the following observations: 1) Acute activation of cAMP phenocopies the tomosyn loss-of-function mutant and manifests as increased synaptic vesicle docking and enhanced release. 2) cAMP activation results in the translocation of tomosyn away from the plasma membrane and 3) cAMP activation combined with tomosyn RNAi shows no additivity, suggesting they act in the same pathway. To definitively establish that the phosphorylation of tomosyn accounts for these cAMP synaptic effects we are generating a tagged tomosyn construct using CRISPR/Cas9 for pull down and subsequent kinase activity assays to determine the most probable PKA binding site. Based on both bioinformatics and molecular evidence potential phosphomimetic and non-phosphorylatable tomosyn fly strains will be generated using CRISPR/Cas9 and will be the subject of electrophysiological and immunohistochemical analyses. 1 Chen, Richlitzki, Featherstone, Schwärzel and Richmond (2011). PNAS 108(45):18482-7 2 Baba, Sakisaka, Mochida and Takai (2005). JCB 170(7):1113-25

Disclosures: S.J. Zinn: None. **D. Featherstone:** None. **J. Richmond:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 124.27/B91

Topic: B.06. Neurotransmitter Release

Title: Quantification *in vivo* of protein SV2A in hippocampus of rats with temporal lobe epilepsy

Authors: *L. PICHARDO-MACIAS[1,2], V. U. DÍAZ[2], G. GÓMEZ LIRA[3], I. J. CONTRERAS[2], S. R. ZAMUDIO HERNÁNDEZ[1], S. GUZMÁN[1], S. M. NAVARRETE[2], J. G. MENDOZA[2];
[1]Inst. Politecnico Nacional, México, Mexico; [2]Inst. Nacional de Pediatría, México, Mexico; [3]CINVESTAV, Mexico, Mexico

Abstract: In recent years, there has been considerable interest in determining the function of the synaptic vesicle protein 2A (SV2A) and its role as target for epileptic drugs. It is know that the SV2A is involved in normal synaptic vesicle function, albeit its participation in synaptic vesicle cycling and neurotransmitter release in normal and pathological conditions is unclear. In some

animal models of epilepsy, SV2A expression decrease in similar way as in the tissue from patients with intractable epilepsy. This implies that the decreased expression of the SV2A is a consequence of seizure activity and could contribute to the progression of epilepsy. However, unfortunately to date, it has not made a detailed quantitative study about SV2A expression in different regions of the hippocampus (epileptogenic region). Thus the aim of this study was to determine the expression of SV2A in animal model of temporal lobe epilepsy (TLE). Male adult Wistar rats were administrated with lithium and pilocarpine to induce status epilepticus (SE). The rats were sacrificed in different periods of the pathology (SE, epileptogenesis and chronic epilepsy) by overdose of sodium pentobarbital and perfused intracardially with 0.9 % saline solution, the brains were removed and process by immunohistochemestry. Randomized SV2A staining was quantified by optical density (OD) using NIH ImageJ software, it was also obtained the volume estimation using Cavalieri's test from twelve different regions of the hippocampus. The result show that the volume of hippocampus was decreased in rats with chronic epilepsy especially in internal molecular layer, CA3 and CA1 Ammon horn, with respect to the OD we observed that during SE, SV2A staining increase in all the regions sampled, while in epileptogenesis, OD increase in external molecular layer but decreased in internal molecular layer without changes in the other regions. Finally in chronic epilepsy there were no significant changes in OD in any region. The preliminary results suggest that a major structural change appears during epileptogenesis and this change was restored in chronic epilepsy whereby SV2A expression could be a possible damage neuronal biomarker.

Disclosures: **L. Pichardo-Macias:** None. **V.U. Díaz:** None. **G. Gómez Lira:** None. **I.J. Contreras:** None. **S.R. Zamudio Hernández:** None. **S. Guzmán:** None. **S.M. Navarrete:** None. **J.G. Mendoza:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 124.28/B92

Topic: B.06. Neurotransmitter Release

Support: National Institute of Neurological Disorders and Stroke (NS014506)

National Institute of Neurological Disorders and Stroke (NS007158)

Title: Localization of Ca^{2+}-channels and their connections to specific 'Active Zone Material' macromolecules at frog neuromuscular junctions

Authors: *J. SZULE, J. JUNG, U. MCMAHAN;
Biol., Texas A&M Univ., College Station, TX

Abstract: A subset of synaptic vesicles docked on the presynaptic membrane at the active zone is triggered by the influx of Ca^{2+} through voltage-gated channels to secrete their neurotransmitter into the synaptic cleft when an electrical impulse arrives at an axon terminal. The cytosolic Ca^{2+} interacts with the vesicle-associated protein synaptotagmin to trigger fusion of the docked vesicle's membrane and presynaptic membrane to form a pore, through which the neurotransmitter is secreted. Ca^{2+}-channels must be located nearby the docked vesicles to provide synaptotagmin with sufficient concentrations of Ca^{2+} to trigger membrane fusion. At frog neuromuscular junctions, a highly ordered network of macromolecules called 'active zone material' is connected to both the docked vesicles and the presynaptic membrane. Transmembrane macromolecules in the presynaptic membrane at the active zone, thought to include Ca^{2+}-channels, are connected to specific macromolecules of the active zone material, called 'pegs'. Each docked vesicle is linked to 4 pairs of pegs; one member of each pair is proximal to the docked vesicle, the other distal to it. In a recent study (see abstract: Jung, Szule and McMahan), we proposed that certain macromolecules of the active zone material regulates the probability of membrane fusion in part by moving the proximal pegs and their associated presynaptic membrane macromolecules toward the docked vesicle, increasing the amount of Ca^{2+} that reaches synaptotagmin when an electrical impulse arrives. Here, we labeled voltage-gated Ca^{2+}-channels at frog neuromuscular junctions with gold colloid (5 nm in diameter) indirectly linked to omega-conotoxin GVIA and, by imaging with electron tomography, tested the hypothesis that pegs proximal to the docked vesicle are connected to voltage-gated Ca^{2+}-channels. We found that although the gold colloid was off-set from the extracellular surface of the presynaptic membrane by its linkers to the Ca^{2+}-channels, it was highly enriched immediately adjacent the region of the presynaptic membrane occupied by the pegs, which would be expected if pegs are connected to the transmembrane macromolecules that contain the voltage-gated Ca^{2+}-channels. Moreover, we found that the distributions of the measurements between the gold colloid to each class of pegs closely matched simulated distributions that assumed the voltage-gated Ca^{2+}-channels were associated with the proximal pegs. From these results, we concluded that the voltage-gated Ca^{2+}-channels are not uniformly associated with all pegs, but rather, most, if not all, are associated with the pegs that are proximal to the docked vesicles.

Disclosures: J. Szule: None. **J. Jung:** None. **U. McMahan:** None.

Poster

124. Presynaptic Structure and Neurotransmitter Release II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 124.29/B93

Topic: B.06. Neurotransmitter Release

Support: Heart and Stroke

CIHR

NSERC

Title: Multiple functions of PKG at the *Drosophila* larval neuromuscular junction

Authors: *J. S. DASON, A. M. ALLEN, M. B. SOKOLOWSKI;
Univ. of Toronto, Toronto, ON, Canada

Abstract: The foraging gene in *Drosophila* encodes a cGMP-dependent protein kinase (PKG). PKG is thought to regulate several aspects of synaptic function, including synaptic plasticity, synaptic vesicle exocytosis and endocytosis, and neurite outgrowth. However, the mechanisms by which PKG regulates these processes are not fully understood. In addition, much of the evidence for its putative role in these processes is based on the use of pharmacological inhibitors. Pharmacological approaches can be limiting because of their non-specific effects and the inability to distinguish between presynaptic, postsynaptic and glial effects. To overcome these limitations, we used a genetic approach to understand the role of PKG. Here, we used a newly created foraging null mutant to characterize the synaptic effects of PKG at the *Drosophila* larval neuromuscular junction. In addition, we used RNAi to selectively knockdown PKG in neurons, glia or muscles to determine where PKG was required for these synaptic effects. Overall, we found that PKG negatively regulates synaptic transmission and nerve terminal growth.

Disclosures: J.S. Dason: None. **A.M. Allen:** None. **M.B. Sokolowski:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.01/B94

Topic: B.07. Synaptic Transmission

Support: UAB CCTS Pilot Grant

Title: Effects of Neuropeptide Y on temporoammonic synapses in hippocampal CA1

Authors: *K. M. CORDER[1], Q. LI[2], A. F. BARTLEY[2], L. E. DOBRUNZ[2];
[1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Neuropeptide Y (NPY) is an endogenous neuropeptide found abundantly in the nervous system. NPY has been implicated in a variety of processes, including epilepsy and feeding behavior. It has also been shown to exhibit robust anxiolytic properties. Anxiety disorders are considered a maladaptive form of learning, and the hippocampus has been implicated in a variety of anxiety disorders, including Posttraumatic Stress Disorder (PTSD). NPY in hippocampus is important for regulating anxiety; hippocampal NPY is reduced in a rodent model of PTSD, and injection of NPY into CA1 has been shown to reduce the anxiety symptoms. However, the effects of NPY on hippocampal synaptic and circuit function are only partially understood. CA1 pyramidal cells receive two major excitatory inputs, the Schaffer collateral (SC) pathway that projects from the CA3 region of hippocampus onto the proximal dendrites of CA1 pyramidal cells in s. radiatum, and the temporoammonic (TA) pathway that projects from entorhinal cortex onto the distal dendrites of pyramidal cells in s. lacunosum-moleculare. While the SC pathway is important for spatial learning, the TA pathway has been shown to be important for memory consolidation and fear learning. NPY is expressed in a subset of GABAergic interneurons in CA1, including Ivy cells that have axons in s. radiatum, and neurogliaform cells that have axons targeting s. lacunosum-moleculare. Previous studies have shown that application of exogenous NPY to acute hippocampal slices decreases excitatory synaptic responses from SC excitatory synapses onto proximal dendrites of CA1 pyramidal cells. However, it had not been shown whether NPY also affects excitatory TA synapses onto the distal dendrites of CA1 pyramidal cells. Here we show that that exogenous NPY application also causes a reduction in synaptic responses at the TA-CA1 synapses. The dose-response curve is different between these two pathways, indicating potential differences in the subtype of NPY receptors. NPY functions as the ligand for a family of G-protein coupled receptors, of which Y1, Y2, and Y5 receptors are found in the central nervous system. Previous studies have shown that NPY reduces synaptic transmission at SC synapses through activation of presynaptic Y2 receptors that inhibit voltage-dependent calcium channels. In contrast, our preliminary results suggest that NPY inhibits TA synapses through activation of Y1 receptors. Investigating the effects of NPY on TA synapses and determining the receptor subtypes involved will be important for advancing our understanding of how endogenously released NPY modulates hippocampal circuit function and behavior.

Disclosures: K.M. Corder: None. **Q. Li:** None. **A.F. Bartley:** None. **L.E. Dobrunz:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.02/B95

Topic: B.07. Synaptic Transmission

Support: NHRI-EX101-10105NI

NSC 100-2320-B-010-014-MY3

Title: Rapid dynamic changes of dendritic inhibition in the dentate gyrus by presynaptic activity patterns

Authors: *Y.-C. LIU, C.-C. LIEN;
Inst. of Neurosci. & Brain Res. Ctr., Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: The dentate gyrus (DG) serves as a primary gate to control information transfer from the cortex to the hippocampus. Activation of incoming cortical inputs results in rapid synaptic excitation followed by slow GABA-mediated (GABAergic) synaptic inhibition onto DG granule cells (GCs). GABAergic inhibitory interneurons (INs) in the DG comprise fast-spiking (FS) and non-fast-spiking (non-FS) cells. Anatomical analyses of DG INs reveal that FS cells are soma-targeting INs, whereas non-FS cells are dendrite-targeting INs. These two IN classes are differentially recruited by excitatory inputs and in turn provide exquisite spatiotemporal control over GC activity. Yet, little is known how FS and non-FS cells transform their presynaptic dynamics into varying postsynaptic response amplitudes. Using paired recordings in rat hippocampal slices, we show that inhibition in the DG is dominated by somatic GABAergic inputs during periods of sparse presynaptic activity, whereas dendritic GABAergic inputs are rapidly shifted to powerful and sustained inhibition during periods of intense presynaptic activity. The variant dynamics of dendritic inhibition is dependent on presynaptic IN subtypes and their activity patterns and is attributed to Ca2+-dependent increases in the probability of release and the size of the readily releasable pool. Furthermore, the degree of dynamic GABA release can be reduced by blocking voltage-gated K+ channels, which increases the efficacy of dendrite-targeting IN output synapses during sparse firing. Such rapid dynamic modulation of dendritic inhibition may act as a frequency-dependent filter to prevent overexcitation of GC dendrites and thus set the excitatory-inhibitory synaptic balance in the DG circuits.

Disclosures: **Y. Liu:** None. **C. Lien:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.03/B96

Topic: B.07. Synaptic Transmission

Support: NHRI-EX104-10105NI

MOST 103-2320-B-010-041-MY3

Title: Specific types of interneurons regulate input pattern-dependent recruitment of dentate granule cells

Authors: *C.-T. LEE, M.-H. KAO, C.-C. LIEN;
Inst. of Neuroscience, Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: The dentate gyrus (DG) serves as a gateway to the hippocampus, filtering and processing incoming afferent information from the cortex and passing output to other hippocampal areas. The DG comprises a heterogeneous population of granule cells (GCs). Among them, mature GCs, the largest neuronal population of the DG, are under tight inhibitory control by various GABAergic interneuron (IN) types. However, the causal link between identified GABAergic INs and mature GC activation in response to afferent activity from entorhinal inputs remains unknown. Here we show that pharmacological GABAA receptor blockade not only greatly enhances the sensitivity of mature GCs to afferent inputs, but also recruits a subset of non-spiking mature GCs. Using cell type-specific optogenetic silencing, we found that parvalbumin-expressing (PV+) INs, but not somatostatin-expressing (SST+) INs, primarily suppress mature GC responses to single-shock stimulation of cortical input. By contrast, PV+ INs and SST+ INs differentially regulate mature GC dynamics in response to θ and γ frequency inputs. Notably, PV+ INs control the onset of the spike series, whereas SST+ INs regulate the late spikes in the series. Together, these results demonstrate that GABAergic IN types differentially regulate mature GC input transformations in response to different cortical input patterns.

Disclosures: C. Lee: None. **M. Kao:** None. **C. Lien:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.04/B97

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Mapping serotonin modulation of excitatory and inhibitory synapses in the limbic mouse brain

Authors: *A. BELMER, O. PATKAR, S. E. BARTLETT;
QUT-IHBI-TRI, Woolloongabba, Australia

Abstract: Serotonin (5-Hydroxytryptamine, 5-HT) is complex neuromodulator acting both at excitatory (glutamate) and inhibitory (GABA) synapses to regulate complex behavioural functions such as mood, appetite, reward and learning. Dysregulations of the 5-HT system have been extensively involved in anxiety, stress, depression, impulsivity and addiction. Serotonin neurons originating from the brainstem Raphe nuclei send broad and diffuse projections all over the brain. However, the exact nature of synapses (excitatory or inhibitory) modulated by serotonin transmission throughout the brain is still unclear. Thus, we have developed a method combining immunohistochemistry, confocal imaging and 3D processing to quantify the density of 5-HT synaptic boutons on excitatory and inhibitory synapses in the limbic system of the mouse brain. Presynaptic boutons within serotonin axons were colocalized with excitatory and inhibitory postsynaptic markers to compare the density of 5-HT appositions on glutamate and GABA synapses throughout several regions of the limbic mouse brain (prefrontal cortex, nucleus accumbens core and shell, bed nucleus of the stria terminalis, basolateral amygdala, hippocampus, ventral tegmental area). This technique will further allow for mapping the changes in 5-HTergic neuroplasticity associated with various psychopathologies (generalized anxiety disorders, acute and post-traumatic stress disorders, major depressive disorders, impulse control disorders and addictive disorders).

Disclosures: **A. Belmer:** None. **O. Patkar:** None. **S.E. Bartlett:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.05/B98

Topic: B.07. Synaptic Transmission

Title: How activation of 5-HT2 receptors increases spontaneous but depresses evoked transmitter release in layer 2/3 pyramidal cells of rat somatosensory cortex

Authors: *F. AGAHARI[1], C. STRICKER[1,2];
[1]The John Curtin Sch. of Med. Res., The Australian Natl. Univ., Canberra, Australia; [2]ANU Med. School, The Australian Natl. Univ., Canberra, Australia

Abstract: Serotonergic modulation of glutamate release in neocortex plays important physiological role in depression. However, the mechanism(s) of action is still not understood. **Objective:** To investigate how serotonin (5-HT) modulates glutamate release in barrel cortex, focusing on signalling downstream of 5-HT G-protein-coupled receptor (GPCR) activation. **Methods:** Recordings were performed in 300 μm thick parasagittal slices of P15-19 male Wistar rats. Miniature excitatory postsynaptic currents (mEPSCs) were recorded from layer 2/3 pyramidal cells in barrel cortex at 36 ± 1°C in the presence of 1 μM tetrodotoxin and 3 μM gabazine. For paired recordings, the pre- and postsynaptic pyramidal cells were current- and voltage-clamped, respectively. Presynaptic action potentials were evoked at 0.2 Hz using short current pulses of ~1.8 nA @ 5 ms. Short-term depression (STD) was induced by 20 or 40 action potentials (APs) at a rate of 50 Hz. Recovery was measured 500 ms after the last AP in STD. **Results:** In 5/12 cells, 10 μM 5-HT increased mEPSC frequency transiently but significantly by 35 ± 3% from 51 ± 4 to 69 ± 5 Hz, without changing its amplitude. 20 μM α-methyl-5-HT (5-mHT, pan-5-HT_2 agonist), but not 1 μM DOB (5-HT_{2C} K_i = 69 nM), produced the same increase (n = 2/9). After application of PKC inhibitor Gö 6983 (100 nM), 5-HT caused a maintained increase in mEPSC frequency by 27 ± 2% from 60 ± 5 to 75 ± 6 Hz (n = 3/7). 5-HT failed to cause any significant change with the PLC inhibitor edelfosine (30 μM; n = 5) or the IP_3 receptor antagonist 2-APB (16 μM; n = 7). In paired recordings, 5-HT depressed EPSC amplitude by 54 ± 3% from -7.4 ±1.3 to -3.4 ± 0.5 pA (p = 0.003; n = 6) independent of $GABA_A$ inhibition. 5-mHT produced the same decrease of 57 ± 4% from -12.1 ± 4.8 to -5.5 ± 2.6 pA (p < 0.05; n = 3). This depression was completely blocked when the Gβγ binding peptide mSIRK (100 μM) was included in the presynaptic patch solute (n = 5). mSIRK also blocked noradrenaline (α_1 adrenergic receptor)-induced EPSC depression (n = 7), suggesting a similar mechanism. 5-HT increased recovery from STD, as the ratio of recovery *vs*. 1st EPSC amplitude increased significantly (n = 8). Quantal analysis for this depression showed that monoamine application presynaptically decreased both quantal content and size by 60 ± 10 and 27 ± 3%, respectively. **Conclusion:** 5-HT activates 5-HT_{2A} G_qPCR to increase mEPSC frequency via signaling downstream of PLC, but concurrently decreases EPSC amplitude (likely) via Gβγ binding to SNAP-25 that results in fusion pore limitation. Our findings establish this mechanism as G_q-mediated pathway, and suggest a common mechanism for monoamine modulation.

Disclosures: F. Agahari: None. **C. Stricker:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.06/B99

Topic: B.07. Synaptic Transmission

Support: NIH DA0171-88

McKnight Foundation

Title: Spontaneous somatostatin cell activity and the regulation of disynaptic, Martinotti inhibition in neocortical networks

Authors: *J. URBAN CIECKO, A. P. GOSWAMI, J. PILLI, A. L. BARTH;
Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: High basal firing of low-threshold-spiking, somatostatin (Sst+)-expressing inhibitory neurons suppresses excitatory transmission between pyramidal cells via tonic activation of presynaptic GABAb receptors. Here we investigate how the spontaneous activity of Sst+ interneurons influences synapses in a ubiquitous cortical module of inhibition, the pyramidal-Martinotti disynaptic loop. Because Martinotti cells themselves are Sst+, we hypothesized that they might suppress their own activity and GABA release via GABAb activation. First, we examined whether disynaptic inhibition might be suppressed when spontaneous Sst+ activity was high. This was not the case. Furthermore, electrophysiological recordings from pairs of synaptically connected pyramidal and Sst+ neurons in layer 2 of primary somatosensory cortex in mice indicate that Sst+ neurons do not show tonic pre- or postsynaptic GABAb receptor activity. Although presynaptic GABAb receptors could be pharmacologically detected at pyramidal to Sst+ cell synapses, spontaneous activity of Sst+ cells was not associated with tonic activation of these GABAb receptors. Optogenetic Sst+-silencing did not significantly change the efficacy of excitatory, pyramidal inputs onto Sst+ cell synapses. Thus, we conclude that disynaptic inhibition through Sst+ Martinotti cells is not regulated by tonic GABAb activity. We propose that the spatial location of Sst+-mediated GABA release is not permissive for the tonic activation of presynaptic GABAb receptors at pyramidal to Sst+ cell connections.

Disclosures: J. Urban Ciecko: None. **A.P. Goswami:** None. **J. Pilli:** None. **A.L. Barth:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.07/B100

Topic: B.07. Synaptic Transmission

Support: NIH RO1 AA020610-01

Title: GABA-B receptor regulation of extracellular glutamate in the prefrontal cortex of adolescent and adult C57Bl/6J mice

Authors: *R. I. MELENDEZ, R. A. J. BROOKS, L. M. MELECIO;
Dept. Anat. and Neurobio. A-543, Univ. Puerto Rico, San Juan, PR

Abstract: The prefrontal cortex (PFC) underlies executive cognitive functions that require elevated capacity for synaptic plasticity, which is mediated by glutamate. However, the *in vivo* processes contributing to extracellular glutamate in the PFC are not well understood. Microdialysis was used to determine the synaptic and/or glial sources contributing to extracellular glutamate release in the PFC of adolescent and adult C57BL/6J (B6) mice (n=4-8/age/drug). A microdialysis probe (1 mm membrane) was inserted into the medial PFC and aCSF was perfused through the probe at 1 μl/min. Following the collection of baseline samples, reverse microdialysis (i.e., local drug infusion) began by replacing the aCSF perfusate with one the following compounds: (a) the GABAB agonist, baclofen (1mM), (b) the AMPA/kainate antagonist, DNQX (100 μM), or (c) system xc- (i.e., glial glutamate) antagonist, carboxyphynylglycine (CPG; 100 μM). Concentrations of glutamate were determined by HPLC analysis. The mean (SEM) basal extracellular glutamate levels in adolescent and adult mice were (in μM) 4.4 + 1.5 and 3.8 + 0.4, respectively. Infusion of baclofen led to a rapid and robust decrease in extracellular glutamate levels in both age groups. Mean (SEM) inhibition of baseline (in percentage) following intra-PFC baclofen was 84.2 ± 1.8 in adolescent and 79.2% ± 3.2 in adult mice, suggesting that GABAB receptor activation is sufficient to regulate nearly 80% of total glutamate release in the PFC. Infusion of DNQX also resulted in a significant reduction in extracellular glutamate levels, and to a greater extent in adolescent (56.6% ± 9.9) compared to adult (38.7% ± 9.7) mice, suggesting greater AMPA and/or kainate receptor glutamate release during adolescence. Interestingly, infusion of CPG resulted in a significant decrease in extracellular glutamate levels in adult mice (23.5 ± 8.0 inhibition), whereas no significant effects were shown in adolescent mice (17.1 ± 3.4 % inhibition), suggesting greater glial-mediated glutamate release during adulthood. In separate mice, we determined the effect of intra-PFC baclofen (1 mM) infusions on the acquisition of spatial learning using a water maze task, and demonstrated a nearly 50% reduction in spatial learning following baclofen relative to aCSF-controls. Together, these findings suggest that the majority of extracellular glutamate levels in the PFC derive from synaptic origins, especially during adolescence. It also appears that GABAB receptors are sufficient for modulating presynaptic glutamate release in PFC, which appears to be necessary for learning and synaptic plasticity.

Disclosures: **R.I. Melendez:** None. **R.A.J. Brooks:** None. **L.M. Melecio:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.08/B101

Topic: D.08. Pain

Support: R01 DA 15438

Title: Analgesic synergy between alpha2A-adrenergic and delta-opioid receptors occurs presynaptically on nociceptive afferent terminals in spinal cord

Authors: D. J. BRUCE[1], J. J. WAATAJA[2], C. A. FAIRBANKS[3], *G. L. WILCOX[4];
[1]Dept Pharmacol., [2]Dept Neurosci., [3]Dept Neurosci, Pharmacol, Pharmaceutics, [4]Dept Neurosci, Pharmacol, Dermatol, Univ. Minnesota Med. Sch., Minneapolis, MN

Abstract: Delta-opioid receptor (DOR) and α2A-adrenergic receptor (α_{2A}AR) agonists produce synergistic antinociception spinally. We have previously shown that i) α_{2A}ARs co-localize with DORs on peptidergic primary afferent terminals in superficial dorsal horn, ii) clonidine (α_{2A}AR-selective agonist) or deltorphin II (DOR-selective agonist) inhibit CGRP release from spinal cord slices stimulated by K^+, and iii) intrathecal co-administration of clonidine and deltorphin II produces PKC-epsilon-dependent analgesic synergy. The literature documents that either DOR or α_{2A}AR agonists reduce evoked excitatory postsynaptic current (eEPSC) size and miniature EPSC (mEPSC) frequency, indicating that they act presynaptically to inhibit glutamate release from primary afferent terminals; however, no study has tested for synergy using these measures. To test this hypothesis, we recorded from rat and mouse substantia gelatinosa neurons in spinal cord slices. In rat spinal cord slices, nociceptive afferent terminals expressing TRPV1 receptors were tonically driven by continuous superfusion with 300 nM capsaicin (in the presence of 1 μM tetrodotoxin (TTX), 100 μM picrotoxin, 100 μM AP5 and 5 μM strychnine); capsaicin treatment increased mEPSC frequency over baseline 2-10-fold, documenting increased vesicular glutamate release from nociceptive afferent terminals. Superfusion with brimonidine (UK-14,304) or deltorphin II decreased capsaicin-driven mEPSC frequency 10-75% in a concentration-dependent manner (range 1-10 nM). Co-administered brimonidine and deltorphin II decreased mEPSC frequency at 100-fold lower concentrations (0.01-0.1 nM), and isobolographic analysis confirmed that this interaction was synergistic. A similar increase in potency to inhibit electrically evoked C-fiber-mediated EPSCs was evident with co-application in mouse spinal cord slices. These data strongly support the hypothesis that DOR- α_{2A}AR synergy takes place presynaptically on nociceptive afferent terminals.

Disclosures: **D.J. Bruce:** None. **J.J. Waataja:** None. **C.A. Fairbanks:** None. **G.L. Wilcox:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.09/B102

Topic: F.02. Animal Cognition and Behavior

Title: Effect of CB1 receptor antagonist on habituation and prepulse inhibition of the acoustic startle response

Authors: *A. G. LOUTTIT, C. DE OLIVEIRA, S. SCHMID;
Anat. and Cell Biol., Univ. of Western Ontario, London, ON, Canada

Abstract: The acoustic startle response (ASR) is a rodent model system used to measure sensory filtering. It consists of a short primary startle pathway modulated by short-term habituation and prepulse inhibition (PPI). The cellular mechanisms underlying short-term habituation and PPI are not fully understood, but a potential role for the cannabinoid type 1 (CB1) receptor exists through inhibition of neurotransmitter release. We hypothesized that CB1 receptors in the caudal pontine reticular nucleus (PnC), the sensorimotor interface of the ASR, are involved in short-term habituation and PPI of the ASR. We investigated the role of CB1 receptors with systemic and intracranial administration of the CB1 receptor antagonist N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) to male Long Evans rats. Bilateral intracranial injections were made into the PnC through chronically implanted cannulae. Following injections, short-term habituation and PPI were monitored using Med Associates startle boxes. Systemic administration of AM251 at the dose of 1 mg/kg had no effect on short-term habituation; however, both the 3 mg/kg and 5 mg/kg dose improved short-term habituation. Local microinfusions of AM251 (0.5 µL per side) had no effect on short-term habituation at both the doses of 10 µM and 1 mM. Both systemic and intracranial injections had no effect on PPI. These findings suggest CB1 receptors modulate either short-term habituation or sensitization through actions outside the PnC and that they are not involved in PPI or PPI modulation.

Disclosures: **A.G. Louttit:** None. **C. de Oliveira:** None. **S. Schmid:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.10/B103

Topic: F.02. Animal Cognition and Behavior

Support: CAPES

Cnpq

FAPESP

Title: Medial pre-frontal cortex CB1 receptors modulate the conditioned emotional response: involvement of the glutamatergic and nitrergic neurotransmissions

Authors: *D. L. ULIANA[1], L. S. ANTERO[2], S. F. LISBOA[2], L. B. M. RESSTEL[2];
[1]Pharmacol. Departament, [2]Pharmachology Departament, Univ. of São Paulo, Ribeirao Preto, Brazil

Abstract: Cannabinoid receptor type 1 (CB1) present in the ventral portion of medial pre-frontal cortex (vMPFC) modulates the conditioned emotional response (CER), in contextual fear conditioning (CFC). CB1 receptor activation has been related with decrease behavior and cardiovascular response during contextual re-exposure. Furthermore, the vMPFC NMDA glutamate receptor and neuronal nitric oxide synthase (nNOS) are involved with CER modulation. Possibly, the response induced by CB1 involves modulation of glutamate release and NO production. Therefore, this study tests the hypothesis that CB1 receptor modulates CER through NMDA activation and NO pathway. Male Wistar rats (250-270g) with bilateral guide cannula targeted to the vMPFC were first exposed to a chamber during 10 min (habituation). In a second exposure to the same chamber, they received 3 electrical footshocks (0.85 mA, 2 s). 24h later, a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. After additional 24h, the behavioral and autonomic responses (mean arterial pressure - MAP, heart rate - HR and cutaneous temperature - CT) were continuously assessed during the 10 minutes of the re-exposition to the same chamber with no footshocks (test session). Vehicle (Saline or DMSO10%) or/and the CB1 antagonist (NIDA; 25, 55, 100pmol); NMDA antagonist (LY 2 nmol); nNOS inhibitor (NPLA; 0.04 nmol); NO scavenger (c-PTIO; 1nmol) or cCG inhibitor (ODQ; 1 nmol) were administered in the vMPFC 10 min before the test session. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 157/2013). CB1 antagonism receptor in the dose of 55 and 100 pmol increased the time spent in freezing behavior ($F_{3,22}=11,14$; $p<0.05$, ANOVA, Tukey pos-hoc), and also induced an enhancement of the rise in MAP ($F_{3,22}=10,23$, $p<0.05$, ANOVA,

Tukey pos-hoc), HR ($F_{3,22}=8,259$, $p<0.05$, ANOVA, Tukey pos-hoc) and the CT decrease ($F_{3,22}=3,276$, $p<0.05$, ANOVA, Tukey pos-hoc) during the re-exposure. LY, NPLA, c-PTIO, ODQ were not able to change either freezing behavior ($p>0.05$) or autonomic response (MAP, $p>0.05$; HR, $p>0.05$; CT, $p>0.05$). However, when administrated previously to the CB1 antagonist, NIDA 55 pmol, prevented the CER enhancement. The present data demonstrate that the CB1 antagonist, NIDA, changed freezing behavior and autonomic response in the re-exposure session, suggesting that CB1 receptor in vMPFC modulate CER, in CFC model. And also, this response seems to be dependent NMDA activation and NO pathway.

Disclosures: D.L. Uliana: None. **L.S. Antero:** None. **S.F. Lisboa:** None. **L.B.M. Resstel:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.11/B104

Topic: B.07. Synaptic Transmission

Support: NHMRC

Title: Neuromodulatory control of intercalated cell-associated synapses within the amygdala: implications for anxiety disorders

Authors: *B. L. WINTERS, E. E. BAGLEY;
Sch. of Med. Sciences/Discipline of Pharmacol., Univ. of Sydney, Sydney, Australia

Abstract: The intercalated cells (ITC) of the amygdala are small, densely populated GABAergic interneurons that form a large nucleus surrounding the basolateral amygdala (BLA) and provide a significant inhibitory interface between the BLA and central amygdala (CEA). ITCs have been found to play an important role in fear memory and the modulation of ITC activity is thought to affect amygdala output and formation of anxiety-related behaviour. In particular, ablation of ITC control has been shown to impede fear extinction, a memory process that is thought to be impaired in anxiety disorders such as post-traumatic stress disorder. These cells can be identified by their high expression of both enkephalin and the mu-opioid receptor. Similarly, ITCs highly express the dopamine D1 receptor, which has been shown to hyperpolarise ITCs through the activity of G-protein inwardly rectifying potassium channels (GIRK). ITCs receive multiple inputs including: glutamate afferents from the BLA and medial prefrontal cortex, dopamine afferents from the ventral tegmental area and local GABAergic inputs within the ITC nucleus.

Convergence of these synaptic inputs and intrinsic opioid signalling likely plays an important role in gating ITC activity. Indeed, both dopamine and opioid-dependent signalling has been implicated in the acquisition and extinction of fear memory. However, the effects of these neuromodulators on ITC-associated synaptic activity are unknown. Using whole-cell, voltage-clamp electrophysiology we recorded electrically evoked synaptic currents from ITCs in coronal slices of the amygdala, isolated from male Sprague-Dawley rats (3-5 weeks). We show that exogenous application of met-enkephalin (ME) reduces the amplitude of excitatory postsynaptic currents (EPSC) at both BLA- and cortical-ITC synapses by decreasing presynaptic glutamate release. Similarly, ME reduced inhibitory postsynaptic currents (IPSC) at local ITC-ITC synapses. Further, following modest field stimulation and inhibition of enkephalin-related peptidases, we show the opioid antagonist naloxone increases EPSCs at BLA-ITC associated synapses indicating endogenous opioids are present and acting to reduce glutamate release. Similarly we find dopamine decreases BLA-ITC synaptic activity via D2 receptor-mediated inhibition of glutamate release. Together, these data indicate endogenous opioids and dopamine have the capacity to be significant modulators of ITC function and may participate in regulating key circuitry underlying fear memory.

Disclosures: **B.L. Winters:** None. **E.E. Bagley:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.12/B105

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant GM094246

AHA 11GRNT7890004

Title: Acid Sensing Ion Channels can follow high frequency 'synaptic' stimuli

Authors: *D. M. MACLEAN, V. JAYARAMAN;
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Abstract: Acid sensing ion channels (ASICs) are trimeric cation-selective ion channels activated by protons in the physiological range. Recently, synapses have been identified which activate ASICs using the protons stored in synaptic vesicles as neurotransmitters. Like virtually all neurotransmitter-gated ion channels, ASICs activate very rapidly and in the continual presence

of agonist they show fast and near complete desensitization with a time constant of approximately 1 second. However during physiological synaptic transmission the acidification of the synaptic cleft does not last for 1 second. Rather, the cleft experiences very transient pH dips of approximately 1 milisecond in duration. Here we examined the responses of ASICs to such physiologically relevant 1 milisecond 'synaptic' acidifications as either single events or as trains of events to mimic high volume synaptic transmission. Whereas virtually every other neurotransmitter receptor desensitizes during such high frequency (50 Hz) trains, we find that ASICs are able to follow these trains with high fidelity and no desensitization. Thus, during the high volume synaptic activity which drives memory formation and synaptic plasticity, when all other neurotransmitter receptors experience desensitization neuronal ASICs may function unimpeded. This unique capacity might underlie ASICs roles in learning, fear memory and drug addiction.

Disclosures: D.M. MacLean: None. **V. Jayaraman:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.13/B106

Topic: B.07. Synaptic Transmission

Support: NIA Grant T32 AG20506

NINDS Grant R21 NS090040

Title: The role of kainate receptors in modulating hilar network excitability

Authors: *T. HEDRICK[1], W. P. NOBIS[2], G. T. SWANSON[2];
[1]Pharmacol., Northwestern University, Feinberg Sch. of Medici, Chicago, IL; [2]Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: Many models of temporal lobe epilepsy are typified by hippocampal hyperexcitability that arises from altered activity and connectivity of hilar neurons, which induces aberrant excitation/inhibition balance and perpetuates seizures. Kainate receptors (KARs) modulate the excitation/inhibition balance elsewhere in hippocampus through actions on synaptic and intrinsic excitability, and recent evidence suggests that enhanced KAR function in the dentate gyrus in part drives hyperexcitability in temporal lobe epilepsy. However, the role of KARs in the healthy hilus is unknown. In this project, we test the hypothesis that KARs modulate synaptic and

intrinsic excitability at defined synapses and in subpopulations of hilar neurons. To address this hypothesis, we are making whole-cell patch clamp recordings of hilar mossy cells (HMC), granule cells (GC), and populations of hilar interneurons (HI) in acute slices from adult mice. We will determine: 1) if KARs are present at various hilar synapses and what subunits and accessory proteins they are composed of, and 2) how KARs impact synaptic or intrinsic excitability. These results will provide a framework for future studies which examine how KAR function is altered in hilar pathology. Our initial results demonstrate that KARs modulate synaptic and intrinsic excitability in the hilus, and that the role of hilar KARs is synapse-specific. At GC-HMC synapses, KARs mediate postsynaptic currents that are impacted by association with neuropillin and tolloid-like (Neto)1 and Neto2 auxiliary proteins (decay kinetics for WT: 45.5 ± 4.4, Neto1 knockout: 21.8 ± 1.9, and Neto2 knockout: 64.8 ± 8.9 ms). Future studies will examine the subunit composition of these KARs and their role in synaptic plasticity. In contrast, at HMC-GC synapses, presynaptic KARs modulate glutamate release (AMPA-mediated current amplitude is decreased to 66.0 ± 5.7% of its original amplitude after kainate application) thus mediating short-term synaptic plasticity. In addition to synaptic effects, KAR activation modulates intrinsic excitability in HMCs by decreasing the Ih current (sag ratio is decreased 4.0 ± 1.2% after kainate application). Thus KARs modulate both synaptic and intrinsic excitability at specific locations within the hilar circuit. Our results show that KARs can help set hilar network excitability at various stages in the hilar circuit via modulation of synaptic and intrinsic excitability. These data shed light on the role of KARs in the healthy hilar circuit, as well as provide a jumping off point for future research which examines alterations in KAR function in hilar pathology.

Disclosures: **T. Hedrick:** None. **W.P. Nobis:** None. **G.T. Swanson:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 125.14/B107

Topic: B.07. Synaptic Transmission

Support: JSPS KAKENHI 15K20561

JSPS KAKENHI 25463150

Title: Propofol facilitates pyramidal cells firing synchrony in rat cerebral cortex

Authors: *Y. KOYANAGI[1], Y. OI[1], N. KOSHIKAWA[2], M. KOBAYASHI[2];
[1]Dept. Anesthesiol., [2]Dept. Pharmacol., Nihon Univ. Sch. Dent., Tokyo, Japan

Abstract: The general anesthetic propofol rises frontal alpha rhythm at the dose that is sufficient to induce loss of consciousness (Purdon at al., 2013; Ching et al., 2010; Feshchenko at al., 2004). However, the neural mechanisms of propofol-induced alpha rhythm in the cerebral cortex remain unknown. We previously demonstrated that inhibitory fast-spiking cells (FS) to excitatory pyramidal cells (Pyr) connections are the most sensitive to propofol-induced facilitation of unitary inhibitory postsynaptic currents (uIPSCs; Koyanagi et al., 2014). Taken together with the finding that inhibitory postsynaptic potentials (IPSPs) promote a high degree of synchrony (Hu et al., 2011), it is reasonable to propose the hypothesis that propofol-induced facilitation of uIPSPs results in firing synchrony among postsynaptic Pyr that receive inhibition from the same presynaptic FS. Triple or quadruple whole-cell patch-clamp recordings were performed from one FS and two or three Pyr in rat insulocortical slices. We recorded the uIPSCs, and the synchronicity was evaluated in the pairs with ≥ one connection of FS◊Pyr. After checking the connections, Pyr were depolarized to generate repetitive spike firing, and we examined how presynaptic FS action potentials modulate the timing of spike firing among Pyr. In control, inhibitory inputs from FS had little effect on spike timing synchrony among Pyr. Bath application of 10 μM propofol significantly potentiated uIPSPs, and reduced the coefficient of variation of spike timing among Pyr during FS activation. Propofol had little effect on spontaneous firing synchronicity. These results suggest that propofol facilitates Pyr firing synchrony by enhancing inhibitory inputs from FS. This synchrony of Pyr may induce the frontal alpha rhythm that associates with propofol-induced loss of consciousness.

Disclosures: **Y. Koyanagi:** None. **Y. Oi:** None. **N. Koshikawa:** None. **M. Kobayashi:** None.

Poster

125. Synaptic Modulation

Location: Hall A

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Program#/Poster#: 125.15/B108

Topic: B.07. Synaptic Transmission

Support: Chateaubriand Fellowship, French Embassy to the US

NIMH grant MH086415

Title: Cyclic-AMP transients in CA1 neurons are greatest in intermediate and distal dendrites during β-adrenergic receptor activation

Authors: *V. LUCZAK[1], T. ABEL[1], J.-A. GIRAULT[2,3], N. GERVASI[2,3];
[1]Biol., Univ. of Pennsylvania, Philadelphia, PA; [2]Inst. Du Fer À Moulin INSERM U839, Paris, France; [3]INSERM UMRs 839, Paris, France

Abstract: In the hippocampus, cyclic-adenosine monophosphate (cAMP) is a critical second messenger required for long-lasting forms of synaptic plasticity, learning and memory. Cyclic-AMP is a direct substrate of protein kinase-A (PKA), exchange protein activated by cAMP (EPAC), and hyperpolarization-activated/cyclic-nucleotide gated channels (HCN channels), and therefore regulates multiple aspects of neurophysiology that include kinase activity, transcription, translation and excitability. To better understand how cAMP operates within the structural complexity of neurons to contribute to synaptic plasticity and memory, we used live two-photon microscopy and a genetically-encoded cAMP FRET sensor to monitor cAMP in live hippocampal slices. Bath application of isoproterenol, a β-adrenergic receptor agonist, produces local cAMP transients with greater amplitude and rate in intermediate and distal dendrites compared to somatic compartments and this effect is most dramatic with 100 nM isoproterenol. Furthermore, NMDA application alone slightly reduced cAMP levels and coapplication of NMDA and 1 μM isoproterenol attenuated cAMP production. Taken together, localized cAMP transients in CA1 neurons suggest that dendritic segments coordinate cAMP signaling and may differentially influence downstream effectors like PKA in specific dendritic compartments.

Disclosures: **V. Luczak:** None. **T. Abel:** None. **J. Girault:** None. **N. Gervasi:** None.

Poster

125. Synaptic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R25 GM 069621

UTEP Faculty Start-up Funds 2G12RR008124

NIH-Funded UTEP Border Biological Research Center Pilot Grant

Title: Investigation of hippocampal pathways in a mouse model of lead exposure relevant to the El Paso region

Authors: A. TENA, E. PERU, *L. E. MARTINETTI, K. FENELON;
Dept. of Biol. Sci., Univ. of Texas At El Paso, El Paso, TX

Abstract: Lead (Pb) is a ubiquitous environmental heavy metal that can cause behavioral and cognitive deficits. Even Low-level Pb induces learning and memory impairments in children from low income neighborhoods found across the United States, including the El Paso-Ciudad Juarez border region. Numerous studies reported that high-levels Pb exposure alters the function of the hippocampus. However, the mechanisms by which lowest-level Pb affects neuronal function are still ill defined, due to the lack of animal models of low Pb exposure. In this study, we compared adult mice that were not exposed to Pb (control) to adult mice exposed from birth to post-natal day 28 to either high Pb levels (330 ppm) or low Pb levels (30 ppm). The 30 ppm exposure was specifically used because it was shown to yield blood Pb levels matching those observed in children of the El Paso region (Sobin et al., 2009 and 2011). Mice exposed to such low-level Pb showed poorer exploratory ambulation (Flores-Montoya and Sobin, 2014) and poorer novel odor discrimination (Flores-Montoya et al, in review), behaviors which are hippocampus-dependent. Using both high and low Pb exposed mice, extracellular field electrophysiological recordings were performed in the CA1 region of acute hippocampal slices. Our results revealed synaptic transmission and short-term synaptic depression abnormalities in both low (N = 9) and high (N = 9) Pb levels exposed mice compared to control mice (N = 17). In addition, previous work in children had shown that low-level Pb exposure is associated with deficits in working memory (Sobin et al, unpublished data), which depends on the functional integrity between the hippocampus and the prefrontal cortex (PFC). Therefore, we performed Optogenetics experiments in acute slices using these Pb exposed mice to determine if the hippocampus-PFC synapses were altered. Our preliminary results suggest that the synaptic strength and short-term synaptic depression are also altered by both high (N = 3) and low Pb (N = 2) levels at hippocampus-PFC synapses. Overall, these results will contribute to better understand the long-term effects of low-level Pb exposure on learning and memory in children living in low-level environmental Pb conditions.

Disclosures: **A. Tena:** None. **E. Peru:** None. **L.E. Martinetti:** None. **K. Fenelon:** None.

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.01/B110

Topic: B.07. Synaptic Transmission

Support: NIH Grant MH099054

Title: Selective cholinergic modulation of layer 5 projection neurons in the mouse prefrontal cortex

Authors: *A. L. BAKER[1], R. J. O'TOOLE[3], A. T. GULLEDGE[2];
[2]Physiol. & Neurobio., [1]Geisel Sch. of Med. at Dartmouth, Lebanon, NH; [3]Biol. Sci., Dartmouth Col., Hanover, NH

Abstract: Pyramidal neurons in layer 5 of the mouse prefrontal cortex comprise two broad classes of projection neurons: commissural/callosal (COM) neurons, and corticopontine (CPn) neurons. These two neuron subtypes have distinct morphological and physiological characteristics, including divergent responses to neuromodulators such as serotonin, acetylcholine (ACh), and norepinephrine. To further characterize the role of ACh in regulating cortical circuits, we compared phasic responses to exogenous and endogenous ACh in labeled COM and CPn neurons in the medial prefrontal cortex (mPFC) of wild-type mice and mice expressing channelrhodopsin-2 in cholinergic neurons (ChAT-ChR2 mice; Jackson Labs). When paired with suprathreshold depolarization, exogenous ACh (100 μM for 100 ms) generated a brief inhibitory response followed by an excitatory response in COM and CPn neurons (data pooled from wild-type and ChAT-ChR2 mice). Apamin-sensitive inhibitory responses were qualitatively similar in COM (n= 22) and CPn (n = 16) neurons, but were of substantially longer duration in COM neurons (1.773 ± 0.176 s vs. 0.807 ± 0.119 s in COM and CPn neurons, respectively; $p < 0.05$). Excitatory responses to exogenous ACh, quantified as the peak increase in firing rate (% over baseline) following ACh application, were preferentially enhanced in CPn neurons. ACh increased firing rates by 191 ± 31% in CPn neurons, and 88 ± 13% in COM neurons ($p < 0.05$, when comparing CPn to COM neurons). Endogenous ACh release triggered by flashes of blue light (100 pulses, 5 ms each, at 59 Hz) had no effect in COM (n = 3) or CPn (n = 5) neurons from wild-type animals, and did not induce inhibitory responses in neurons from ChAT-ChR2 mice, but increased firing rates (by 27 ± 8% and 38 ± 10%, respectively) in both COM (n = 10) and CPn (n = 9) neurons ($p = 0.39$) from ChAT-ChR2 mice. Remarkably, single flashes of light (5 ms duration) enhanced action potential generation selectively in CPn neurons. In baseline conditions, periodic current steps (1.5 s duration) generated 9.2 ± 0.6 and 8.5 ± 0.4 action potentials in CPn neurons and COM neurons, respectively. Single flashes of blue light increased the number of action potentials in CPn neurons by 33 ± 5% (n = 9; $p < 0.05$), but had negligible impact (6 ± 6%; $p = 0.14$) on action potential generation in COM neurons (n = 13). Together, our results demonstrate cell-type selectivity in phasic cholinergic signaling in projection neurons in the mPFC, with ACh preferentially promoting excitation in CPn neurons.

Disclosures: **A.L. Baker:** None. **R.J. O'Toole:** None. **A.T. Gulledge:** None.

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.02/B111

Topic: B.07. Synaptic Transmission

Title: The role of acetylcholine in neural circuit modulation, behavior and development in *Drosophila* melanogaster

Authors: *C. MALLOY, C. ENGLISH, R. L. COOPER;
Biol., Univ. of Kentucky, Lexington, KY

Abstract: Acetylcholine (Ach) is the major excitatory neurotransmitter within the CNS of many insects, including the model organism, *Drosophila* melanogaster. The cholinergic system is integral in regulating specific behaviors in this animal, including the medial fiber escape response, in stimulating grooming and jumping, and in hyperactive geotaxis ability. This suggests that this system is important in mediating distinct sensory-CNS-motor circuits; however, the specific cholinergic receptor subtypes that are involved in regulation of these circuits is currently not well-defined. The larval CNS is readily accessible for direct recordings of identifiable sensory and motor neurons associated with the locomotive behaviors. In addition, sensory-CNS-motor circuits are able to be driven for pharmacological studies associated with acetylcholine receptors (AChRs) and genetic manipulation of cholinergic receptors is highly amenable. Thus, a combined genetic and pharmacological approach can be taken in order to examine the functional role of specific cholinergic receptor subtypes in modulating a defined sensory-CNS-motor circuit. In this study, we use electrophysiological and behavioral analyses to study neural circuit modulation in the presence of classic AChR agonists and antagonists. In addition, genetic knockdown of individual receptor subunits are used in order to identify specific receptor subunits that are integral in mediating these behaviors. Analysis has shown that AChR agonists, nicotine and muscarine, enhance motor activity when exposed directly to a larval CNS, while the classic muscarinic acetylcholine receptor (mAChR), scopolamine decreases motor activity. In addition, exposure to Ach and nicotine enhance larval locomotion after long-term exposure but decrease mouth hook movements providing evidence that these agonists have varying modulatory effects on two distinct circuits. We are now using these techniques in order to advance our knowledge regarding the role of this system in various developmental processes. Developmental assays can be performed in order to deduce the role of specific receptor subtypes in regulating nervous system development. Loss of specific AChR receptor subunits may prove detrimental in neural circuit formation and associated behaviors; however, compensatory mechanisms may act to override the loss of function of individual subunits. Thus, investigation into neuronal plasticity can be used in this model, and this analysis can act as a building block for these future studies.

Disclosures: **C. Malloy:** None. **C. English:** None. **R.L. Cooper:** None.

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.03/B112

Topic: F.02. Animal Cognition and Behavior

Support: NIAAA DICBR

Title: Removal of D2 receptors on striatal cholinergic interneurons impairs sequence learning

Authors: *J. H. CHANCEY[1], D. M. LOVINGER[2];
[1]LIN, [2]Lab. for Integrative Neurosci., NIH-NIAAA, Rockville, MD

Abstract: The dorsal striatum (dStr) plays a critical role in encoding action initiation and action sequences. Projection neurons from dStr, medium spiny neurons (MSNs), encode the start and stop of learned action sequences and are able to encode entire action sequences as a single event through sustained changes in firing throughout the sequence. Acetylcholine (ACh) plays an important role in striatal function by regulating MSN excitability, presynaptic glutamate release, GABAergic interneuron excitability, and plasticity. ACh can also drive the release of dopamine and possible co-neurotransmitters through nicotinic receptors on presynaptic terminals. Cholinergic interneurons (CINs) are the main source of ACh in the striatum and are tonically active, thereby establishing a cholinergic tone. Previous studies show that CINs in the dStr pause their firing during action sequences, but the significance of this physiology for behavioral performance has not been explored. We hypothesize that the pausing behavior of CINs is critical for mediating learning by regulating dopamine and glutamate release, and MSN excitability, allowing for the plasticity necessary for learning. Activation of D2 receptors (D2Rs) on CINs drives pauses in firing. Here we test how removing D2Rs from CINs affects learning a sequence of actions to obtain a sucrose reinforcer. We selectively eliminated D2Rs from CINs by crossing $D2^{flox/flox}$ with ChatCre mice (D2F-ChatCre) and tested sequence learning using an adapted fixed-ratio lever-pressing task that produces reliable action sequences in mice. Mice lacking D2Rs in CINs were able to learn the sequence task, but at a slower rate than $D2^{flox/flox}$ littermates. Trained D2F-ChatCre mice also demonstrated fewer lever presses and received fewer reinforcers per session than controls. The behavioral deficits were not due to overall deficits in motor skill learning or general motivation, because D2F-ChatCre mice perform similarly to controls in accelerated rotarod and progressive ratio breakpoint tasks and showed similar levels of homecage sucrose intake. Thus, the deficits we see in sequence learning may be specific to

action initiation, action chunking or behavioral flexibility required for the sequence task. We are currently performing, *in vivo* recordings of unit activity in dStr throughout acquisition of the sequence to determine if D2F-ChatCre mice have deficits in sequence encoding.

Disclosures: J.H. Chancey: None. **D.M. Lovinger:** None.

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.04/C1

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: The role of the cholinergic midbrain in sensory filtering and sensorimotor gating

Authors: *E. C. AZZOPARDI[1], S. SCHMID[2];
[2]Anat. and Cell Biol., [1]Univ. of Western Ontario, London, ON, Canada

Abstract: Acetylcholine (ACh) is an important neurotransmitter that is involved in many aspects of cognition, but perhaps most notably in attention. Here, we aim to investigate the role of ACh in the pre-attentive processes of sensory filtering and sensorimotor gating. Sensory filtering is the removal of unnecessary sensory information from our conscious perception, freeing cognitive resources for more important information, whereas sensorimotor gating suppresses sensory evoked motor responses in favor of an orienting response towards a sensory stimulus. We study this using habituation and prepulse inhibition (PPI) of the acoustic startle response, respectively. Habituation is the exponential decrease in startle magnitude after repeated presentation of the startling stimulus. PPI occurs when the presentation of a prepulse inhibits the processing of the startling stimulus, resulting in a reduced startle magnitude. Traditionally, it has been assumed that cholinergic projections from the pedunculopontine tegmental nucleus (PPT) mediate PPI. To test this, we bilaterally injected 8 transgenic rats (Chat-Cre) with the DREADD vector rAAV8-hSyn-DIO-hM4Di-mcherry (or control) in the PPT. After 21 days of recovery, rats received an IP injection of CNO (3 or 10 mg/kg in 7% DMSO) or vehicle prior to sensory filtering testing. This specifically silenced cholinergic neurons of the PPT. Preliminary data shows that our highest dose of CNO did not disrupt short-term habituation or PPI at both prepulse levels (75 or 85 dB SPL) or across all interstimulus intervals tested (15, 30 or 100 ms). We did see a trend toward reduced baseline startle with CNO administration compared to vehicle. Preliminary immunohistochemistry showed good expression of the viral tag, mCherry, in the PPT and LTD.

Moreover, there was a very high overlap between mCherry and the cholinergic marker, Choline Transporter (ChT). Our ongoing experiments are investigating cholinergic PPT stimulation during sensory filtering tasks through optogenetics to continue to address this question. Additionally, we are using chemogenetic cholinergic PPT silencing in to investigate the role of this structure in orienting responses and attention.

Disclosures: E.C. Azzopardi: None. **S. Schmid:** None.

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.05/C2

Topic: F.02. Animal Cognition and Behavior

Support: NIH T32-NS058280

NIMH Grant RO1 62122

Narsad Distinguished Investigator Award 18667

Title: Optogenetic activation of medial septum cholinergic neurons improves contextual fear learning and alters choline levels in hippocampus

Authors: *S. J. HERSMAN, J. CUSHMAN, K. WASSUM, M. S. FANSELOW, S. LOTFIPOUR;
UCLA, Los Angeles, CA

Abstract: Cholinergic inputs to hippocampus, primarily from medial septum through the fimbria fornix, have long been thought to play an important role in contextual learning. Hippocampal blockade of muscarinic receptors using scopolamine prevents contextual learning, and inhibition of cholinesterase can lead to memory improvements in a variety of tasks. However, the effects of selective depolarization of cholinergic medial septal neurons on acetylcholine release in hippocampus and hippocampal-dependent learning and memory is not well understood. Using newly developed choline biosensors, we activated channel rhodopsin in medial septum cholinergic neurons (ChAT-Cre rats and mice) and measured light-evoked choline release in hippocampus. Choline release was timed to laser onset and offset and scaled with pulse width and laser power. Furthermore, pulse trains at moderate power levels led to sustained increases in choline throughout the duration of the pulse train stimulation. In order to test the selective effect of these pulse trains on contextual learning, we separated the contextual learning epoch from the

fear conditioning epoch using the context pre-exposure facilitation paradigm. ChAT-ChR2 mice and littermate controls were surgically implanted with fiberoptic ferrules targeting medial septum, and allowed to explore a novel environment while receiving concurrent blue light stimulation. The following day, mice were returned to the same environment and given a mild foot shock after 10 seconds in the environment. During a test of fear the following day, mice that received ChR2 stimulation during training demonstrated significant enhancements in freezing. These findings demonstrate the correspondence between choline increases detectable by the choline biosensor and changes to behavior, and suggest that increased acetylcholine in hippocampus during training facilitates contextual processing.

Disclosures: S.J. Hersman: None. **J. Cushman:** None. **K. Wassum:** None. **M.S. Fanselow:** None. **S. Lotfipour:** None.

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.06/C3

Topic: B.07. Synaptic Transmission

Support: NRF Grant 2013-R1A1A2053280

Title: Cholinergic modulation of the gain of hippocampal CA1 pyramidal neuron is synaptic input layer-dependent

Authors: *K. PARK, J. KWAG;
Dept. of Brain and Cognitive Engin., Korea Univ., Seoul, Korea, Republic of

Abstract: The spike outputs of hippocampal CA1 pyramidal cell (PC) contain neural information important for memory and spatial information processing. Thus, understanding how CA1 PC transforms its synaptic input into spike output and how neuromodulators change the sensitivity of the CA1 PC to its input could help us elucidate the principles underlying hippocampal neural computation. Among many different neuromodulators, acetylcholine (ACh) is known to play critical roles in hippocampal function, but its role in modulating CA1 PC's input-output transformation and its gain is not well understood. Therefore, we characterized the gain of CA1 PC in response to CA3-stimulated excitatory inputs via two synaptic layers, stratum oriens (SO) and stratum radiatum (SR), and investigated how the gain of CA1 PC is cholinergically modulated in the hippocampal circuit using whole-cell patch-clamp recording *in vitro*. Either SO or SR in the CA1 area were electrically stimulated at 1, 3, 5, 10, 20 and 30 Hz

and the corresponding spike outputs were analyzed to quantify the input-output (IO) curve and its gain. All experiments were conducted in the presence of D-AP5 (50 μM) to block the NMDA receptor-mediated plasticity. Cholinergic modulation of gain was investigated by applying ACh antagonist, atropine (10 μM) and agonist, carbachol (5 μM). When the stimulation frequency of SO was increased, the firing rate of CA1 PC increased sub-linearly, while the same stimulation protocol in the SR resulted in the spike output frequency of CA1 PC being buffered at 6 Hz. The gain of CA1 PC by SO stimulation was significantly higher than that by SR stimulation (gain: SO = 0.55 ± 0.07, SR = 0.14 ± 0.05, n = 8, $p < 0.001$), suggesting that CA1 PC gain is modulated in a synaptic layer-dependent way. Next, to investigate the effect of ACh on such synaptic input layer-dependence of gain modulation, we applied atropine. Atropine abolished the difference in gain (gain: SO = 0.27 ± 0.06, SR = 0.16 ± 0.02, n = 4, $p > 0.05$). Interestingly, atropine significantly decreased the gain of CA1 PC with inputs from SO only. When we applied carbachol, it increased the gain of CA1 PC with inputs from the SO but had little effect on the gain with inputs from the SR (gain: SO = 0.67 ± 0.10, SR = 0.23 ± 0.06, n = 4, $p < 0.01$). These results demonstrate that cholinergic modulation of CA1 PC's gain is synaptic layer-dependent and specific to input from SO. Such hippocampal circuit and neuromodulatory control of CA1 PC's gain may contribute to dynamically modulate the computational modes of CA1 PC to efficiently process the spatio-temporally dynamic inputs.

Disclosures: **K. Park:** None. **J. Kwag:** None.

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.07/C4

Topic: B.07. Synaptic Transmission

Title: Dissecting the roles of M1 and M4 receptors in CA1 excitatory transmission

Authors: ***C. A. THORN**, J. EDGERTON;
Pfizer Inc., Cambridge, MA

Abstract: Xanomeline, a muscarinic M1/M4 preferring agonist, has been shown to exhibit pro-cognitive and antipsychotic effects in patients with Alzheimer's Disease (Bodick et al., 1997) and schizophrenia (Shekhar et al., 2008); however, the mechanisms by which it exhibits these effects remain unknown. While the etiologies of Alzheimer's Disease and schizophrenia differ dramatically, both diseases are associated with severe dysregulation of hippocampal circuitry, which is thought to underlie psychotic symptoms and deficits in cognitive function. The CA1

region of the hippocampus integrates information from two excitatory pathways - the temporoammonic (TA) pathway from entorhinal cortex and the Schaffer collaterals (SC) from CA3. In the CA1 area, increased levels of acetylcholine are thought to bias transmission toward the more direct sensory input from entorhinal cortex over the more processed input from CA3 by acting at both muscarinic and nicotinic receptors in these two pathways (Hasselmo et al., 1996). In Alzheimer's Disease, reduced cholinergic tone in CA1 is thus thought to bias transmission toward SC inputs over the TA. Hyperdopaminergic function, seen in schizophrenia, has likewise been shown to produce selective inhibition of TA input with sparing of SC transmission (Otmakhova & Lisman, 1999). Within CA1, M1 receptors are expressed post-synaptically on pyramidal cells and interneurons, whereas M4 receptors are expressed pre-synaptically on SC terminals. Co-activation of these two receptor subtypes with compounds such as xanomeline could therefore normalize excitatory transmission within CA1 by selectively inhibiting SC pathway signaling while simultaneously enhancing TA signaling. Muscarinic acetylcholine receptors have been shown to modulate synaptic transmission in CA1, but determining the role of specific receptor subtypes has been historically difficult due to a lack of specificity of available agonists and antagonists. Recently, however, highly selective M1 and M4 agonists and positive allosteric modulators have become available to address this question. Using novel selective compounds, we have begun testing the effects of M1 or M4 activation in CA1 using whole cell *ex vivo* recordings from adult rat hippocampal slices. Here, we present preliminary results suggesting that M4 activation selectively reduces SC transmission in CA1 while sparing TA transmission.

Disclosures: C.A. Thorn: A. Employment/Salary (full or part-time):; Pfizer Inc. **J. Edgerton:** A. Employment/Salary (full or part-time):; Pfizer Inc..

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.08/C5

Topic: B.07. Synaptic Transmission

Title: Effects of M1 and M4 muscarinic receptor activators on rat hippocampal neuron activity

Authors: E. UYKAL[1], S. J. NEAL[2], S. GRIMWOOD[1], *J. R. EDGERTON[1];
[1]Neurosci. & Pain Res. Unit, Pfizer, Inc, Cambridge, MA; [2]Novartis, Inc, Cambridge, MA

Abstract: Hippocampal dysfunction is implicated in many neurological and neuropsychiatric disorders. Both hyperexcitability and hypoactivity of hippocampal neurons have been associated with learning and memory deficits. Acetylcholine (ACh) is a key modulator of neural excitability and synaptic transmission in the hippocampus, and loss of cholinergic innervation has also been implicated in episodic memory deficits associated with ageing and disease. Muscarinic receptors (mAChRs) of subtypes M1, M2, M3 and M4 are expressed in the hippocampus, and non-selective mAChR antagonists induce severe learning and memory deficits in both humans and animals. The respective functional roles of the mAChR subtypes have been partially addressed using knockout mice and relatively non-selective modulatory compounds, but the development of highly selective agonists and positive allosteric modulators (PAMs) for M1 and M4 in recent years is enabling more precise dissection of individual mAChR contributions. To gain a better understanding of M1 and M4 regulation of hippocampal circuitry, we have used multi-electrode arrays to record synaptic field potentials and neuronal action potentials from acute hippocampal slices prepared from 7 to 10 week-old rats. Field potential responses were recorded in CA1 stratum radiatum during Schaffer collateral stimulation, and in CA3 stratum radiatum during stimulation of the longitudinal association fibers. Extracellular spike rates were measured in CA1 and CA3. Activation of M1 receptors with the allosteric agonist GSK1034702 increased CA1 neuron firing by a factor of 32 ± 8 fold over baseline, with an EC_{50} of 443 ± 89 nM (n=9). M1 PAMs were active at significantly lower concentrations when applied in the presence of a low dose of an orthosteric agonist, compared to when applied alone, correlating with the results of cell-based assays. Activation of M4 receptors with the M4 PAM VU-0152100 potently inhibited glutamatergic transmission from CA3 to CA1 (EC_{50} = 77 nM $\pm$ 15.3 (n=3); max = 55%). Another M4 PAM, PT-1148, also inhibited the CA3 auto-associative network. These results suggest that M1 activators will increase hippocampal pyramidal neuron excitability and potentially benefit disorders of hippocampal hypofunction, while M4 activators will dampen the CA3 auto-associative network and reduce CA3-to-CA1 transmission, making them attractive therapeutics for disorders of hippocampal hyperexcitability.

Disclosures: **E. Uykal:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **S.J. Neal:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **S. Grimwood:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **J.R. Edgerton:** A. Employment/Salary (full or part-time):; Pfizer, Inc.

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.09/C6

Topic: B.07. Synaptic Transmission

Title: Characterization of a novel M1 mAChR positive allosteric modulator radioligand, [^{3}H]PF-1284

Authors: *D. L. SMITH[1], J. E. DAVOREN[2], J. T. LAZZARO[3], L. ZHANG[2], S. GRIMWOOD[1];
[1]Neurosci., [2]Medicinal Chem., Pfizer Inc, Cambridge, MA; [3]Pharmacokinetic and Dynamics, Pfizer Inc, Groton, CT

Abstract: The orthosteric muscarinic acetylcholine receptor (mAChR) agonist xanomeline achieved a proof of concept in human clinical trials for schizophrenia and Alzheimer's disease. Although reportedly selective for mAChR M1 and M4-subtypes, xanomeline caused adverse effects presumed to be mediated through mAChR M2 and M3 subtypes, which led to a high drop-out rate in these clinical trials. In 2009 the highly M1-selective positive allosteric modulator (PAM) BQCA was disclosed. Based on distribution studies, activation of M1 subtypes should bestow a pro-cognitive effect. The development of an M1 PAM radioligand will be a useful tool in characterizing the allosteric binding site as well as a probe to explore effects of M1 PAMs. PF-1284 is a novel M1 mAChR PAM shown to have an EC_{50} of 21.5 ± 4.37 nM (mean ± SEM; n=6) for stably expressing human M1 CHO cells (hM1), measured using a Ca^{2+} mobilization assay (FLIPR), in the presence of an EC_{20} concentration of ACh. PF-1284 also reduced the affinity of ACh required to inhibit [^{3}H]NMS binding to hM1, left-shifting the ACh EC_{50} by approximately 12-fold at 10 μM. The affinity and binding capacity of [^{3}H]PF-1284 were found to increase in the presence of increasing concentrations of ACh. In the presence of 1 mM ACh (EC_{max}), [^{3}H]PF-1284 was demonstrated to have saturable binding to hM1 with K_d = 6.18 ± 0.097 nM (n=3) and a maximal binding capacity of 6583 ± 373 fmol/mg protein (n=3). The M1 selective PAMs PF-8862 and PT-8345 were shown to inhibit [^{3}H]PF-1284 binding to hM1 with Ki values of 23 ± 3.31 and 68 ± 17 nM, respectively (mean ± SEM; n=3). Conversely, the M1 mAChR allosteric agonist, GSK1034702, showed weak affinity (>30 μM) in the [^{3}H]PF-1284 binding assay. M1 FLIPR EC_{50} values for a cohort of >20 compounds correlated well (r=0.8) with K_i values generated using the [^{3}H]PF-1284 binding assay in the presence of EC_{max} ACh. The [^{3}H]PF-1284 binding assay has been shown to be a useful tool for the investigation of M1 PAMs.

Disclosures: **D.L. Smith:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **J.E. Davoren:** A. Employment/Salary (full or part-time):; Pfizer Inc. **J.T. Lazzaro:** None. **L. Zhang:** None. **S. Grimwood:** None.

Poster

126. Cholinergic Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 126.10/C7

Topic: B.07. Synaptic Transmission

Support: NIH intramural research program

Title: Interplay between cholinergic and galaninergic modulation of GABA release in the basal forebrain

Authors: *J. C. DAMBORSKY, J. L. YAKEL;
Neurobio., NIEHS, Research Triangle Park, NC

Abstract: The basal forebrain (BF) is an important regulator of hippocampal and cortical activity. In Alzheimer's disease, there is significant dysfunction of cholinergic neurons within the BF, along with a hypertrophy of fibers containing the neuropeptide galanin. Despite its potential impact on BF function in Alzheimer's disease, little is known about the role that galanin plays in regulating BF activity under normal or pathological states. To examine the role of galanin in modulating synaptic transmission in the basal forebrain, we performed whole-cell patch clamp recordings from medial septum/diagonal band of broca (MS/DBB) neurons in acute brain slices from 6-10 week old C57/Bl6 mice. Galanin (500 nM) significantly decreased the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) with no effect on spontaneous excitatory postsynaptic currents (sEPSCs). Miniature (m)IPSCs were not significantly altered by galanin, indicating that galanin is working through an action potential-dependent mechanism. In addition to decreasing baseline inhibition within the MS/DBB, galanin also attenuated the increase in sIPSC frequency induced by the nonspecific cholinergic agonist carbachol (50 μM). These results suggest that galanin decreases GABA release in the MS/DBB, and may also interfere with the function of cholinergic receptors on GABAergic neurons. In order to selectively record from and stimulate cholinergic cells within the BF we injected AAV9-dfloxed hChR2(H134R)mCherry into the MS/DBB of 5-7 week old ChAT-cre mice. Whole-cell recordings were performed in acute slices 2-3 weeks following virus injection. Similar to what was seen in WT mice, we found that galanin significantly decreased sIPSC frequency onto identified cholinergic neurons in injected ChAT-cre mice. Activation of cholinergic neurons in the MS/DBB using 470 nm light to stimulate ChR2 increased sIPSC frequency in a subset of cholinergic neurons, an effect that was reduced by galanin. Together, our data point to an intricate relationship between galaninergic and cholinergic signaling in the MS/DBB, whereby galanin decreases inhibitory input onto cholinergic neurons and also attenuates cholinergic-mediated increases in GABA release. This balance between cholinergic and galaninergic modulation of GABA release is likely to be altered in patients with Alzheimer's disease, who have increased galanin expression and diminished cholinergic function in the BF.

Disclosures: **J.C. Damborsky:** None. **J.L. Yakel:** None.

Poster

127. Signal Propagation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 127.01/C8

Topic: B.09. Network Interactions

Support: Foundation for Anesthesia Education and Research Mentored Research Training Grant-Basic Science

Title: Firing statistics of parvalbumin neurons are selectively altered under isoflurane anesthesia

Authors: *A. E. HUDSON[1], J. T. TRACHTENBERG[2];
[1]Anesthesiol., [2]Neurobio., David Geffen Sch. of Medicine, UCLA, Los Angeles, CA

Abstract: General anesthetics suppress neuronal firing rates *in vivo*, yet unconsciousness is produced long before electrical quiescence. As volatile anesthetic concentration increases, population electrical activity becomes dominated by large amplitude, low-frequency oscillations. Using the genetically-encoded calcium indicator GCAMP-6 in mouse prefrontal cortex, we show that firing rates of parvalbumin-expressing inhibitory cells are less suppressed than pyramidal cells in layer 2/3 for isoflurane concentrations greater than 1.25%. Moreover, correlations between parvalbumin cells are higher than between pyramidal cells. This effect persists whether the continuous fluorescence signal or quantal fluorescent events corresponding to calcium fluxes are considered. This suggests that individual members of the cortical microcircuit are differentially affected by isoflurane and that the synchronization observed in population electrical activity may be, at least in part, mediated by increased correlation of firing in parvalbumin expressing inhibitory interneurons.

Disclosures: **A.E. Hudson:** None. **J.T. Trachtenberg:** None.

Poster

127. Signal Propagation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 127.02/C9

Topic: B.09. Network Interactions

Support: JSPS KAKENHI Grant 26630089

JSPS KAKENHI Grant 26-10399

Title: Coordinated activation of neuronal patterns in synchrony of dissociated cortical neurons

Authors: *Y. YADA[1,2,3], R. KANZAKI[1,2], H. TAKAHASHI[1,2];
[1]Res. Ctr. for Advanced Sci. and Technol., [2]Mechano-Informatics, The Univ. of Tokyo, Tokyo, Japan; [3]JSPS Res. Fellow, Tokyo, Japan

Abstract: Coordinated neuronal activity in spontaneous synchrony has been observed in various types of neuronal networks. Even population bursting of dissociated cortical neurons has stable activity patterns, and it has been reported that some neurons act like “leaders” of bursting. Cortical neurons derived from rat embryos were cultured on CMOS-based microelectrode arrays. Spontaneous activity was recorded, and reproducible spatial patterns and their activity were extracted with non-negative matrix factorization. Then, activity of a specific spatial pattern always appeared before occurrence of burst; the pattern was defined as a leader pattern. We expressed the activity propagation from spatial patterns to spatial patterns with the state-space representation, where the activity of leader patterns acts as input to the model and the others as internal state variables. With only the activity of a leader pattern, spatiotemporal activity in population bursts was reconstructed from the model. The results suggested that activation patterns through neuronal synchrony are highly stable and leader neurons play important roles in generation of spontaneous synchrony.

Disclosures: Y. Yada: None. **R. Kanzaki:** None. **H. Takahashi:** None.

Poster

127. Signal Propagation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 127.03/C10

Topic: B.09. Network Interactions

Title: The dependence of the structural and functional changes of inhibitory neurons and glial cells of the human neocortex during chronic ischemia

Authors: *V. AKULININ[1], S. S. STEPANOV[2], A. SERGEEV[2], A. MYTSIK[2];
[1]Omsk State Med. Acad., Omsk, Russian Federation; [2]Omsk State Med. Univ., Omsk, Russian Federation

Abstract: The study of brake interneurons and glial cells of different lobes of the human neocortex during chronic ischemia. The actual data obtained on the intraoperative material (removal of tumors, n=19). For immunofluorescence studies of brake interneurons used primary rabbit polyclonal antibodies to calbindin D28k and neuropeptide Y (NPY), for glial cells to glial fibrillary acidic protein (GFAP). Visualization of the immune reaction was performed using goat polyclonal secondary antibody to rabbit immunoglobulin, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (TR) No. ab6719; Abcam, Cambridge, England), diluted 1:200. Antibodies associated with the fluorescent dye Texas Red® Sulfonyl Chloride (molecular weight 625 daltons). On the microscope, Axioskop 40, Karl Zeiss, equipped with a mercury lamp HBO 100, camera on the CCD sensor - Axio CamMRc and lens EC Plan-Neofluar x40, aperture of 0.9, was done digital micrograph image size x pixels, the actual size h μm (38280 μm2). Morphometric image analysis was performed using the ImageJ 1.46. We determined the overall numerical density (per 1 mm2) and total area (μm2) of the fluorescent marker pellets NPY and GFAP in the field of view of the drug. The statistical hypotheses were carried out using the software StatSoft Statistica 8.0. Used the nonparametric Mann-Whitney test and Spearman correlation analysis. The results are presented as median (lower, upper quartiles). The null hypothesis was rejected at $p < 0.05$. It is established that chronic ischemia in all fractions of neocortex in the brake interneurons activated synthesis calbindin and NPY, and in glial cells GFAP. The relative size of the labels NPY in the frontal cortex, in comparison with the control, increased from 2.20 (1.87; 2.50) to 4.28 (3.80; with 4.86) %. parietal - 2.13 (1.66; 2.76) to 3.93 (3.07; 4.61) %. temporal - 1.80 (1.50; 2.34) to of 3.91 (3.47; 4.67) %. occipital - 1.91 (1.71; 2.41) to 4.11 (3.30; 4.24) %. The area of GFAP on average increased in to 1.93 times - from 5.9 (4.0-8.3) % to 11.4 (8.7-14.5) % field of view. Revealed a strong positive stochastic relationship ($r = 0.82$; $p = 0.002$) between the relative area of the field of view of the brake interneurons and astrocytes. High level of expression of a specific protein in inhibitory interneurons was accompanied by high levels of expression of GFAP in glial cells. Probably, in the aggregate, it provides the regulation of distribution etc. excitatory impulses and the removal of excess glutamate from the extracellular space.

Disclosures: V. Akulinin: None. **S.S. Stepanov:** None. **A. Sergeev:** None. **A. Mytsik:** None.

Poster

127. Signal Propagation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 127.04/C11

Topic: B.09. Network Interactions

Support: CIHR

NSERC

MRC

Title: A long-range disinhibitory circuit between the hippocampal area CA1 and the subiculum

Authors: R. FRANCAVILLA[1], E. P. MUNOZ[1], S. CHAMBERLAND[1], X. LUO[1], O. CAMIRE[1], K. WAGNER[2], P. SOMOGYI[2], *L. TOPOLNIK[1];
[1]CRIUSMQ, Laval Univ., Quebec, QC, Canada; [2]MRC Brain Network Dynamics Unit, Dept. Pharmacol., Oxford Univ., Oxford, United Kingdom

Abstract: Hippocampal long-range GABAergic projection (LRP) neurons comprise a diverse population of cells projecting to the medial septum, the subiculum, the medial entorhinal cortex, the retrosplenial cortex, the contralateral hippocampus as well as to the indusium griseum. Despite their potentially important role in coordinating rhythmic activity among functionally relevant brain areas, very little is currently known about their physiological parameters and cellular synaptic targets. Using a combination of retrograde labeling, dual patch-clamp recordings, anatomical analysis and optogenetics, we identified a subtype of vasoactive intestinal polypeptide (VIP)/muscarinic acetylcholine receptor type 2 (soma and dendrites) coexpressing interneuron with somata and dendrites located within stratum oriens and alveus of the hippocampal CA1 region, and an axon making local collaterals within CA1 as well as projecting to the subiculum (VIP-LRPs). Locally, VIP-LRPs could establish type 2 synapses with perikarya and dendrites of CA1 nonpyramidal neurons. In particular, VIP-LRPs innervated oriens-lacunosum moleculare interneurons via dendritically located synapses with a high probability and large unitary conductance. Furthermore, VIP-LRPs were coupled through GABAergic synapses and gap junctions. Interestingly, the electrotonic delay changed little with coupling ratio, pointing to phase compensation between electrically coupled neurons depending on dendritic gap junction location. In summary, the molecular profile and local targets of VIP-LRPs distinguish them from other hippocampo-subicular projecting nonpyramidal cells. The functional significance of this synaptically and electrically coupled disinhibitory circuit is currently under investigation.

Disclosures: R. Francavilla: None. **E.P. Munoz:** None. **S. Chamberland:** None. **X. Luo:** None. **O. Camire:** None. **K. Wagner:** None. **P. Somogyi:** None. **L. Topolnik:** None.

Poster

127. Signal Propagation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 127.05/C12

Topic: B.09. Network Interactions

Title: Investigation of theta-gamma coupled cortical traveling waves based on electrocorticography

Authors: Y. PARK[1], T. KIM[1], J. KIM[1], J. PARK[1], J. KANG[2], I. KIM[1], *D. JANG[1];
[1]Biomed. Engin., Hanyang Univ., Seoul-City, Korea, Republic of; [2]Neurol., Asan Meidcal Ctr., Seoul, Korea, Republic of

Abstract: In recent studies, coupled oscillations between the theta- and gamma-frequency bands have been investigated by electrocorticography (ECoG) signals in humans. Cortical propagation of low-frequency activity has also been shown in several studies. In this study, we observed the cortical traveling theta waves coupled with gamma power and showed regions that gamma activities coordinated by traveling theta waves. ECoG data were used that were acquired from grid electrodes covered frontal, temporal and parietal regions. To remove line noise, a 60Hz notch filter was used. Independent component analysis and common average reference were also applied to noise rejection. In order to verify the center frequencies of coupled low-frequency phase and high-frequency power, we obtained the phase-amplitude coupling (PAC) index that is calculated by entropy difference between the power distribution for each phase and the uniform distribution. Low- and high-frequency of maximum PAC index were selected as coupled frequencies. To observe traveling theta waves, we used concepts of reference location and motifs (Bahramisharif et al., 2013). Motifs consisted of four or more consecutive electrodes that have a linear increasing (or decreasing) pattern of phase respect to a reference location. In addition, only electrodes with a phase-locking value (PLV) over 0.1 with respect to the reference location. The band-pass filtered data with theta center frequency were used to calculate PLV and count motifs. To estimate PLV, the averaged phase difference between different electrodes over time was calculated and the angle of averaged phase difference was applied to confirm motifs respect to reference locations. We counted the number of motifs with respect to all reference locations and higher counted motifs were considered as main motifs of traveling theta waves. Furthermore, in order to identify which motifs coordinate some gamma activities, we also considered PAC index of motif electrodes with theta and gamma center frequency, As results of this study, we observed two streams of traveling theta waves coupled with gamma power activities. First coupled traveling waves propagated from anterior to posterior middle temporal lobe. The other traveling waves propagated from dorsal to ventral posterior parietal lobe.

Disclosures: **Y. Park:** None. **T. Kim:** None. **J. Kim:** None. **J. Park:** None. **J. Kang:** None. **I. Kim:** None. **D. Jang:** None.

Poster

127. Signal Propagation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 127.06/C13

Topic: B.09. Network Interactions

Support: The Hartwell Foundation

Title: Abnormal cell-intrinsic excitability and cortical network activity in serotonin-deficient mice

Authors: *R. FERNANDEZ GALAN[1], P. A. PUZEREY[2], N. X. KODAMA[2];
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Abstract: Neurons originating from the raphe nuclei of the brainstem are the exclusive source of serotonin (5-HT) to the cortex. Their serotonergic phenotype is specified by the transcriptional regulator *Pet-1*, which is also necessary for maintaining their neurotransmitter identity across development. Transgenic mice in which the *Pet-1* gene is genetically knocked out (KO), show a dramatic reduction (ca. 80%) in forebrain 5-HT levels, yet no investigations have been carried out to assess the impact of such severe 5-HT depletion on the function of target cortical circuits. Using whole-cell patch clamp techniques, 2-D electrode arrays, morphological reconstructions of cortical neurons, and animal behavior, we investigated the impact of 5-HT depletion on cortical cell-intrinsic and network excitability. We found significant changes in several parameters of cell-intrinsic excitability in cortical pyramidal cells, as well as an increase in spontaneous synaptic excitation through 5-HT3 receptors. These changes are associated with increased cortical network excitability and oscillatory activity in a 5-HT2 receptor-dependent manner, consistent with previously reported hypersensitivity of cortical 5-HT2 receptors in mutant mice. In contrast, recordings of network activity with 2-D arrays (120 electrodes; 100 um pitch) revealed that neuronal activity propagates less and more slowly in the neocortex of *Pet-1* KO mice than in wild-type controls. A possible, mechanistic explanation for these results is provided by the analysis of pyramidal cell morphology, which reveals a significant reduction in the complexity of their dendritic arbors. This likely leads to reduced network connectivity, acting as a compensatory mechanism for increased excitability at the single-cell level. Consistent with this interpretation, when we carried out experiments with convulsant-induced seizures to asses cortical excitability *in vivo*, we observed no significant differences in seizure parameters between wild-type and *Pet-1* KO mice. Our findings provide the first evidence for functional changes in neuronal and network excitability in target structures of serotonergic neurons in mice lacking the *Pet-1* gene.

Disclosures: **R. Fernandez Galan:** None. **P.A. Puzerey:** None. **N.X. Kodama:** None.

Poster

127. Signal Propagation

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Topic: B.09. Network Interactions

Support: The Hartwell Foundation

Title: Imaging neuronal network activity with 2D electrode arrays

Authors: ***N. KODAMA**[1], P. PUZEREY[3], R. FERNANDEZ GALAN[2];
[1]Dept. of Neurosciences, [2]Case Western Reserve Univ., Cleveland, OH; [3]Cornell Univ., Ithaca, NY

Abstract: Current approaches to visualizing and analyzing neuronal activity at the circuit and microcircuit levels are mostly based on optical imaging techniques, using calcium and voltage-sensitive dyes. The former offers good spatial resolution to identify cell clusters and microcircuits but relatively poor temporal resolution, which is limited by the dissociation time constant of the dyes. In contrast, voltage-sensitive dyes offer high temporal resolution, comparable to that of electrophysiological recordings, but poor visualization of neuronal circuits, since the dyes mostly localize in dendritic arbors. For both techniques, the multiplexing of data acquisition hardware also imposes a trade-off between spatial and temporal resolutions. We present an alternative approach to imaging neuronal activity, not with optical signals but electric ones, which has both high temporal and spatial resolutions. To this end we use high-density, two-dimensional electrode arrays (120 electrodes; 1.2 sq. mm). Simultaneous recordings from all the electrodes over a patch of brain tissue are plotted as dynamic maps of the electric potential across neuronal networks, from which we can quantify the emergence, propagation speed and direction of neuronal activity, either evoked or spontaneous. Also, a current source density analysis allows us to track over time the current sources and sinks in the network, and determine their co-localization with different cell groups and types. We demonstrate how this approach to investigating neuronal network activity allows us for the first time to detect abnormalities of cortical circuits in animal models of disease in an accurate and highly efficient manner.

Disclosures: **N. Kodama:** None. **P. Puzerey:** None. **R. Fernandez Galan:** None.

Poster

127. Signal Propagation

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Support: NIMH Grant MH071739

NINDS Grant NS074785

NINDS Grant NS024067

Title: Disinhibitory control of CA1 signal propagation through 5HT3a+ interneurons

Authors: *B. SUUTARI, R. W. TSIEN;
NYU Neurosci. Inst., New York, NY

Abstract: Signal transduction and information flow through the hippocampus is dependent on the strength and timing of excitatory and inhibitory transmission. Inhibitory neurons (INs) regulate the synaptic integration window and spike threshold of pyramidal (pyr) neurons. Given the variety of IN types in the hippocampus, the interplay between excitation and inhibition is dynamic and complex. One recently described aspect of the hippocampal excitatory-inhibitory circuit is disinhibition, or the inhibition of INs by other INs. Some CA1 INs that express the 5HT3a receptor (5HT3aR) inhibit parvalbumin (PV+), 5HT3a- INs. PV+ INs exert the greatest influence on feed-forward, perisomatic inhibition, which is responsible for regulating the ability of CA1 pyr cells to spike in response to excitatory synaptic input. Thus, 5HT3aR+ interneurons could in principle affect the entire CA1 microcircuit through disinhibition. Indeed we found that application of the 5HT3aR specific agonist 1-(m-chlorophenyl)-biguanide (mCPBG) caused a significant increase in the responsiveness of pyr neurons to Schaffer collateral (SC) inputs, consistent with the disinhibitory hypothesis. To dissect the mechanism of this improvement in spike throughput, we analyzed the interactions between 5HT3aR+ INs, PV+ INs and CA1 pyr neurons, specifically focusing at first on possible disinhibitory effects. To isolate the effect of the 5HT3aR+ IN→PV+ IN→Pyr neuron pathway, we held pyr neurons at 0 mV to trigger endocannabinoid (EC) release and thus depress the 5HT3aR+ IN→ Pyr synapse. Under these conditions, we observed a significant decrease in IPSC frequency in Pyr cells. Direct recordings from PV+ INs demonstrated a corresponding increase in the frequency but not the amplitude of sIPSCs. The situation was more complex when the 5HT3aR+ IN→ Pyr connections were also allowed to participate, either by holding pyr neurons at their resting potential or by blocking CB1Rs with the antagonist AM251 while holding at 0 mV. In either case, mCPBG caused a slight, transient increase in sIPSC frequency, suggesting that 5HT3aR+ INs directly inhibit pyr

cells. The direct influence on pyr neurons may provide a useful inhibitory tone to suppress spontaneous pyr activity, even as the disinhibitory influence of 5HT3aR+ INs on PV+ neurons is dominant in enhancing spike throughput. In future experiments, we plan to use optogenetic stimulation in transgenic mouse lines with labeled 5HT3aR INs to clarify the role of 5HT3aR+ synapses on the CA1 microcircuit in both disinhibition and direct inhibition. These studies will provide insight on microscopic and mesoscopic aspects of CA1 information processing.

Disclosures: **B. Suutari:** None. **R.W. Tsien:** None.

Poster

127. Signal Propagation

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Program#/Poster#: 127.09/C16

Topic: B.09. Network Interactions

Support: NIMH IRP

Title: Neuronal avalanches during motor and cognitive tasks in macaque monkeys

Authors: ***S. YU**[1,3], T. LINS RIBEIRO[1], S. CHOU[1], A. MITZ[2], R. SAUNDERS[2], D. PLENZ[1]; [1]Section on Critical Brain Dynamics, [2]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Brainnetome Ctr., Inst. of Automation, Beijing, China

Abstract: Ongoing neuronal dynamics has been shown to be maintained at a phase transition between an inactive (subcritical) and a self-sustained state (supercritical), through a precise balance of excitation/inhibition. At this critical point, spatiotemporal clusters of synchronized firing (as measured through large negative deflections of local-field potentials, or nLFPs) emerge as a scale-free activity, called neuronal avalanches, and characterized by power-law size distributions. Those were studied in many different experimental setups, but mostly restricted to ongoing dynamics (avalanches in freely-behaving animals were studied in the context of spiking activity). Here we analyze neuronal avalanches using chronically implanted 10x10 microelectrode arrays in monkeys (adult Macaca mulatta) subjected to either 1) self-initiated touching of a sensor pad for rewards (premotor cortex) or 2) a visual-motor mapping task in which food rewards were retrieved from dispensers according to a visual cue (dorsal-lateral prefrontal cortex): they had to reach for the feeder either at the left or at the right side, depending on which cue was presented. We found that there was a modulation of the rate of nLFPs before the touching (task 1) and during the cue presentation (task 2), but only for trials in which the correct feeder was the left one (we call these left trials). Together with that increase in the

activity rate, we observed a decrease in the Fano factor, a measure of the variance in the signal over different trials, in agreement with what is found in the literature. This period with increased activity rate also lead to larger avalanches which was reflected in the corresponding size distributions, suggesting the conclusion that the system transiotions to the supercritical state during processing of information. However, when a normalization of the temporal binning employed in the avalanche analysis was performed the size distributions recovered their power-law nature, indicating that the critical state is maintained even though there is an evoked response.

Disclosures: **S. Yu:** None. **T. Lins Ribeiro:** None. **S. Chou:** None. **A. Mitz:** None. **R. Saunders:** None. **D. Plenz:** None.

Poster

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Support: NRF Grant 2013-R1A1A2053280

Title: Dynamic activation of feedforward and feedback inhibition enables reliable propagation of sparse *in vivo* spike patterns in feedforward network

Authors: *H. JANG, J. KWAG;
Dept. of Brain and Cognitive Engin., Korea Univ., Seoul, Korea, Republic of

Abstract: Spike patterns *in vivo* are sparsely-activated in subsets of neurons and are temporally complex, containing precisely timed spikes with instantaneously varying spike rate. Understanding how these neural code-containing *in vivo* spike patterns propagate in neural network is critical for elucidating the principles for neural information processing. Feedforward network (FFN) consisting of sequentially connected layers of neural network has been used as a general framework in investigating the network conditions for neural code propagation. While most studies focused on FFNs composed of only excitatory (EX) neurons, the function of inhibition in neural code propagation has never been investigated. By including two distinct inhibitory networks, feedforward (FF) or feedback (FB) inhibitory circuit, to FFN, we investigated the network requirements for the reliable propagation of sparse *in vivo* spike patterns. Six-layer EX FFN was constructed with 200 single compartment Hodgkin-Huxley EX neurons per layer. Three different types of FFNs were built with 50 inhibitory neurons per layer:

EX FFN with intra-layer FB (EX-FB), with inter-layer FF (EX-FF) and with both FB and FF (EX-FB-FF) inhibition. To make the *in vivo* input sparse, the *in vivo*-recorded spike train from thalamus was simulated to 20% of layer 1 EX neurons while the remaining 80% EX neurons were made to fire asynchronously. Similarity ratio (*SR*) between *in vivo* spike train and the output-layer spike train was used to quantify the reliability of propagation. Instantaneous firing rate (IFR) was used to measure rate code propagation. The sparse *in vivo* spike train could not reliably propagate to the final layer of the EX FFN model (*SR* = 0.43). In contrast, EX-FB and EX-FF FFNs could reliably propagate the *in vivo* spike train (EX-FB: *SR* = 0.56; EX-FF: 0.63), suggesting that inclusion of inhibitory network enhances spike pattern propagation. Analysis of the relationship between IFR of *in vivo* pattern and *SR* revealed that spikes with high IFR (α and β band) reliably propagated in EX-FB FFN (α: *SR* = 0.78; β: 0.64), while spikes with low IFR (δ and θ band) reliably propagated in EX-FF FFN (δ: *SR* = 0.79; θ: 0.81). Due to the complementary frequency-selective band-pass filtering feature of FB and FF, the sparse *in vivo* spike pattern could be reliably propagated in EX-FB-FF FFN (*SR* = 0.86), especially with FF:FB ratio of 0.66:0.34. Our results demonstrate for the first time that critical balance between FB and FF inhibition is essential for reliable propagation of sparse *in vivo* spike patterns containing neural codes and the dynamic recruitment of FB and FF inhibition might serve as a key gating mechanism for neural code propagation *in vivo*.

Disclosures: **H. Jang:** None. **J. Kwag:** None.

Poster

127. Signal Propagation

Location: Hall A

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Program#/Poster#: 127.11/C18

Topic: B.09. Network Interactions

Support: Intramural Research Program, NIH

Title: Exactly solved models of neuronal spike statistics with absolute refractory period

Authors: ***S. CHANDRA**, B. RICHMOND;
NIMH, Lab. of Neuropsychology, NIH, Bethesda, MD

Abstract: An accurate description of the statistics of spike trains in the human brain is essential to understand how the information in the brain is coded and processed. As a first approximation the spike trains in the cortical neurons have a statistics resembling a Poisson process. An obvious reason for deviations from Poisson statistics is the refractory period of a neuron which prevents

two consecutive spikes from occurring very close to each other. Here we present several simple exactly solvable models of neuronal spike dynamics. We start with dynamical rules for spike dynamics without any refractory period that gives a Poisson spike train as the output when a Poisson spike train is given to it as an input. This model is exactly equivalent to an analytically solvable queueing model called M/M/1 queue. We then present three more models of neuronal spike dynamics which take into account the absolute refractory period of a neuron. Due to finite decay time of conductances, an EPSP during the absolute refractory period may cause a spike after the absolute refractory period is over. Alternately, an EPSP during the absolute refractory period may simply be lost. Our models for the absolute refractory period have dynamical rules that take in account these various possibilities. We solve them exactly using Markov chains and equivalence to queueing models to obtain simple analytic expressions for the quantities of interest.

Disclosures: **S. Chandra:** None. **B. Richmond:** None.

Poster

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Topic: B.09. Network Interactions

Support: NEUROSCAFFOLDS-FP7-NMP-604263

CARBONANOBRIDGE-ERC-2008-227135

PRIN-MIUR n. 2012MYESZW

Title: A novel Carbon Nanofibers-based 3D nanoscaffold to promote regrowth and bridging between cultured spinal explants

Authors: *S. USMANI[1], E. AURAND[1,3], D. SCAINI[3], S. BOSI[3], M. MEDELIN[3], M. PRATO[3], L. BALLERINI[2];
[1]Neurobio., [2]SISSA, Trieste, Italy; [3]Univ. of Trieste, Trieste, Italy

Abstract: Functional rehabilitation of injury in the Central Nervous System (CNS) is a major challenge in the field of neuroscience. Carbon-based materials made up of multi walled carbon nanotubes (MWCNTs) interfaced to neural networks have previously been shown to impact synapse formation and neuronal growth [1,2,3,4,5,6]. In this work, we demonstrate an innovative, implantable scaffold, which could effectively reconstruct neural growth *in vitro*.

However, issues such as biocompatibility, structural framework, and functional effectiveness of the material pose serious questions as to their practical uses [7]. We tested a carbon-based freestanding self-assembly of nanotubes [8] in the form of three-dimensional carbon nano-fibers scaffold (3DCNF) to mimic the native CNS tissue topography. We obtained embryonic organotypic spinal slices, which were then cultured on 3DCNF in pairs at a distance known to cause impaired functional re-connection in basal conditions [9]. Electrophysiological recordings of the local field potential were performed on 10-17 days *in vitro* cultures. Simultaneous assessment of rhythmic activity triggered by Strychnine and Bicuculline application [10,11] revealed a higher cross correlations between each pair of slices on 3DCNF samples compared to controls (88% in 3DCNF vs 27% in Control). Moreover, 77% of stimulated 3DCNF samples were cross entrained by dorsal horn stimulation of one of the two slices, while this effect was seen in only 29% of stimulated Control samples. Immunohistochemistry demonstrated rewiring of neurites in a complex mesh of neuronal network outgrowth throughout the scaffold, including into the third dimension. Furthermore, 3DCNF scaffolds were implanted into adult rat brain and showed a lack of significant glial scar response, assessed by GFAP immunostaining, demonstrating good *in vivo* biocompatibility. Moreover, the presence of β-tubulin III-positive neurons within the scaffold suggests that neurons were able to migrate into the 3DCNF implant. Our results show that these 3DCNF materials provide an innovative scaffold for increased neuronal reconnection and a promising tool for future interfaces developments. References: 1.Fabbro Adv Drug Deliv Rev 65, 2034 (2013) 2.Fabbro ACS Nano 6, 2041 (2012) 3.Cellot J Neurosci 31, 12945 (2011) 4.Cellot Nat Nanotechnol 4, 126 (2009) 5.Mazzatenta J Neurosci 27, 6931 (2007) 6.Lovat Nano Lett 5, 1107 (2005) 7.Straley J Neurotrauma. 27, 1 (2010) 8.Camilli Nanotechnology. 25, 065071 (2014) 9.Heidemann Neuroscience 262, 40 (2014) 10.Ballerini J Physiol. 517, 459 (1999) 11.Streit J Neurophysiol 70, 871 (1993)

Disclosures: **S. Usmani:** None. **E. aurand:** None. **D. Scaini:** None. **S. Bosi:** None. **M. medelin:** None. **M. Prato:** None. **L. ballerini:** None.

Poster

127. Signal Propagation

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Topic: B.09. Network Interactions

Support: SFB 1089

Title: Recurrent neuronal microcircuits of the medial septum control locomotor activity

Authors: *L. SOSULINA[1], H. KANEKO[1], F. FUHRMANN[1], D. JUSTUS[1], T. BEUTEL[1], D. FRIEDRICHS[1], S. SCHOCH[2,3], M. K. SCHWARZ[4], M. FUHRMANN[5], S. REMY[1,2]; [1]Neuronal Networks Group, German Ctr. for Neurodegenerative Dis., Bonn, Germany; [2]Dept. of Epileptology, Univ. of Bonn Med. Ctr., Bonn, Germany; [3]Section of Translational Epilepsy Research, Dept. of Neuropathology, Univ. of Bonn Med. Ctr., Bonn, Germany; [4]Functional Neuroconnectomics Group, Dept. of Epileptology, Life & Brain Center, Univ. of Bonn, Med. Sch., Bonn, Germany; [5]Neuroimmunology and Imaging Group, German Ctr. for Neurodegenerative Dis., Bonn, Germany

Abstract: We have shown an important role of the medial septal nucleus and the diagonal band of Broca (MSDB) in the control of locomotion and theta oscillations. Three major cell types have been described in the MSDB: Excitatory glutamatergic VGluT2 neurons, inhibitory PV interneurons and cholinergic ChAT neurons. To better understand the role of each of these neuron types during locomotor behavior, we first attempted to characterize their properties and their synaptic connectivity within the medial septal network. Using VGluT2-cre, PV-cre and ChAT-cre mouse lines and a medial septal injection of pAAV2.1-EF1a-double floxed ChR2-EYFP-WPR (H134R) we could identify the respective cell types in acute brain slices via eYFP expression. In whole-cell patch-clamp recordings of eYFP labeled neurons we could clearly separate the three neuron types according to their electrophysiological parameters. Thus, a discriminant analysis allowed the discrimination of non-labeled neurons. Using light activation of ChR2 expressing neurons, we could assess the connection probabilities. We found a strong non-selective connectivity of VGluT2 neurons onto all three cell types. Following VGluT2 stimulation 87 % neurons received monosynaptic EPSPs with an average latency of 3.9±0.17 ms (n=100). PV stimulation led to monosynaptic IPSPs in 54 % of all neurons (n=62). In ChAT-cre mice single light stimulation (15 ms) or 2 s train stimulation at 10 Hz did not evoke synaptic responses in standard experimental conditions (n=29). With increasing the stimulation duration to 6 s a slow depolarization was observed in 7 neurons, consistent with volume transmission. We concluded that VGluT2 neurons represent a main source of excitation within the MSDB, whereas PV interneurons suppress network activity via recurrent inhibition. To understand the role of recurrent PV mediated inhibition during locomotor behavior, we then blocked the glutamatergic excitation of recurrent PV interneurons using intraseptally applied NBQX/D-AP5 in head-fixed mice performing o a linear treadmill. We hypothesized that this manipulation would lead to higher activity of VGluT2 neurons due to the loss of inhibitory control during VGluT2 stimulation. Higher activity of VGluT2 neurons then should in turn result in increased locomotor activation. Indeed, with reduced recurrent inhibition VGluT2-stimulation-induced locomotion was performed at higher speed and initiated more promptly. Strikingly, in the presence of NBQX/D-AP5 locomotion of the mice persisted even after the cessation of stimulation confirming an important role of recurrent MSDB PV interneurons in the control of locomotor activity.

Disclosures: **L. Sosulina:** None. **H. Kaneko:** None. **F. Fuhrmann:** None. **D. Justus:** None. **T. Beutel:** None. **D. Friedrichs:** None. **S. Schoch:** None. **M.K. Schwarz:** None. **M. Fuhrmann:** None. **S. Remy:** None.

Poster

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Support: FWO (Fonds Wetenschappelijk Onderzoek - Vlaanderen) post-doc grant 12L5112L

Title: Emergent frequency components by non-linear dynamics of pyramidal cells in visual cortex

Authors: *N. KOGO;
Lab. of Exptl. Psychology, Univ. of Leuven, Leuven, Belgium

Abstract: Frequency tagging technique is a powerful tool applied in the “steady state visual evoked potential” (SSVEP, Regan, 1966, Electroen Clin Neuro 20, 238-248) in EEG recording. It allows detecting specific neural responses to specific (tagged) input components from entangled populations of neural activities. Furthermore, non-linear frequency components (intermodulation components, IMs) can be detected which indicate the neural interaction between the signals carrying separate frequencies. Recent advance has shown that IM can be used as “neural signature” of interactions that are linked to perceptual organization such as figure-ground organization, multi-stable perception, illusory surface perception and face perception (Aissani et al., 2011, Brain Res 1408, 27-40; Appelbaum et al., 2006, J Vision 8(9), 1-19; Boremans et al., 2013, J Vision 13(11), 1-18; Gundlach et al., 2013, Biol Psychol 94(1), 55-60; Sutoyo et al., 2009, Brain Res, 1251, 245-255). As this approach to investigate the neural activities underlying visual perception advances, it is quite important to investigate the non-linear neural dynamics that give rise to IMs. Magnai et al. (2011, J Comput Neurosci, 31(3), 595-607; 2014, Frontiers Cellular Neurosci, 8(239)) showed non-linear neural responses to multi-frequency current injection to stellate cells in rat entorhinal cortex. To further investigate the phenomenon, I recorded the frequency responses of pyramidal cells in layer 2/3 of mice primary visual cortex to a combined frequency inputs. Patch (or double patch) clamp recordings in whole cell configurations were obtained from pyramidal cells. In the first experiment, a current with combined sine waveforms (+/-50pA, f1=7.50Hz and f2=5.45Hz) was injected to the cell at -70mV (subthreshold condition) and at -50mV (suprathrehold condition, that evokes series of

action potentials). In the second experiment, extracellular electrical stimulation was applied at two locations proximal to the cell's apical dendrite. The frequency components of the responses were analyzed. While double spectrum peaks, reflecting the input frequency components (f1, f2), were found in all conditions, extra frequency components were found only in the suprathreshold condition. In addition to the harmonics of f1 and f2, discrete peaks were identified at the frequencies of IMs (most prominently at f1-f2, 2f2-f1, 2f1-2f1, and f1+f2). When only one of the two input frequencies was given at the suprathreshold condition, the power spectrum showed peak at the given frequency and at its harmonics. Hence, at the depolarized state, nonlinear frequency components emerge that are likely the origin of IMs in SSVEP.

Disclosures: N. Kogo: None.

Poster

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Support: NEUROSCAFFOLDS-FP7-NMP-604263

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PRIN-MIUR n. 2012MYESZW

Title: 3D appealing: novel nanostructured CNT scaffolds induce three-dimensional neuronal network reconstruction and potentiated signalling

Authors: *D. SCAINI[1], R. RAUTI[1], S. BOSI[1], M. PRATO[1], L. BALLERINI[1,2];
[1]Univ. of Trieste, Trieste, Italy; [2]S.I.S.S.A., Trieste, Italy

Abstract: Modern neuroscience increasingly relies on material science and nanotechnology tools to engineer 3D neuronal network models. *In vitro* recreated 3D neuronal circuits will substantially increase the relevance of results from cultured to whole-brain networks and will promote enabling technologies for neuro-engineering applications. Recently we develop a novel composite materials obtained entrapping carbon nanotubes (CNTs) in an elastomeric porous material able to instruct *genuine* 3D growth and organisation of living neurons inside it. Such system allows investigating the emerging activity, in terms of calcium signals, of small clusters of neurons as a function of the interplay between the 2D or 3D architectures and network dynamics. We observed an intrinsic ability of the 3D geometry to improve functional

organization and synchronization in small neuronal assembly and we propose a mathematical model of network dynamics that supports such a result. Finally, scaffolds nanostructured *via* CNTs insertion remarkably affected network behaviour, allowing for the first time to exploit nanomaterial/cell interfacing in a 3D growth support. Our 3D system represents not only a simple, reliable construct able to improve the complexity of current tissue culture models but opens to the development of new tissue engineering tools and paradigms for unconventional neuroprosthetic and neuromedicine applications. **References:** [1] Cellot G, Cilia E, Cipollone S, Rancic V, Sucapane A, Giordani S, Gambazzi L, Markram H, Grandolfo M, Scaini D, Gelain F, Casalis L, Prato M, Giugliano M, Ballerini L. Carbon nanotubes might improve neuronal performance by favouring electrical shortcuts. Nat Nanotechnol. 2009, 4(2):126-33. [2] Fabbro A, Villari A, Laishram J, Scaini D, Toma FM, Turco A, Prato M, Ballerini L. Spinal cord explants use carbon nanotube interfaces to enhance neurite outgrowth and to fortify synaptic inputs. ACS Nano. 2012, 6(3):2041-55 [3] Bosi S, Rauti R, Laishram J, Turco T, Lonardoni D, Nieus T, Prato M, Scaini D, Ballerini L From 2D to 3D: novel nanostructured scaffolds to investigate signalling in reconstructed neuronal networks. Sci. Rep. 2015, 5:9562

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Poster

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Support: NIH Grant NS052233

Title: Spatial motifs in a living engineered hippocampal circuit

Authors: ***G. J. BREWER**[1], A. BHATTACHARYA[1], T. B. DEMARSE[2], B. C. WHEELER[2], H. DESAI[1];
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Abstract: To encode memories, the tri-synaptic loop in the mammalian hippocampus exhibits unidirectional feed-forward communication from the dentate gyrus (DG) region to the next region, CA3. The signal provided for these feed-forward connections in functional organization of the different regions is yet to be understood. One way to gain insight is to study the number

and types of functional blocks or patterns of activity (motifs). Here we reconstructed two regions of the hippocampus in a unique cell culture system. Neurons were dissected from the DG and the CA3 regions of P3 rats and cultured in distinct wells or chambers connected by microtunnels carrying only axons for communication between the regions. The system sits on top of an electrode array to be able monitor each subnetwork and the communication between them. Microfabrication (MEMS) technology was used to create two wells connected by micro-scale tunnels. The tunnels facilitate growth of axons between wells loaded with cultured cells from different hippocampal subregions. Spike trains obtained from each electrode were smoothed using a 32-ms Gaussian kernel to yield an activity profile at each electrode. A dendrogram clustering algorithm revealed motifs or patterns of spatial activity. Spike shuffling across electrodes was used to estimate the chance occurrence of such patterns in each of 25 trials as the statistical control. We examined the number of spontaneous spatial motifs in CA3 when CA3 is connected to DG (DG-CA3), when CA3 is connected to other CA3 neurons (CA3-CA3) and in DG connected to DG networks (DG-DG). The higher number of CA3 events identified in DG-CA3 compared to CA3-CA3 and DG-DG suggests longer-range connectivity in the natural configuration. The percentage of CA3 events requiring $\geq$75% of the tunnels active preceded by DG or opposing compartment events was higher in DG-CA3 than CA3-CA3 or DG-DG, suggesting robust wiring of DG to CA3. The percentage of CA3 events preceded by tunnel events (70%) was higher in DG-CA3 networks than for the reverse direction (20%), remarkably different from the percentages for the control CA3-CA3 and DG-DG networks. This shows the connection bias of the natural DG-CA3 network. Overall, motifs in DG-CA3 networks occurred more frequently (3.6 ± 0.9 motifs in 3 min) than expected by chance in shuffles (1.7 ± 0.4) ($p<0.001$) and at only chance frequencies in DG-DG networks. This suggests that the anatomically accurate feed-forward connections result in significant spontaneous spatial configurations of activity at measured times.

Disclosures: **G.J. Brewer:** None. **A. Bhattacharya:** None. **T.B. Demarse:** None. **B.C. Wheeler:** None. **H. Desai:** None.

Poster

127. Signal Propagation

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National Science Foundation CAREER Award # 095286

National Science Foundation Grant # DGE-0903637 IGERT: Integrative research in motor control and movement

Title: Ensemble methods enhance the identification of synaptic connections from multineuronal activity patterns

Authors: *D. Y. LITTLE, B. CHAMBERS, J. DECHERY, J. N. MACLEAN;
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Abstract: If we are to mechanistically understand information processing in the neocortex, it is crucial to map synaptic connectivity to cortical circuit dynamics. Circuit spike activity, however, is sparse and diverse in ways that are not easily predicted from connection patterns alone (Barth and Poulet 2012). Functional connectivity maps are an important bridge between static connectivity and dynamic information processing. Previously we have shown that statistical dependencies in the spiking between two neurons have the capacity to reveal a select subset of causal monosynaptic connections. However, the ability to identify a connection is in part determined by the inference algorithm employed. Different inference methods, such as lagged correlation, mutual information, and transfer entropy, capture different statistical correlations in population dynamics and identify distinct, though overlapping, sets of connections. This is the optimal situation in which an ensemble method that combines multiple inference algorithms should obtain better predictive performance than can be achieved using any of the constituent inference algorithms separately. Here, we show that an ensemble of linearly combined inference methods can infer synaptic connectivity with greater precision than any of the underlying methods alone. Using conductance based leaky integrate-and-fire network models we demonstrate that a simple random search algorithm with cyclically annealing step sizes can quickly identify effective ensemble weights that in turn identify synaptic connections. Learned weights show little variance across repeated randomizations supporting the optimality of the resultant ensemble score. Most importantly, we demonstrate that ensemble weights learned for one network can be transferred to improve the prediction accuracy for a novel network. While ensemble learning can be transferred between networks, we find that different weights must be learned for different temporal resolutions. This is not surprising and likely reflects variability in the optimal time-scales of the underlying measures. In developing these ensemble methods, we also introduce new refinements on mutual information and transfer entropy that account for polysynaptic inhibition and heteroskedasticity. These innovations significantly improve inference of synaptic connectivity in neuronal network models.

Disclosures: **D.Y. Little:** None. **B. Chambers:** None. **J. Dechery:** None. **J.N. MacLean:** None.

Poster

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National Science Foundation Grant # DGE-0903637 IGERT: Integrative research in motor control and movement

Title: A small world of synaptic integration

Authors: *B. CHAMBERS, J. N. MACLEAN;
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Abstract: Linking cortical population activity to the synaptic structure that instantiates and constrains it is a critical goal for neuroscience. A major challenge to understanding structure-function relationships is that they depend on large numbers of synaptic interactions which combine in a complex and non-linear fashion. In this work, rather than focusing on individual connections, we compare the global features of two networks: (1) a functional wiring diagram that summarizes pairwise correlations, and (2) its underlying synaptic network. To study their interrelationship with known ground truth, randomly connected LIF networks were generated with conductance-based synapses and heterogeneous connection strengths. Despite the fact that underlying synaptic connectivity was random, functional wiring diagrams were marked by a global statistical structure: the small-world property. We find that small-worldness is the result of a dramatic elevation in fan-in triangle clustering. Functional wiring diagrams generated from 2-photon imaging of circuit activity in mouse neocortex were similarly marked by an overrepresentation of fan-in triangular clustering, which was not present in rate-matched Poisson nulls. In the LIF model, the clustering of presynaptic activity increased with depolarization in the postsynaptic neuron, linking clustering with effective postsynaptic integration. Synaptic weights in the model were increased to lessen the requirement for integration of multiple inputs to achieve threshold. In these strong-synapse models, functional wiring diagrams were significantly more faithful to the actual underlying connectivity than were models with naturalistic weights. Compared to relationships of simple convergence, activity at fan-in triangle motifs was marked by stereotyped presynaptic timing. Together, our results demonstrate how fan-in triangular clustering improves the reliability of postsynaptic recruitment. As a result, fan-in triangle motifs are especially salient in functional maps, relevant for mapping structure to function in cortical circuits. The integrative properties of individual neurons impose structure in functional maps by

favoring presynaptic coincidence. When applying graph theory to neural data, it is necessary to consider the computational properties of the nodes themselves.

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Poster

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Title: Optical adapter for two photon deep brain imaging

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Abstract: Current imaging systems are suitable to functionally image neural tissue elements from the area range down to the synaptic elements, thereby being an indispensable tool to understand brain function. A common drawback of these systems though is the depth limit they all face. The usual working depth of optical imaging systems do not exceed a few hundred micrometers, hardly the width of the rodent cortex. There are engineering approaches to solve this problem, since deeper neural tissue elements also play crucial role in shaping cognition and behavior. So far these solutions all suffer from one or several constraints of being highly invasive, not precise, or of limited scope. Here we describe a possible solution along with *in vivo* test measurements to use with two photon laser scanning imaging of genetically encoded calcium indicators allowing to image deep tissue functions in considerable volumes.

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Poster

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Topic: B.09. Network Interactions

Support: ERC starting grant

Wallenberg academy fellowship to G.S.

Title: Intrastriatal inhibition by somatostatin expressing neurons

Authors: *M. C. DORST[1], M. ZHOU[2], I. LAZARIDIS[2], S. KIAN TAI[2], K. MELETIS[2], G. SILBERBERG[2];
[2]Dept. of Neurosci., [1]Karolinska Institutet, Stockholm, Sweden

Abstract: A select population of neostriatal interneurons express somatostatin (SOM). These interneurons are tonically active and provide GABA-mediated inhibition onto neighboring projection neurons. Little is known, however, about their connectivity with other types of striatal interneurons. We used a combination of electrophysiological and optogenetic methods to study the synaptic connectivity from SOM neurons to neighboring striatal neurons. Following transduction of AAV DIO ChR2(H134R)-mCherry in SOM-cre mice, we could selectively record from and photostimulate SOM neurons. SOM neurons displayed the electrophysiological characteristics of Low Threshold Spiking interneurons, including high membrane resistance, a long membrane time-constant and a depolarized resting membrane potential resulting in tonic activity. Photostimulation of SOM neurons evoked inhibitory synaptic responses in neighboring projection neurons as well as interneurons, with strongest responses observed in cholinergic interneurons (ChINs). Direct synaptic connections between individual SOM neurons and ChINs were weak and sparse, suggesting that part of the optogenetic response was mediated by axons originating from more distal presynaptic neurons. Bath application of GABAzine (SR-95531) significantly increased the spontaneous firing rate of ChINs, and photostimulation of SOM neurons induced biphasic changes in ChIN firing rate, composed of an immediate pause followed by an increase in discharge frequency. Our results suggest that SOM expressing neurons play an important role in sculpting striatal activity, in particular by regulating the spontaneous activity of ChINs.

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Poster

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Support: NIMH Intramural Research Program

Maryland Biophysics Graduate Program

Title: Non-parabolic unfolding of neuronal avalanches recorded in the awake monkey

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Abstract: Agreement between experimental neuroscience and the theory of criticality has been established in terms of observed scale-invariance for neuronal avalanches, which constitute spontaneous activity cascades in the cortex. Pharmacologically-validated optimization of certain aspects of information processing, such as dynamic range, information capacity, and the transient formation of phase-coupled neuronal ensembles, are in line with a balanced critical regime of cortical activity. It is therefore worthwhile to consider how the brain might leverage neuronal avalanches to store, transmit, and process information. A major information aspect introduced by neuronal avalanches is related to their instantaneous spatial spread, e.g. the number of sites visited per time step. From neural network studies, we find that critical dynamics on a network with homogeneous random connectivity generates avalanches with a parabolic spread in time; avalanches will typically initiate at a single site, engage more and more sites as they unfold, and eventually contract spatially and terminate. As reported previously, scale-invariance has been observed for event size and duration probabilities in both simulation and *in vivo* data. However, experimental findings presented here illustrate that not all neuronal avalanches recorded in cortical layers 2/3 of awake Macaque monkeys (n=2) can be described as having a parabolic temporal profile shape. Instead, neuronal avalanches recorded *in vivo* have a flattened spread such that (1) they visit significantly fewer sites during their unfolding than would be expected from homogeneous network architecture, and (2) long-lasting avalanches display considerable variability around a characteristic mean function that is independent of avalanche lifetime. Avalanche data collected *in vivo* did not collapse well, as short-duration avalanches had a steeper curvature than avalanches with long lifetimes (>10ms). Simulated avalanches on homogeneous topology always collapsed well. We altered our network model to capture the *in vivo* profile shape by adding a series of strong directed links to an otherwise random graph. This topological "highway" increased the inhomogeneity in the network and had a flattening effect on the average shape of avalanches of all durations. However, such inhomogeneity did not replicate the poor scaling collapse seen for *in vivo* avalanches. We conclude that additional inhomogeneities beyond network topology (e.g. initiation sites frequency) may contribute to the absence of scaling collapse for avalanche waveforms despite robust power laws in avalanche sizes, a hallmark of neuronal avalanche dynamics.

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Poster

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Support: FQRNT

CIHR

Title: Neural correlations mediate feature invariant responses

Authors: ***M. G. METZEN**[1], M. J. CHACRON[1,2];
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Abstract: Feature invariant coding has been observed across sensory modalities and species. Such coding strategies, often associated with sparse coding, are believed to have advantages over more dense responses as they provide a stable perception of behaviorally important stimuli. Invariant responses at the single neuron level to a given feature are generally observed in higher brain areas such as the midbrain and it has been shown that such a global invariance develops in stages by integrating the responses of neurons that display a local invariance. However, the mechanisms that lead to locally feature invariant responses in central neurons are still largely unknown. Here we focused on feature invariant coding of communication signals by electrosensory primary afferents (P-units) in the weakly electric fish, *Apteronotus leptorhynchus*. These fish generate an electric field (electric organ discharge, EOD) surrounding their body. If two fish are in close proximity, their electric fields interfere creating a beat. Furthermore, these fish can produce communication signals (chirps) that can occur on every phase of the beat and thus appear as very heterogeneous stimuli waveforms. We show that correlated activity of P-units can provide a feature invariant representation of small chirp waveforms over a behavioral relevant range of beat frequencies. In contrast, the single neuron activities were not invariant as they faithfully represented the detailed timecourse of the different stimulus waveforms. To understand the mechanisms leading to such responses we used a generic leaky-integrate-and-fire model. By varying model parameters we were able to identify the regimes by which feature invariant responses of P-units are optimum. The model predicts a strong relationship between invariant coding in correlated activity of P-units and stimulus intensity as well as their baseline variability. Correlated activity of the receptors could then be used by downstream neurons such

as ON and OFF cells to generate locally invariant responses. Physiological results will be supplemented with behavioral assays to test whether there is a behavioral correlate to the feature invariant responses seen in correlated activity of P-units. Therefore, this study gives important insights as to how the brain can generate feature invariant responses starting as early as the periphery through correlated activity and how this is decoded by downstream areas to give rise to perception/behavior.

Disclosures: **M.G. Metzen:** None. **M.J. Chacron:** None.

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Title: An activity dependent feedback inhibitory loop in mouse presubiculum

Authors: ***J. SIMONNET**[1,2], M. NASSAR[2], B. MATHON[2], I. COHEN[3], C. N. BOCCARA[4], R. MILES[2], D. FRICKER[2];
[1]Bernstein Ctr. for Computat. Neurosci., Humboldt Univ. zu Berlin, Berlin, Germany; [2]INSERM U1127 / CNRS UMR7225, Inst. du Cerveau et de la Moelle Epinière, Paris, France; [3]CNRS UMR 8246 / INSERM U1130, Neurosci. Paris Seine, Paris, France; [4]IST Austria, Am Campus 1, Klosterneuburg, Austria

Abstract: The presubiculum is a cortical region located between the hippocampus and the entorhinal cortex. It contains head direction cells that fire as a function of an animal's directional heading. Presubiculum plays a major role in controlling the accuracy of the head direction signal used for hippocampal-based landmark navigation. The presubicular head direction signal arises mainly from Anterior Thalamic (ATN) inputs but its regulation in presubiculum is poorly understood due to the lack of knowledge of presubicular computation. We used dual patch clamp recordings in the acute slice preparation from adult mice (P20 - P60) to highlight an inhibitory

feedback circuit and some of its functional properties in presubicular superficial layers that are important for the accuracy of the head direction signal. We focused on two neuronal populations, Pyramidal Cells (PC) and Martinotti Cells (MC), using transgenic mice containing fluorescent SST positive interneurons (X98-SST or SST-cre::dtTomato). MC axons target mainly layer 1 and 3, where PC-dendrites receive thalamic inputs. PCs send many axon collaterals to layer 3, where soma and dendrites of MC cells are located. Using stereotaxic viral injections to transduce channerhodopsin2-YFP into ATN, we showed that ATN axons target layers 1 and 3 of the presubiculum and make direct synaptic contacts with PCs but not MCs. The PC-MC connection probability was high: 58% for MC-to-PC, 37% for PC-to-MC and 28% for reciprocally connected pairs. We showed that inhibitory postsynaptic events in PCs were reliable (transfer rate = 0.85 ± 0.05) but small (mean amplitude = 9 ± 1.2 pA), consistent with dendritic inhibition. The efficacy of the PC-to-MC synapse was activity dependent: excitatory post-synaptic events were rarely detected in MCs upon single spike firing of PCs (transfer rate = 0.11 ± 0.02), but synaptic efficacy (initially -2.4 ± 0.6 pA) increased with the duration and frequency of the presynaptic discharge. Several seconds were necessary for this enhanced efficacy to decay down to its initial value. We then showed how these properties conditioned MC recruitment during head directional activity recorded *in vivo*, and injected as spike trains into PCs *in vitro*. The delay of MC recruitment was short, so that self-induced inhibition was time-locked to the PC spike. Time-locked inhibition had little effect on PC firing frequency and even was rather facilitating compared to lateral inhibition, likely to be randomly timed. The PC-MC feedback loop may therefore function as a lateral inhibition process and reinforce the accuracy of specific head direction signals in the presubiculum.

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Poster

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Title: Spike frequency adaptation regulates network sensitivity to synaptic heterogeneities in a cortical model of cholinergic modulation

Authors: *J. P. ROACH[1], L. M. SANDER[2,3], E. BEN-JACOB[5,6], M. ZOCHOWSKI[2,4,3]; [1]Neurosci. Grad. Program, [2]Dept. of Physics, [3]Ctr. for the Study of Complex Systems, [4]Biophysics Program, Univ. of Michigan, Ann Arbor, MI; [5]Sch. of Physics and Astronomy, Tel-Aviv Univ., Tel Aviv, Israel; [6]Ctr. for Theoretical Biol. Physics and Dept. of Biochem. and Cell Biol., Rice Univ., Houston, TX

Abstract: Acetylcholine is a major regulator of cortical network functions such as arousal state and attention. ACh is associated with the transition between slow wave sleep (SWS; where ACh is absent) and rapid eye movement (REM) sleep or waking states (where ACh is high). Patterns of neural activity within the cerebral cortex change drastically when between these states. During bouts of SWS neurons oscillate between high frequency up-states and quiescent down states, which manifest as traveling frequency waves on the macroscopic level. On the other hand, during REM sleep and waking neurons fire tonically and network activity is likely to be organized on a more local scale. Locally driven activity is could underlie working memory and attention dynamics while traveling waves may be needed for synaptic renormalization. The network level mechanisms that translate the cellular effects of ACh into differing activity patterns are not well characterized. In this study we use a model for cholinergic modulation of a network of neurons of the Hodgkin-Huxley type, which recreates the reduction of spike-frequency adaptation (SFA) induced by ACh. During periods of high ACh (SFA is minimal) network activity is localized and highly sensitive to synaptic heterogeneity (i.e. high frequency activity is centered around regions defined by enhanced recurrent excitatory synapses). Increasing the modeled ACh level (SFA is present) leads to traveling waves of activity. Within this activity state ACh level continues to regulate sensitivity to synaptic heterogeneity. At moderate levels of ACh regions defined by synaptic heterogeneity are occupied by frequency wave longer. We show that a mechanism for these divergent states is that the balance of excitation and inhibition (E/I balance) sets the spatial extent of network activity and that SFA defines the temporal scope, which is directly modulated by ACh in the model. Synaptic heterogeneity disrupts E/I balance with decreasing effectiveness as SFA increases. The model simulations give unique insights into the role and significance of ACh in determining patterns of cortical activity and functional differences arising from these patterns.

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Poster

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Support: NSERC CREATE BIP U of L

Title: Optical flow platform for analyzing voltage sensitive dye imaging of evoked and spontaneous activity to quantify velocity and direction, source and sink points, and tracking activity trajectory

Authors: ***N. AFRASHTEH**[1], M. KYWERIGA[2], M. HEIDARI MOHAJERANI[2];
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Abstract: The Voltage-Sensitive Dye (VSD) imaging technique is a powerful method to study the subthreshold activity of neuronal depolarizations. The VSD imaging method provides an excellent opportunity to examine the spatiotemporal dynamics of the activity spread over the cortex. However, analysis of these spatiotemporal patterns has been mostly qualitative to date. Plotting fluorescence intensity changes over time was used mainly in previous studies to quantify the dynamics of neural depolarization. The Optical Flow (OF) analysis is a well-known method in computer vision for quantitative motion (velocity and direction) estimation of moving objects. Here we developed an open-source software package to quantify fast local flow patterns of cortical population activation, as measured by VSD imaging. The toolbox can be used to quantify neural depolarization waves in VSD signal in term of their velocity, orientation, localize activity sources and sinks, and track a source to its corresponding sink. Using this toolbox, we analyzed the images to quantify spontaneous and stimulus evoked activity in the isoflurane anesthetized mouse. First, by analyzing VSD signals for stimulus-evoked activity, we found that the global spreading of the neural activity throughout the cortex for whisker stimulation was faster than for forelimb stimulation (n=3). The distributions of velocity and direction throughout the cortex were non-uniform and had multi-modal functions. Second, to assess differences between evoked and spontaneous activity, we compared evoked forelimb activity to forelimb-evoked like pattern in spontaneous activity. We found that the spread of evoked activity was faster than spontaneous activity throughout the cortex. Third, using source and sink analysis we found that the distribution of sources and sinks were constant over time in spontaneous activity. These points were more frequent over the anteromedial and posterolateral regions of the cortex, and along the path connecting them. This result is in line with the results from direction analysis where the majority of activities are traveling along anteromedial posterolateral pathway. We

believe this analysis toolbox will be of broad interest to researchers involved in systems neuroscience, especially those that are interested in the organization of cortical circuits in healthy brain and those that model disease such as Autism or Alzheimer's.

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Title: Mechanosensitivity of isolated enteric neuronal networks

Authors: ***G. MAZZUOLI**[1], E. M. KUGLER[1], K. MICHEL[1], F. ZELLER[2], M. SCHEMANN[1]; [1]Technische Univ. München, Freising, Germany; [2]Dept. of Surgery, Klinikum Freising, Freising, Germany

Abstract: The gastrointestinal tract contains a number of neurons comparable to the spinal cord. These neurons are embedded into the gut wall and constitute the enteric nervous system (ENS). The ENS controls gut functions independently from the central nervous system. The wall of gastrointestinal tract is constantly under mechanical stress, which results from the movements of its muscle layers. The enteric neurons, sandwiched between these muscle layers, are the local controller of peristalsis. Direct activation of myenteric neurons by mechanical stimuli has been shown. However, the precise stimulus modalities (shear or normal stress) that excite myenteric mechanosensors are so far unknown. The aim of the present study was to describe the properties of mechanosensitive enteric neurons and to compare them with other first order sensory neurons. Primary cultured myenteric neurons were used to exclude non-neuronal influences. Neuronal activation was recorded with an ultra-fast neuroimaging technique with voltage-sensitive dye that made it possible to record from multiple neurons simultaneously. Normal stress was applied by probing with an ultra-fine von Frey hair. Shear stress was applied by fluid flow through a micro channel. Mechanosensitive myenteric neurons had no specialized regions for mechanotransduction. Instead, this occurred along the entire neurites (45%) and somata (14%). The electrical signal originates at the site of the mechanical stimulation. Neurite signals propagated towards the soma and invaded other neurites. Mechanosensitive neurites not only transmit sensory information but also function as motor neurites spreading the signal to other

cells. This supports the theory of multifunctionality of mechanosensitive myenteric neurons. We proved that the excitability state of the soma is critical for the transduction of mechanical signals. This suggests that neuronal responses can be modulated, which would be crucial to provide appropriate responses to environmental signals. Myenteric neurons share some properties with other sensory neurons but also exhibit specific features, very likely reflecting adaptation to their physiological function in the gut. Mechanosensitive properties are conserved across species as shown for guinea pig and human. Responses to normal stress were reproducible, while shear stress showed only a marginal effect. Thus, normal stress appeared to be the primary stimulus for myenteric mechanosensitive neurons. The present study helps to understand the complex process of neuronal mechanotransduction and to clarify different neuro-motoric patterns involved in the regulation of peristalsis.

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Poster

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Title: Neural network properties can be inferred from electrophysiological power spectral geometry

Authors: *T. J. NOTO, R. GAO, E. PETERSON, B. VOYTEK;
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Abstract: The study of the biophysical and cognitive role of neural oscillations has become a cornerstone of modern neuroscience. These oscillations are inferred from the power spectral density (PSD) of the neurophysiological signal of interest. The PSD of electrophysiological neural activity assumes a general $1/f^{\chi}$ form. Although changes of power in narrow frequency bands (alpha, beta, etc.) have been related to a variety of cognitive and behavioral states, and the broadband power (the offset) of this process has been shown to reflect aggregate population spiking activity, there is scant evidence for how other global properties of power spectral

geometry relate to the underlying neural network activity. Treating the neural power spectra as a holistic entity affords the application of different analyses that may provide novel insights that would not be evident in narrow bands. Presumably, neural networks produce characteristic changes in the full spectrum (aside from narrowband oscillations) under different operational modes, so it may be possible to estimate certain features of the network from its geometry. Concurrent single cell and local field recordings from rat hippocampus and recordings directly from human cortex allow us to probe these relationships. The slope of the broadband spectrum (10-100 Hz) had a positive correlation with spike count (r=0.35) and a negative correlation with the fano factor of the inter-spike interval (r= -.35). Additionally, the slope of high gamma (80-125 Hz) negatively correlated with phase/amplitude coupling (r = -0.1). These results draw a link between spectral geometry, network properties, and neurobiology, and support the idea that the power spectrum should be considered as a holistic entity that contains a wealth of information about the network that produces it.

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Title: Dynamic shifts in large-scale functional connectivity predict transitions in brain state

Authors: ***I. M. STITT**[1], K.-J. HOLLENSTEINER[1], E. GALINDO-LEON[1], F. PIEPER[1], E. FIEDLER[2], T. STIEGLITZ[2], G. ENGLER[1], A. K. ENGEL[1];
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Abstract: Throughout each day, the brain typically transitions several times between varying states of vigilance and unconsciousness. While the electroencephalographic spectral correlates of brain states have been studied for some time, it remains unclear how large-scale cortico-cortical functional connectivity systematically reorganizes across brain states. In this study, we investigate the time-varying nature of large-scale cortical functional connectivity across brain

states by recording micro-electrocorticographic (μECoG) activity during spontaneous sleep/wake transitions in the ferret. Data were obtained from 64-channel μECoG arrays chronically implanted in 3 female ferrets. Continuous local field potential (LFP) recordings of up to 3 hours duration were made during spontaneous behaviours in the open field. To quantify brain states, we use a data driven approach that projects time-varying LFP spectral properties into lower dimensional state-space. Trajectories of neural data in state space suggest that the temporal evolution of brain state across the sleep/wake cycle resembles a multistable process, defined by a recurring set of neural conditions, with some stages displaying a strong degree of stability (e.g., slow-wave sleep), while others displays a greater dynamical range of freedom (e.g., awake state and rapid eye movement sleep). Distinct brain states displayed markedly different patterns of large-scale functional connectivity, with phase synchronization in slow, theta, and spindle frequencies differing the most across brain states. The largest between-state difference in functional connectivity was observed between presumed slow-wave and rapid eye movement sleep, which were characterized by periods of cortical network fragmentation and global hypersynchronization, respectively. Changes in large-scale functional connectivity at the end of sleep/wake cycles also reliably predicted animal movement associated with waking. We conclude that large-scale cortico-cortical functional connectivity dynamically reorganizes with brain state across the sleep/wake cycle, and suggest that further investigation should focus on the functional connectivity correlates of more subtle state transitions associated with spontaneous drifts in mental activity during resting periods.

Disclosures: I.M. Stitt: None. **K. Hollensteiner:** None. **E. Galindo-Leon:** None. **F. Pieper:** None. **E. Fiedler:** None. **T. Stieglitz:** None. **G. Engler:** None. **A.K. Engel:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.01/C36

Topic: B.11. Glial Mechanisms

Title: Protein arginine methyltransferase1 regulates astrocyte differentiation of neural stem cells through the modification of STAT3

Authors: *M. HONDA, S. KATADA, K. NAKASHIMA;
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Abstract: Members of the interleukin-6 family of cytokines, such as leukemia inhibitory factor (LIF), induce astrocyte differentiation of neural stem cells (NSCs) by activating the transcription

factor signal transducer and activator of transcription-3 (STAT3). It is well known that the activation of STAT3 is induced by its tyrosine-phosphorylation, however, other posttranslational modifications such as methylation and acetylation are also important. For example, several reports have shown that the phosphorylation and acetylation of STAT3 positively regulate its activity, whereas lysine methylation has the opposite effect. Nevertheless, the effect of STAT3-arginine methylation remains elusive. Arginine methylation is catalyzed by the members of protein arginine methyltransferase (PRMT) family enzymes, yet the role of PRMTs in NSCs is not well understood. Interestingly, our microarray analyses revealed that PRMT1 is highly expressed in mouse embryonic NSCs. To examine whether PRMT1 methylates STAT3, we first transfected STAT3-expressing construct with or without that of PRMT1 to HEK293T cells, and performed western blot using anti-methylated arginine antibody. We found that PRMT1 overexpression enhanced STAT3 arginine methylation. Furthermore, *in vitro* arginine methylation assay using recombinant proteins demonstrated that PRMT1 methylates STAT3. Next, to examine the effect of STAT3 arginine methylation by PRMT1 in NSCs, we performed knockdown experiments of PRMT1 using Prmt1-shRNA in embryonic day (E) 14.5 NSCs. When PRMT1 was knocked-down in E14.5 NSCs, LIF-induced gfap-promoter activation was significantly decreased. Moreover, PRMT1 knocked-down NSCs generated less GFAP-positive astrocytes upon LIF stimulation compared to control. We report here for the first time, the arginine methylation of STAT3 by PRMT1 enhances transcription of a astrocyte specific gene, promoting astrocyte differentiation of NSCs.

Disclosures: **M. Honda:** None. **S. Katada:** None. **K. Nakashima:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.02/C37

Topic: B.11. Glial Mechanisms

Support: VA merit review grant

Title: Neuroprotective effect of 7,8-dihydroxyflavone on serum deprived C8D1A astrocyte cells

Authors: *V. J. SHUKLA[1], D. PATEL[1], A. TRIPATHI[1], S. MAKAR[1], P. GUDA[1], J. BHUJU[1], C. BEVER[1,2], D. TRISLER[1,2], S. JUDGE[1,2], S. RAY[1];
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Abstract: Astrocytes play a major role in the central nervous system (CNS). Astrocytes secrete pro-inflammatory cytokines in different neurodegenerative diseases. Astrocytes grown in serum-deprived (SD) media can cause apoptosis by elevating different apoptotic proteins. Furthermore, when astrocytes are grown in oxygen-,glucose- and serum deprived media apoptosis is induced via endoplasmic reticulum (ER) stress. 7,8-dihydroxyflavone (DHF), a BDNF receptor (Trk-B) agonist is used for neuroprotection in different animal models of neurodegenerative diseases such as Parkinson's, Alzheimer's and Epilepsy. In PC12 cells, it is found that DHF effectively prevented cell death, apoptosis and mitochondrial dysfunction induced by 6-hydroxydopamine. Our objective is to determine out the neuroprotection role of DHF on serum free C8D1A astrocyte cell line where cell death occurs in an apoptotic manner. 50 μM DHF was used in serum free media of C8D1A cell culture. The cells were incubated for 4 hours in SD. Interestingly, we found that level of interleukin1 (IL1), tumor necrosis factor α (TNFα), and Interferon γ (IFN-γ) were significantly increased in serum free media and that cytokine levels were decreased after DHF treatment. We also found that Bax, caspase 3 and caspase 9 were increased in serum deprived cells and that all of these parameters were reduced after DHF treatment. Furthermore, we observed that the ER stress marker Protein kinase R [PKR]-like ER kinase (PERK) expression was significantly increased in SD cells and was suppressed by DHF treatment. In the same experimental condition, Endothelin receptor type A (ETA) receptor expression was upregulated in SD cells whereas DHF treatment suppressed the expression. Pro-inflammatory cytokines are involved in inflammation and causing cell death. In our study we found that IL-1, TNFα and IFN-γ increased in SD media which may cause the induction of different inflammatory proteins that lead to induced ER stress by activating the ETA receptor. As a result, the apoptotic proteins Bax, Caspase3 and caspase-9 were increased. However, DHF showed its neuro-protective activities by down-regulating the above mentioned parameters. Collectively, our findings suggest That DHF can be used for anti-inflammation and neuroprotection.

Disclosures: V.J. Shukla: None. **D. Patel:** None. **A. Tripathi:** None. **S. Makar:** None. **P. Guda:** None. **J. Bhuju:** None. **C. Bever:** None. **D. Trisler:** None. **S. Judge:** None. **S. Ray:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.03/C38

Topic: B.11. Glial Mechanisms

Support: EU-Marie Curie (OLIMPIA)

Title: Effects of hypotonicity on primary rat astrocytes

Authors: S. B. RAO, H. B. BOLDT, D. S. Y. LEUNG, *R. TORP, M. R. AMIRY-MOGHADDAM;
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Abstract: Hyponatremia is associated with a number of clinical conditions and may cause serious consequence such as brain edema with significant mortality rate. Despite several decades of research, there is still no treatment preventing edema formation. Studies in the last decade have shown that Aquaporin-4 (AQP4) water channels anchored to perivascular astrocytic endfeet through an interaction with the dystrophin associated protein complex (DAPC) are involved in development and resolution of hyponatremic brain edema. An earlier study has shown an increased density of the perivascular AQP4 following systemic hyponatremia in rat. However, the increase in AQP4 protein was not accompanied by an increase in mRNA level. The mechanisms involved in the increased AQP4 protein following hyponatremia are still unknown. In this study, we analysed the expression pattern of AQP4 and other members of DAPC in primary rat astrocytes exposed to hyponatremia. The cells were exposed to 1 or 6 hours of hyponatremia, followed by 6 or 12 hours of incubation in the normal media. Our data shows a significant increase in the expression level of DAG1, the gene encoding for the transmembrane proteins alpha- and beta- dystroglycan. Expression levels of the other DAPC members such as AQP4, dystrophin, alpha syntrophin were unchanged. Since dystroglycan link the extracellular matrix to the intracellular cytoskeleton, we hypothesize that it is the gene regulated by the hypotonic stress and its upregulation leads to increased mobilization of AQP4 to the perivascular membrane domains. This hypothesis is currently being tested in animal models.

Disclosures: S.B. Rao: None. **H.B. Boldt:** None. **D.S.Y. Leung:** None. **R. Torp:** None. **M.R. Amiry-Moghaddam:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.04/C39

Topic: B.11. Glial Mechanisms

Title: Microfluidic platform for the study of water transport in astrocytes

Authors: *J. M. LECHOWICZ[1], J. XU[2], S. T. ALFORD[3], A. A. LINNINGER[1];
[1]Bioengineering, [2]Mechanical and Industrial Engin., [3]Biol. Sci., Univ. of Illinois At Chicago, Chicago, IL

Abstract: Recently, the glymphatic system has been proposed as the mechanism for soluble waste clearance in the central nervous system parenchyma. This system facilitates the exchange between cerebrospinal fluid and interstitial fluid. A decreased efficiency or perturbation of this system has been speculated as an etiology of neurodegenerative disorders such as Alzheimer's disease, as well as a major contributor in traumatic brain injuries. Aquaporin-4 is the principal water channel in central nervous system acting as a bidirectional passive conduit. It is expressed by astrocytes and localized to perivascular endfoot processes. Aquaporin-4 appears to be an important component of the glymphatic system as its absence results in more than a 60% reduction of tracer clearance from the parenchyma. Little however is known about the mechanisms at the cellular level. Using soft lithography we have developed a microfluidic platform which enables us to induce rat cortex astrocytes to develop the polarized perivascular endfoot-like processes. This is achieved by developing a nutrient gradient and by patterning of the basal lamina extracellular matrix components, laminin and agrin. We are able to indirectly view and quantify water movement in the cell using calcein quenching and the efflux of water through the use of fluorescence dilution. This allows us to interrogate the water transport in astrocytes and determine whether ion transport plays a role.

Disclosures: J.M. Lechowicz: None. **J. Xu:** None. **S.T. Alford:** None. **A.A. Linninger:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.11. Glial Mechanisms

Support: NIH Grant R01 NS061953

Title: Mechanisms responsible for limited astrocytic glutamate release during pathological cell swelling

Authors: A. L. SCHOBER, *A. A. MONGIN;
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Abstract: Hyponatremia, epilepsy, stroke, and several other CNS pathologies are associated with substantial swelling of astrocytes. To counteract such swelling, astrocytes engage volume regulated anion channels (VRAC) to release cytosolic ions and small organic molecules, including L-glutamate and taurine. This protective mechanism is problematic because it leads to a buildup of the extracellular levels of the excitotoxic transmitter glutamate. Paradoxically, cultured astrocytes lose disproportionally less intracellular glutamate, as compared to taurine and several other molecules, which share the same VRAC release pathway. In the present study, we explored potential mechanisms that allow astrocytes to conserve intracellular glutamate in a cellular model of hyponatremia. To induce cell swelling, we exposed primary rat astrocytes to hypoosmotic medium (with [NaCl]o reduced by 58 mM). Initial model experiments, revealed fairly similar permeability of VRAC to radiolabeled [14C]taurine and L-[3H]glutamate. However, when we used an HPLC assay to determine the endogenous amino acid content, we found that within 30 min swollen astrocytes lose 54% of the intracellular taurine, but only 19% of the intracellular glutamate. This dramatic difference was eliminated after inhibition of both glutamate reuptake (with the glutamate transporter blocker 300 μM TBOA) and mitochondrial glutamate synthesis (with the inhibitor of aminotransferases 1 mM aminooxyacetic acid, AOA). Treatment with TBOA+AOA made reductions in the intracellular taurine and L-glutamate levels approximately equal. Taken together, these data suggest that swollen astrocytes actively conserve intracellular glutamate via reuptake and de novo synthesis. Our findings also likely explain why in animal models of acute hyponatremia, extracellular levels of taurine are dramatically elevated with minimal impact on extracellular L-glutamate.

Disclosures: **A.L. Schober:** None. **A.A. Mongin:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.11. Glial Mechanisms

Support: Slovenian Research Agency P3 310

Slovenian Research Agency J3 4051

Slovenian Research Agency J3 3632

Slovenian Research Agency J3 6790

Slovenian Research Agency J3 4146

Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins

COST Action BM1002

Title: Attenuation of astrocyte swelling by adrenergic activation: a new strategy for rescuing cells in neural edema

Authors: *N. VARDJAN[1,2], A. HORVAT[1], J. E. ANDERSON[3], D. YU[3], D. CROOM[4,5], X. ZENG[3], Z. LUŽNIK[1], M. KREFT[1,2,6], Y. D. TENG[3,7], S. A. KIROV[4,5], R. ZOREC[1,2];
[1]Fac Med, Univ. Ljubljana, Ljubljana, Slovenia; [2]Celica BIOMEDICAL, Ljubljana, Slovenia; [3]Harvard Med. Sch., Boston, MA; [4]Brain and Behavior Discovery Institute, Georgia Regents Univ., Augusta, GA; [5]Med. Col. of Georgia, Georgia Regents Univ., Augusta, GA; [6]Biotechnical Faculty, Univ. Ljubljana, Ljubljana, Slovenia; [7]Div. of SCI Research, VA Boston Healthcare Syst., Boston, MA

Abstract: Edema in the central nervous system develops in a wide range of pathologic conditions, rapidly resulting in life-threatening complications. Vasogenic edema is clinically manageable, but there is no established medical treatment for cytotoxic edema, which affects astrocytes and is a primary trigger of acute post-traumatic neuronal death. To test the hypothesis that adrenergic receptor agonists, including the stress molecule epinephrine protects neural parenchyma from damage, we characterized its effects on hypotonicity-induced cellular edema in cortical astrocytes by *in vivo* and *in vitro* imaging. After epinephrine administration, hypotonicity-induced swelling of cortical astrocytes was markedly reduced and cytosolic 3'-5'-cyclic adenosine monophosphate (cAMP) was increased, as shown by a fluorescence resonance energy transfer nanosensor. Interestingly, in swollen primary cortical astrocytes, the kinetics of cAMP signaling was slowed. To ascertain effects on downstream cytoplasmic processes, we analyzed Ca2+ concentration. We found that epinephrine, via modifying cAMP-signaling, reduced hypotonicity-induced cytosolic Ca2+ excitability, which may be the key to prevent astrocyte swelling. Furthermore, in a rat model of spinal cord injury, epinephrine applied locally markedly reduced neural edema around the contusion epicenter. Together, these findings reveal new targets for the treatment of cellular edema in the central nervous system.

Disclosures: **N. Vardjan:** None. **A. Horvat:** None. **J.E. Anderson:** None. **D. Yu:** None. **D. Croom:** None. **X. Zeng:** None. **Z. Lužnik:** None. **M. Kreft:** None. **Y.D. Teng:** None. **S.A. Kirov:** None. **R. Zorec:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

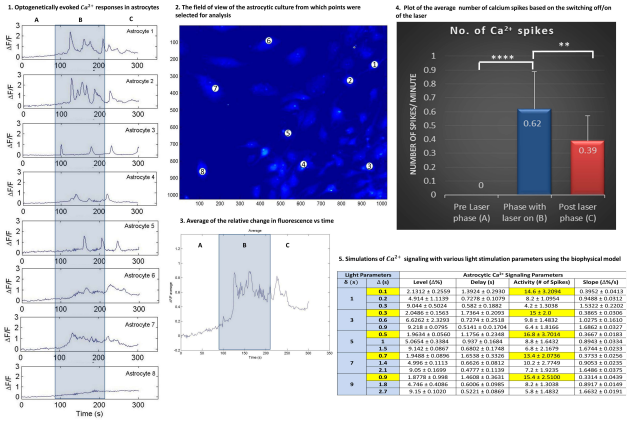
Program#/Poster#: 128.07/C42

Topic: B.11. Glial Mechanisms

Title: Quantitative evaluation of optogenetically-induced calcium signaling in astrocytes

Authors: *L. BALACHANDAR[1,2], A. RAYMOND[3], A. OROZCO[2], J. RIERA DIAZ[2];
[2]Biomed. Engin., [3]Immunol., [1]Florida Intl. Univ., Miami, FL

Abstract: Optogenetics, a modern technique in neuroscience to control excitable cells with light, has been recently expanded to astrocytes (Figueiredo et-al., 2010). However, the mechanisms by which light-activated channelrhodopsins affect the behavior of such non-excitable cells haven't been clearly identified / quantified. The purpose of this study is to establish a methodology to stimulate astrocytes with light *in vitro*, which included an evaluation of viral parameters for proper cell viability/activity, quantification of experimental parameters for optimal Ca2+ indicator loading and development of a mechanistic biophysical model to predict transient Ca2+ responses in astrocytes. We were able to evoke and quantify Ca2+ signaling in cultured astrocytes (Fig.1.1) and found that Ca2+ signaling is highly dependent on light stimulation parameters. As indicated in Fig. 1.4, after the stimulation laser was switched on astrocytes start firing Ca2+ spikes in a random fashion (Phase B), which trailed off once the laser was turned off (Phase C). As predicted by our biophysical model, low percentage of sustained light was not enough to reach the calcium basal level needed for firing Ca2+ spikes, whereas high percentage brought Ca2+ baseline to a level that blocked Ca2+ activity. We concluded that in order to induce sustained excitability in astrocytes, the overall proportion of sustained light (δ/α %) should be kept within the range of 5-25% (Fig 1.5). These light stimulation parameters are crucial, as it can adversely affect cellular activity by different mechanisms (e.g. phototoxicity, IP3R saturation, ER Ca2+ depletion), if subjected to high frequencies/ time periods of stimulation. We will evaluate this protocol on *in vivo* situations in the near future.



Disclosures: **L. Balachandar:** None. **A. Raymond:** None. **A. Orozco:** None. **J. Riera Diaz:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.08/C43

Topic: B.11. Glial Mechanisms

Support: Margolis Foundation Award

NIH R01 NS078331

Title: Calcium signaling in astrocytes quantified through experimental measurements and mathematical modeling

Authors: ***M. TAHERI**[1,2], G. HANDY[3], A. BORISYUK[3], J. A. WHITE[4];
[1]Univ. of Utah, Salt Lake City, UT; [2]Dept. of Bioengineering, Univ. of Utah, Salt lake city, UT; [3]Dept. of Mathematics, Univ. of Utah, Salt Lake City, UT; [4]Dept. of Biomed. Engin., Boston Univ., Boston, MA

Abstract: Increasing evidence suggests that astrocytes, through their intracellular calcium signaling, are involved in synaptic plasticity, buffering of extracellular ions, regulation of blood flow in the brain, and several other important physiological functions in the CNS. However, the underlying mechanisms of astrocyte calcium signaling, as well as how calcium signaling contributes to these functions, remain unclear. By pursuing coordinated experimental and modeling studies, our goal is to provide insight into the mechanisms and effects of astrocyte calcium signaling. The resulting mathematical model will allow us to evaluate interpretations from experimental results, test hypotheses, resolve some of the controversies in the field, and make predictions to guide future experiments. Experimentally, we use two-photon microscopy to measure calcium activity in astrocytes from crosses based on our novel PC::G5-tdT transgenic mice (available from JAX labs, stock no. 024477). These crosses express the genetically-encoded calcium indicator GCaMP5G in astrocytes. For *in vivo* preparations, we evoke astrocyte calcium activity with physiological sensory stimulation, such as startling or whisker stimulation. In acute brain slices, we evoke calcium activity through focal application of different GPCR (G-protein coupled receptor) agonists with varying application durations and time intervals. These experiments are being carried out in the absence or presence of blockers for certain calcium dynamic components, such as store-operated calcium channels (SOCCs). Through analyzing

these recordings, we are categorizing the different types of calcium responses and quantifying calcium dynamics in terms of duration, amplitude, latency, as well as rise and decay times. In parallel with experiments, we have developed a mathematical model for astrocyte calcium dynamics. We are currently using the model to make experimentally verifiable predictions and explore causes of apparent inconsistencies in the field.

Disclosures: **M. Taheri:** None. **G. Handy:** None. **A. Borisyuk:** None. **J.A. White:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.11. Glial Mechanisms

Support: NIH NINDS Grant RO1 NS077773

NIH NICHD Grant P30 HD26979

Title: Calcium regulates mitochondrial mobility and mitochondria regulate calcium signaling in astrocyte processes

Authors: ***J. G. JACKSON**[1], D. A. COULTER[2,4], M. B. ROBINSON[3,5];
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Abstract: We recently showed that inhibition of neuronal activity, glutamate uptake, or reversed-Na+/Ca2+-exchange increases mitochondrial mobility in astrocytic processes. In neurons, cytosolic Ca2+ decreases mitochondrial mobility in a Miro-dependent fashion, and mitochondria in turn shape Ca2+ signaling. In the present study, we examined the inter-relationships between mitochondrial mobility and Ca2+ signaling in astrocyte processes in organotypic cultures of rat hippocampus. All three treatments that increase mitochondrial mobility (tetrodotoxin, TFB-TBOA, YM-244769) decreased basal Ca2+ as assessed using a genetic Ca2+ indicator (GCaMP). As has been recently reported, we observed spontaneous Ca2+ spikes with half-lives of ~1 sec that spread ~6 µm and are almost abolished by a TRPA1 channel antagonist. Virtually all of these Ca2+ spikes overlap mitochondria (98%), and 62% of mitochondria are overlapped by these spikes. The spread and frequency of these spikes was inversely related to the density of mitochondria in a process. Although tetrodotoxin, TFB-TBOA, or YM-244769 also increased Ca2+ signaling; the specific effects on peak, decay time, and/or

frequency were different. To more specifically manipulate mitochondria, we explored the effects of Miro proteins. We show that Miro1 & Miro2 are both expressed in astrocytes and that exogenous expression of Ca2+-insensitive Miro mutants (KK) nearly doubles the percentage of mobile mitochondria. Expression of Miro1KK had a modest effect on the frequency of these Ca2+ spikes, but nearly doubled the decay half-life. The mitochondrial proton ionophore, FCCP, caused a large prolonged increase in cytosolic Ca2+ followed by an increase in the decay time and the spread of the spontaneous Ca2+ spikes. Photo-ablation of individual mitochondria in astrocyte processes resulted in a large, long-lasting increase in Ca2+ and increased the amplitude, spatial spread, and time constant of individual Ca2+ transients. Together these studies show that Ca2+ regulates mitochondrial mobility and mitochondria in turn regulate Ca2+ signals in astrocyte processes. Positioning of mitochondria at sites of elevated activity within the astrocyte might influence local signaling and energy supply within the astrocyte processes.

Disclosures: **J.G. Jackson:** None. **D.A. Coulter:** None. **M.B. Robinson:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.10/C45

Topic: B.11. Glial Mechanisms

Support: Kaken-hi (22115003; 2625000)

The Japan Prize Foundation

Title: Astrocyte calcium dynamics during early and late juvenile periods in the mouse hippocampus

Authors: *R. NAKAYAMA, T. SASAKI, Y. IKEGAYA;
Grad. Sch. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: Astrocytes generate calcium signals throughout their fine processes, which are assumed to locally regulate neighboring neurotransmission and blood flow. The intercellular morphological relationships mature during juvenile periods when astrocytes elongate highly ramified processes. In this study, we examined how protoplasmic astrocytes in the hippocampus change intracellular calcium activity with development using a transgenic mouse line in which astrocytes selectively express a genetically encoded calcium indicator, Yellow Cameleon-Nano50. Compared with postnatal day 7, astrocytes at postnatal day 30 showed (1) larger

subcellular calcium events, (2) a greater proportion of somatic events, and (3) hotspots of calcium events more largely involved in the somatic region. Such calcium activity in late juvenile astrocytes was not affected by spontaneously occurring sharp waves that trigger a large number of neuronal spikes, implying the independence of astrocyte calcium signals from neuronal synchronization. These inherent features of calcium activity may be crucial for maintaining astrocyte functions during juvenile development.

Disclosures: **R. Nakayama:** None. **T. Sasaki:** None. **Y. Ikegaya:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.11/C46

Topic: B.11. Glial Mechanisms

Title: Astrocyte microdomain Ca^{2+} transients arise from transient opening of the mitochondrial permeability transition pore

Authors: ***A. AGARWAL**[1], P.-H. WU[2], E. G. HUGHES[1], M. FUKAYA[4], M. A. TISCHFIELD[3], J. NATHANS[3], D. WIRTZ[2], D. E. BERGLES[1];
[1]Dept. of Neurosci., [2]Dept. of Chem. and Biomolecular Engin., [3]Dept. of Mol. Biol. and Genet., Johns Hopkins Univ., Baltimore, MD; [4]Dept. of Anat., Kitasato University, Sch. of Med., Kitasato, Sagamihara, Japan

Abstract: Astrocytes exhibit spontaneous increases in intracellular Ca2+ that are spatially confined within their highly ramified processes. However, the cellular mechanisms responsible for triggering these “microdomain” Ca2+ transients and the relevance of this signaling have remained elusive. To facilitate analysis of Ca2+ signaling in astrocyte processes, we generated a knock-In mouse line (ROSA26-lsl-mGCaMP3) in which a membrane-anchored variant of the genetically encoded calcium indicator GCaMP3 (mGCaMP3), can be conditionally expressed. A nested neomycin resistance gene (Neo) cassette flanked by FRT sites was added between loxP sites to lower recombination efficiency and increase specificity. Mice exhibited much higher recombination efficiency when the Neo cassette was removed, demonstrating that this mouse line can be adapted to maintain efficiency and specificity of mGCaMP3 expression with different levels of Cre expression. In GLAST-CreER;ROSA26-lsl-mGCaMP3 mice exposed to tamoxifen, mGCaMP3 was expressed in astrocytes throughout the brain and silver enhanced immunogold electron microscopic analysis showed that mGCaMP3 was localized to the plasma membrane and was abundant in the thinnest astrocyte processes. In acute brain slices from these mice,

spatially restricted, spontaneous fluctuations in Ca2+ levels were observed within astrocyte processes. To identify active microdomains and define the characteristics of their Ca2+ transients we developed a machine-learning based algorithm in MATLAB, called CaSCaDe (Ca2+ Signal Classification and Decoding). Ca2+ transients in microdomains were not coincident, suggesting that these regions are functionally uncoupled, and they persisted in bafilomycin A1, indicating that they do not result from neurotransmitter release. These Ca2+ transients were reduced in mice lacking IP3R2 or after treatment with thapsigargin, but events with similar characteristics (amplitude, duration) were still present, indicating that a proportion of these events do not reflect Ca2+ release from the endoplasmic reticulum. Using mice in which EGFP can be specifically expressed in astrocytic mitochondria, we found that the majority of microdomain Ca2+ transients co-localized with mitochondria. Furthermore, pharmacological inhibition of the mitochondrial permeability transition pore (mPTP) inhibited these events, while enhancing mPTP opening potentiated these events. Thus, transient opening of the mPTP induces spatially restricted Ca2+ transients in astrocytes processes, providing a means to link astrocyte respiration rates and Ca2+ dependent effector pathways.

Disclosures: **A. Agarwal:** None. **P. Wu:** None. **E.G. Hughes:** None. **M. Fukaya:** None. **M.A. Tischfield:** None. **J. Nathans:** None. **D. Wirtz:** None. **D.E. Bergles:** None.

Poster

128. Astrocytes: Profiling and Modulation

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Topic: B.11. Glial Mechanisms

Support: CIHR CGS-M

University of Calgary Donald Burns and Louise Berlin Graduate Award in Dementia Research

Canadian Institutes of Health Research

Title: Specific patterns of synaptic activity alter astrocyte resting calcium

Authors: *E. MEHINA, G. GORDON;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Astrocytes act as important regulators of synaptic function and brain blood vessel tone, carrying out these functions in response to changes in intracellular calcium. While much

research has sought to examine the effects of large, transient increases in astrocyte calcium, the investigation of the role of resting calcium in astrocytes remains untouched. We endeavored to address this deficiency by using two-photon calcium imaging to examine changes in resting astrocyte calcium in acute brain slices of the somatosensory cortex in male Sprague-Dawley rats. Astrocytes were co-loaded with the Ca2+ indicator Rhod-2/AM and the morphological dye calcein-green/AM. A ratio was taken of the two signals to control for any volume or optical changes to the tissue during experiments. In response to electrical theta burst stimulation of neural afferents astrocytes showed a decrease in resting calcium, with these levels becoming reset at a new, lower baseline level. This effect was compounded by subsequent stimulations. Application of a glutamate receptor antagonist cocktail, designed to target metabotropic glutamate receptors (mGluRs), ionotropic N-methyl-D-aspartate receptors (NMDARs) and ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), suppressed the effect suggesting a glutamatergic mechanism. These results indicate that resting astrocyte calcium levels change in response to specific patterns of synaptic activity. This observation is significant because it may have functional consequences to the control of blood vessel tone by astrocytes, as well as their effects at the synapse, providing further insight into the means by which these cells carry out their vascular and synaptic roles.

Disclosures: E. Mehina: None. **G. Gordon:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.13/C48

Topic: B.11. Glial Mechanisms

Support: NHRI NP-103-PP-09

Title: LPA-LPAR1 signaling modulates astrocyte reactivation in neurodegeneration

Authors: L.-Y. TSAI[1], Y.-H. LEE[2], *C.-Y. WANG[3], J.-J. LIANG[1], P.-C. HSU[2], F.-S. SHIE[1]; [1]Ctr. for Neuropsychiatric Res., Natl. Hlth. Res. Inst., Miaoli County, Taiwan; [2]Inst. of Physiol., Natl. Yang-Ming Univ., Taipei, Taiwan; [3]Chang Gung Univ., Tao-yuan/kwei-Shan, Taiwan

Abstract: Lysophosphatidic acid (LPA) is involved in the regulation of various cellular functions largely mediated through LPA receptor-1 (LPAR1). Here, we show that LPAR1 was increased in reactive astrocytes in the brain of mice with neuroinflammation induced by systemic administration of lipopolysaccharide (LPS) and its expression was in paralleled with the

pathological progression in mice genetically engineered to develop Alzheimer's disease. We also found that genetic silence against LPAR1 mRNA in primary astrocytes led to an increase of LPS-activated immune responses, while overexpression of LPAR1 or treatment of LPA *in vitro* suppressed astrocyte reactivation as evidenced by the reduction of many pro-inflammatory indications. Importantly, the reduction of body temperature and the expression of hippocampal inducible nitric oxide synthase (iNOS) in mice received intraperitoneal injection of LPS can be ameliorated by co-treatment of LPA, which effects were abolished by an LPAR1 inhibitor, Ki16425. These data suggest that LPA-LPAR1 signaling could play an anti-inflammatory role during neuroinflammation. However, our findings also indicate that intraperitoneal injection of LPA promoted LPS-induced GFAP expression and astrocyte IL-6 in the hippocampus in an LPAR1-dependent manner. In summary, LPA-LPAR1 signaling exerts multiple functions in the modulation of astrocyte reactivation under the circumstances of pathological insults. Because of the pivotal roles of astrocyte-mediated neuroinflammation in the pathogenesis of many neurodegenerative diseases, further understanding the impacts of astrocyte LPA-LPAR1 signaling in the diseased brain may confer the molecular basis for the development of the disease therapy.

Disclosures: **L. Tsai:** None. **Y. Lee:** None. **C. Wang:** None. **J. Liang:** None. **P. Hsu:** None. **F. Shie:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.14/C49

Topic: B.11. Glial Mechanisms

Support: DP1MHI04069

Title: Neural microcircuit specific astrocyte transcriptomes and proteomes

Authors: *B. DÍAZ CASTRO, H. CHAI, F. GAO, E. MONTE, G. COPPOLA, J. WHITELEGGE, T. M. VONDRISKA, B. S. KHAKH;
UCLA, Los Angeles, CA

Abstract: Astrocytes are found throughout the brain and are involved in diverse processes including synapse formation/removal, ion homeostasis, neurotransmitter uptake, regulation of neuronal function, metabolic support of neurons, and blood flow regulation. In addition, astrocytes respond to all forms of injury and trauma. A common assumption has been that

astrocytes serve identical functions throughout the brain, i.e. that they are a largely homogenous population of cells. However, recent studies raise the possibility that astrocytes may mediate a diverse range of responses in a manner that is tractable and suggestive of astrocyte diversity. In order to systematically compare astrocytes from two different regions of the brain, we performed side-by-side evaluations of astrocyte transcriptomes and proteomes from the hippocampus and striatum. To this end, we refined protocols to enzymatically dissociate brain tissue and then to isolate astrocytes by fluorescence activated cell sorting from P7 and P30 Aldh1L1-eGFP mice, which express eGFP in essentially all astrocytes. We performed basic Western blot analyses to confirm that pure populations of astrocytes were isolated from each area. We used Illumina MouseRef-8 v2.0 Expression BeadChip microarrays and a Thermo LTQ Orbitrap XL mass spectrometer to carefully document RNA and protein expression profiles for hippocampal and striatal astrocytes. Here, we report 70 astrocyte-enriched molecular markers that are common between the hippocampus and striatum, and maintained through postnatal brain development. In addition, our data reveal 89 genes displaying differential expression in astrocytes from striatum and hippocampus at P30 and up to 108 such genes at P7. Finally, we compare results from transcriptomic and proteomic evaluations for astrocytes from hippocampus and striatum at P7. Our data and analyses so far suggest that astrocytes from striatum and hippocampus are largely similar and that most expressed transcripts are common to both cell populations. However, astrocytes from these two regions can be classified as distinct populations based on the expression of several unique genes, supporting the idea that astrocyte function is dependent on the local neuronal microcircuit. Our results will serve as a valuable resource to perform hypothesis-driven experiments to explore astrocyte physiology in the hippocampus and striatum.

Disclosures: **B. Díaz Castro:** None. **H. Chai:** None. **F. Gao:** None. **E. Monte:** None. **G. Coppola:** None. **J. Whitelegge:** None. **T.M. Vondriska:** None. **B.S. Khakh:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

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Topic: B.11. Glial Mechanisms

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Larry L. Hillblom Foundation

NIH Grant NS087783

Title: Morphologic, phenotypic, and transcriptomic analyses of post-stroke reactive astrocyte heterogeneity: bridging structure and function

Authors: *A. J. GLEICHMAN[1], R. KAWAGUCHI[2], M. V. SOFRONIEW[2], G. COPPOLA[2], S. CARMICHAEL[1];
[1]Neurol., [2]UCLA, Los Angeles, CA

Abstract: It is increasingly clear that astrocytes have diverse and complex roles in both the healthy and injured CNS, such as regulating blood flow, mediating synapse formation and elimination, and responding to and releasing cytokines and chemokines. Many of these functions have clear implications for post-stroke repair, yet little is known about how astrocytes respond to stroke or how these responses may vary. Using both cortical and white matter models of ischemic stroke, we have analyzed the morphologic, phenotypic, and transcriptomic changes astrocytes undergo in these different regions as a function of their distance from the site of injury. The most commonly used marker of reactive astrocytosis is GFAP, which allows only visualization of the main branches of astrocytes. In order to more completely elaborate the fine processes of astrocytic arbors, which comprise the majority of the astrocyte and are crucial for many astrocytic functions, we have developed lentiviral vectors that drive expression of advanced reporters. Using these reporters, we have delineated multiple distinct zones of astrocytes by varying morphology. By developing similar tools for other cell populations, we can obtain a detailed view of the interactions between astrocytes, neurons, and oligodendrocytes. As many important astrocytic functions are dependent on the interaction of astrocytes with other cell types, this detailed morphologic assessment of not only astrocytes but also their position relative to other cell types can yield important clues as to their changing functions post-stroke. Furthermore, morphologically discrete zones have then been further characterized by phenotypic differences, including differences in Ki67, aquaporin-4, glutamate transporters, potassium channels, and others. Together, these data support the existence of multiple discrete zones of reactive astrocytes post-stroke. We have used the morphologic and phenotypic delineation of astrocytic zones to inform transcriptomic analyses of these regions. Using the Ribotag mouse model with a GFAP Cre, we have developed a model in which astrocytic ribosomes express an HA tag, allowing immunoprecipitation of ribosomes and their associated mRNA. Discrete astrocytic zones are specifically isolated using laser capture microdissection, ribosomes are immunoprecipitated, RNA isolated and analyzed by RNAseq. These data provide us a clear view of the differences between these astrocytic populations, which may afford insight into their different roles and the ways in which those roles may be manipulated to promote post-stroke repair. Supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

Disclosures: A.J. Gleichman: None. **R. Kawaguchi:** None. **M.V. Sofroniew:** None. **G. Coppola:** None. **S. Carmichael:** None.

Poster

128. Astrocytes: Profiling and Modulation

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Topic: B.11. Glial Mechanisms

Support: NIH Grant R01NS081703

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Title: Acute purification and transcriptome profiling of human astrocytes

Authors: *Y. ZHANG, S. A. SLOAN, L. E. CLARKE, C. CANEDA, M. HAYDEN-GEPHART, G. A. GRANT, S. H. CHESHIER, M. S. EDWARDS, G. LI, G. K. STEINBERG, H. VOGEL, P. BLUMENTHAL, B. A. BARRES;
Stanford Univ., Stanford, CA

Abstract: Astrocytes play critical roles in the development and function of the central nervous system. However, our understanding of astrocyte physiology is largely predicated on research in rodent models. Surprisingly, the degree to which rodent and human astrocytes are comparable remains relatively unknown. We developed an immunopanning-based method to acutely purify astrocytes from fetal and postnatal human brains without the need to expand these cells *in vitro* via exposure to serum. Functionally, we found that similar to rodent astrocytes, human astrocytes are capable of promoting neuronal survival, inducing synapse formation, and engulfing synaptosomes. Furthermore, human astrocytes exhibit distinct calcium responses to ATP and glutamate. To uncover the human astrocyte transcriptome, we performed RNA-sequencing of acutely purified astrocytes and compared these data to purified human neurons, oligodendrocytes, microglia and endothelial cells. Our purification method and transcriptome dataset provide new resources for investigating the function of human astrocytes in healthy and diseased brains. This work is supported by NIH grants R01NS081703 and R01MH099555 to B.A.B., K99NS089780 to Y.Z., and F30MH106261 to S. A. S. Y.Z. and S.A.S. contributed equally to this work.

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Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.17/C52

Topic: B.11. Glial Mechanisms

Support: NSF IOS 1354913

Title: Diurnal influences on regional astrocyte morphological functional plasticity

Authors: *S. IRVING[1], M. GILLETTE[2], J. MITCHELL[2];
[1]Mol. and Integrative Physiol., [2]Cell and Developmental Biol., Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Astrocytes in the brain are critical to maintaining homeostasis and modulating synaptic signaling and strength. These cellular activities occur in the context of regional differences in brain function that vary with respect to time of day. When challenged osmotically, hypothalamic astrocytes undergo extreme morphological changes that alter relationships with surrounding cells and coordinate neurohormone release. We asked whether under resting physiological conditions morphological change occurs on a daily basis, the period over which brain physiology oscillates normally. To evaluate whether astrocytic morphology varies diurnally, we measured branch complexity of the GFAP cytoskeleton and membrane surface area in two brain regions, the hypothalamic suprachiasmatic nucleus (SCN) and the hippocampal gyrus (DG), throughout the day-night cycle. Unbiased analysis using Imaris software found that GFAP branch complexity and astrocyte surface membrane area of in the SCN are greatest in the early morning (zeitgeber time 2, ZT2) and smallest in the early evening (ZT14). The hippocampal DG showed the opposite changes, with the greatest complexity and surface area in early night and least in early morning. Additionally, utilizing a hyperosmotic challenge via intraperitoneal (IP) injection at ZT2 and ZT14, we identified diurnal differences in GFAP branching changes in response to osmotic stress. In addition to the SCN and DG, we also analyzed GFAP branching in the supraoptic nucleus (SON), a hypothalamic region known to respond to osmotic stress. Results suggest that time-of-day influenced the magnitude of GFAP branching in the SON in response to osmotic stress, with a greater change in early night v*s.* early day, whereas osmotic stress did not change the GFAP branching morphology significantly in either DG or SCN. In conclusion, astrocytes exhibit diurnal regional heterogeneity in GFAP branching complexity and differences in the magnitude of the branching changes, along with differential responses to osmotic challenge. Funding: NSF IOS 1354913

Disclosures: **S. Irving:** None. **M. Gillette:** None. **J. Mitchell:** None.

Poster

128. Astrocytes: Profiling and Modulation

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Program#/Poster#: 128.18/C53

Topic: B.11. Glial Mechanisms

Support: Research Support Center (Graduate School of Medical Sciences, Kyushu University)

Title: Impact of thyroid hormone on glial function and morphology

Authors: ***M. NODA**, J. LIU, Y. YOSHII, Y. YOSHIOKA;
Kyushu Univ., Fukuoka, Japan

Abstract: L-tri-iodothyronine (3, 3', 5-triiodothyronine; T3) is an active form of the thyroid hormone (TH) essential for the development and function of the central nervous system (CNS). Circulating thyroxine (T4) crosses blood-brain barrier via specific transporters. T4 in the brain is taken up by astrocytes, de-iodinated to produce T3, and then taken by other cells. In adult CNS, both hypo- and hyper-thyroidism may affect psychological condition and potentially increase the risk of cognitive impairment and neurodegeneration including Alzheimer's disease (AD). We have reported non-genomic effects of T3 on microglial functions and its signaling *in vitro* (Mori et al., GLIA, 2015). To investigate whether or not change in THs level affects glial cells *in vivo*, the effects of hyper- and hypothyroidism on microglia and astrocytes were investigated. The morphological changes in microglia and astrocytes in the cerebral cortex and hippocampus in C57/BL6 mice were investigated by immunohistochemical analysis. Hyper- or hypothyroidism were induced by intraperitoneally injecting T4 (0.3 mg/kg) 4 times during 2 weeks or propylthiouracyl (60 mg/kg/day) for 21 days. Interestingly, effects of hyper- and hypothyroidism on glial cells were sex- and age-dependent. Behavioral changes are now under investigation. These results may help to understand physiological and/or pathophysiological functions of THs in the CNS.

Disclosures: **M. Noda:** None. **J. Liu:** None. **Y. Yoshii:** None. **Y. Yoshioka:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: B.11. Glial Mechanisms

Support: NIH Grant HS2694

Title: Molecular heterogeneous atlas of adult mouse and fly astroglia

Authors: *Y. YANG, L. MOREL, Y. HUANG, S. NG, M. TOLMAN, F. NG, N. BOWENS, S. SENGUPTA, L. IYER, R. JACKSON;
Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: *In vivo* molecular evidence to support astroglia heterogeneity is largely lacking, as is information regarding conservation of astroglia-expressed genes between important biological models. Employing TRAP-mediated isolation of translating mRNAs from adult astroglia of mouse forebrain and subcortical regions, and subsequent RNA-seq analysis, we provide the first *in vivo* evidence for a genome-wide molecular heterogeneity of brain astroglia. Our current study also reveals region-specific expression patterns for genes that are selectively expressed and functionally important in astroglia, providing novel insights about the specificity of their functions. Using similar TRAP-based methods, we identify *Drosophila* genes showing enriched expression in astroglia that are evolutionally conserved between the fly and mouse. A subsequent RNAi-based screen in the fly shows that astroglial AP-2σ expression is required for normal circadian behavior. Overall, our *in vivo* results suggest important functions for certain mouse and fly astroglial genes and provide the first molecular heterogeneous atlas for adult astroglia.

Disclosures: **Y. Yang:** None. **L. Morel:** None. **Y. Huang:** None. **S. Ng:** None. **M. Tolman:** None. **F. Ng:** None. **N. Bowens:** None. **S. Sengupta:** None. **L. Iyer:** None. **R. Jackson:** None.

Poster

128. Astrocytes: Profiling and Modulation

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Topic: B.11. Glial Mechanisms

Support: Brain Science Institute

Robert Packard Center for ALS Research

National Science Foundation GRFP Fellow

Title: Molecularly defined astrocyte subpopulations in adult CNS and their response to neurodegenerative disease injury

Authors: *S. J. MILLER[1], T. PHILIPS[2], M. ROBINSON[3], R. SATTLER[2], J. D. ROTHSTEIN[1];
[1]Neurology/Cellular & Mol. Med., [2]Neurol., Johns Hopkins Sch. of Med., Baltimore, MD;
[3]Pharmacol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Astrocytes are the most abundant cell types in the central nervous system. Historically, astrocytes have been considered a homogenous population of cells. However, accumulating evidence has shown that astrocytes are indeed a heterogeneous cell type that exhibits distinct gene and protein expression profiles dependent on their anatomical location. To date, there is a very limited collection of tools available to study different astrocyte populations. This is due to a lack of known markers unique to each subtype. Previously our group has generated a transgenic mouse model containing a genetically encoded fluorescent reporter that labels a specific astrocyte subpopulation in the adult spinal cord and neocortex. We employed this model to study astrocyte subpopulations and show that the distribution of these astrocytes is enriched in Layers II and V of the neocortex and in the ventral horn of the spinal cord grey matter. We also utilized multiphoton *in vivo* imaging and were able to track individual astrocytes over a time period of weeks in the adult mouse neocortex thus establishing that this fluorescent reporter is static. Finally, to understand the role of this astrocyte subpopulation in neurodegenerative disease context, we crossed our transgenic astrocyte reporter mice with G93A SOD1 ALS mouse model. We show that this astrocyte subpopulation and markers enriched in this subpopulation are remarkably affected in both the spinal cord and neocortex of the ALS mouse. Taken together, these results shed light on the heterogeneous population of astrocytes in health and the susceptibility of certain astrocyte subpopulations to disease.

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Poster

128. Astrocytes: Profiling and Modulation

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Topic: B.11. Glial Mechanisms

Support: WUSTL Neurosciences Program T32 GM008151

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NIH R21 DA038458-01

NIH R21 MH099798-01

WUSTl CDI MD-II-2013-269

Title: Locally translated proteins in peripheral astrocyte processes

Authors: *K. SAKERS, J. D. DOUGHERTY;
Genet., Washington Univ. Sch. of Med. in St Lou, Saint Louis, MO

Abstract: Within the last two decades, the critical role of peripheral astrocyte processes (PAPs), especially those surrounding synapses, have become increasingly more apparent. Of their many functions, PAPs serve to maintain proper neurotransmission by buffering ions and neurotransmitters in the synaptic cleft. To do so, PAPs must express specific ion channels and solute carrier proteins at their distal ends - at or near the synapse. PAPs are the third component of the “tripartite synapse,” a dynamic structure in which rapid responsiveness to stimuli is critical. Neurons, for example, have adapted to locally synthesize proteins in their dendrites to compensate for the distance from the cell body. This mechanism is important during prolonged periods of activity, such as long-term potentiation, and is required for processes of learning and memory. As a fundamental part of the tripartite synapse, PAPs must have the ability to rapidly adapt to synaptic activity as well. Because PAPs meet, and exceed, length requirements of neuronal dendrites, we hypothesize that PAPs locally synthesize proteins. In order to test this, we have developed PAP-TRAP (peripheral astrocyte processes-translating ribosome affinity purification) in which we can isolate PAPs and purify mRNA bound to ribosomes in order to determine which, if any, mRNAs may be undergoing local translation in these distal processes. Coupling this method to qPCR for candidate mRNAs, we have found evidence for the local translation of the glutamate transporters, Glt1 and Glast, and the inwardly-rectifying potassium channel, Kir4.1.

Disclosures: K. Sakers: None. **J.D. Dougherty:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TRAP Licensing.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.22/C57

Topic: B.11. Glial Mechanisms

Support: NS 0075062

Title: Novel applications of magnetic sorting to analyze cell-type specific gene and protein expression in a mouse model of Rett Syndrome

Authors: *L. HOLT, N. PACHECO, M. OLSEN;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: The isolation and study of cell-specific populations in the central nervous system (CNS) has gained significant interest in the neuroscience community. The ability to examine cell-specific gene and protein expression patterns in healthy and pathological tissue is critical for our understanding of CNS function. Here we demonstrate that distinct cell populations can be isolated in rodents through adulthood using magnetic bead sorting. We found this technique to be easily amendable to customization using commercially available membrane targeted antibodies, allowing for cell-specific isolation across development and animal species. High quality RNA which can be utilized for downstream applications including quantitative PCR and RNA sequencing was obtained at relatively low cost and without the need for specialized equipment or fluorescently labeled cells. Adding to its utility we demonstrate that cells were isolated largely intact, retaining their processes, enabling analysis of extrasomatic proteins. We used magnetic cell sorting to examine changes in astrocyte gene/protein expression and function in the neurodevelopmental disorder Rett Syndrome. Rett Syndrome, which is caused by mutations in the transcriptional regulator MeCP2, has long been considered a neuronal disease. However, recent work highlights a role for astrocytes in disease pathogenesis. Preliminary data suggests that BDNF is significantly decreased in astrocytes in a mouse model of Rett Syndrome. Work in progress is directed at elucidating both the autonomous and non-autonomous contribution of aberrant astrocytic BDNF regulation in Rett Syndrome.

Disclosures: L. Holt: None. **N. Pacheco:** None. **M. Olsen:** None.

Poster

128. Astrocytes: Profiling and Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 128.23/C58

Topic: B.11. Glial Mechanisms

Title: Defining gene expression and protein secretion signatures in murine primary glia

Authors: *Y. HE[1], N. TAYLOR[1], X. YAO[2], T. LOVENBERG[1], A. BHATTACHARYA[1]; [1]Neurosci., [2]Discovery Sci., Janssen Res. & Develop. LLC., San Diego, CA

Abstract: Growing evidence shows depression involves a complex and bidirectional interaction between the immune system and the central nervous system (CNS). Immune dysfunction plays an important role in the etiology and pathogenesis of major depression by increasing production of pro-inflammatory cytokines. Like peripheral immune cells, glia in the brain are the main source and target cells for cytokines/chemokines (gliotransmitters), which regulate neuronal survival, synaptic plasticity, and neurogenesis. However, the effects of these factors on glia during depression are not clear. To that end, we measured gene expression and protein secretion of isolated glia upon depression-related cytokine stimulation (IL-1b, TNFa, IL-6; 10 ng/ml; 24 hrs). IL-1b was the most sensitive cytokine in the microglia when assessed for upregulation of a panel of genes (iNOS, TSPO, COX-2, P2RX7, CCR2, CSF1R, Arg-1, IL-6, TNFa, IL-1b). Contrary to this, astrocytes were more sensitive than microglia in upregulating expression of iNOS, TSPO, TNFa, IL-6, and CSF-1R upon TNFa stimulation. In conditions that drove M1 (LPS + IFNg) polarization, astrocytes specifically secreted chemokines CXCL1 and CCL2, while microglia secreted CCL3, CXCL2, GCSF, IL-12, TNFa and IL-6. It is apparent that microglia and astrocytes respond to neuroinflammation differentially; the signatures defined at the murine glia will be used to compare and contrast human glial signatures that may shed insight into disease biology and aid in novel target discovery.

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Poster

128. Astrocytes: Profiling and Modulation

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Topic: B.11. Glial Mechanisms

Support: China NSF Grants 81101899

China NSF Grants 81372698

JiangSu PSF Groject 1202119C

Title: The significance and mechanism of differential expression of GDNF splice variants in glioma cells

Authors: H. LI[1], *D. GAO[1,2];
[1]Xuzhou Med. Col., Jiangsu, China; [2]Neurosurg., Affiliated Hosp. of Xuzhou Med. Col., Xuzhou, China

Abstract: Glial cell line-derived neurotrophic factor (GDNF) is highly expressed in glioma cells, and there is evidence that two different GDNF splice variants may be present during transcriptional splicing in eukaryotic cells. It is unclear whether this alternative splicing is related with high GDNF expression in glioma cells or whether different GDNF splice variants produced as a result impact glioma pathogenesis. To shed light on these issues, this study was designed to measure the differential expression of GDNF splicing isoforms in glioma cells, analyze the relationship between these differences and the characteristics of different types of glioma cells, and explore possible mechanisms for these differences. Our results indicate that the two GDNF isoforms, α- and β-pro-GDNF, are expressed in different types of glioma cells, but α-pro-GDNF was preferentially expressed in the most highly invasive glioblastoma tissue. In addition, histone acetylation assays revealed high H3K9 acetylation at the GDNF Δ78 alternative splicing site in glioma cells. Collectively, these results demonstrate differential expression of GDNF alternative splice variants in glioma cells and a possible relationship between preferential expression of α-pro-GDNF and high tumor invasiveness; they also serve as preliminary evidence that differential GDNF expression may be related to changes in histone acetylation in the gene's coding region.

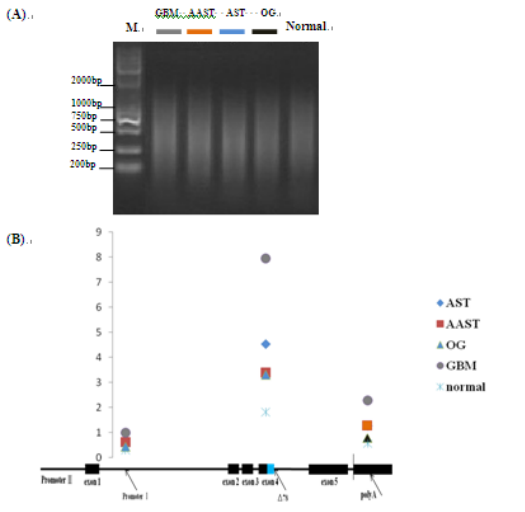

Figure. H3K9 acetylation at the GDNF Δ78 site in gliomas. (A) Electrophoresis of DNA after ultrasonic chromatin shearing. M indicates Marker. (B) H3K9 acetylation at the Δ 78 site compared to the promoter and polyA region. The ordinate indicates the GDNF gene mode, exons are shown as black boxes, and horizontal lines indicate the intron. The corresponding polyA region is behind the vertical bar. The site selected for H3K9 acetylation measurement is denoted with an arrow. Normal represents normal brain tissue. In both panels, AST, OG, AAST, GBM, and Normal indicate the four glioma samples and normal brain tissue, respectively.

Disclosures: H. Li: None. **D. gao:** None.

Poster

128. Astrocytes: Profiling and Modulation

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Topic: B.11. Glial Mechanisms

Support: NIH P50GM068762

NMSU Manasse Endowment

Title: Tridimensional culture induces neuronal differentiation in a commercial normal human astrocyte cell line (NHA)

Authors: *V. B. KNIGHT, E. E. SERRANO;
Biol., New Mexico State Univ., Las Cruces, NM

Abstract: Tridimensional environments modulate the fate and morphology of many different cell types. When a primary human cell line derived from embryonic brain tissue was cultured in 3D (Normal Human Astrocytes; NHA; Lonza), its morphology resembled that of mixed neuronal-glial cultures. The characteristic groupings of phase-bright neuronal somata were not visible in monolayer culture where instead, cells assumed the flattened uniform appearance of glial cultures. Next Generation Sequencing technology was used to explore the genetic basis for the observed structural differences. RNA (RIN > 8.9) from replicate NHA samples grown in monolayer or 3D was sequenced at the MIT BioMicro Center using Illumina Tru-Seq protocols. Sequences were mapped to the human genome (UCSC build hg 19; Genome Reference Consortium GRCh37) using TopHat pipeline software running on GenePattern. DeSeq was used to determine whether genes were differentially expressed between tridimensional and monolayer culture conditions. The gene ontology of differentially expressed genes was explored with DAVID (Database for Annotation, Visualization and Integrated Discovery v6.7) and GeneCards. We found significant (enrichment score > 1.3) annotation clusters that included ontological terms such as “neuron development”, “axonogenesis”, “cognition”, and “learning or memory”. Functional analysis suggests that the morphological differences observed in 3D could be due to key genes that include UNC5B and NPTX1. Outcomes may reflect the degree of genetic and cellular variation in primary tissue-derived lines as compared to clonally-derived lines. Results imply that tissue engineering and targeted cellular differentiation can be undertaken with this commercially available cell line. Supported by NIH P50GM068762; NMSU Manasse Endowment.

Disclosures: **V.B. Knight:** None. **E.E. Serrano:** None.

Poster

128. Astrocytes: Profiling and Modulation

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Topic: B.11. Glial Mechanisms

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G11 HD052352

USDE Title V PPOHA P031M105050

RCMI-SRDU

NIGMS-SC1GM088019

NIGMHD-G12MD007583

Title: TAMRA-conjugated spermine is selectively taken up by glial cells in the rodent brain and retina

Authors: *J. BENEDIKT[1], A. ZAYAS-SANTIAGO[2], Y. RIVERA[2], Y. KUCHERYAVYKH[2], L. KUCHERYAVYKH[2], L. CUBANO[2], M. EATON[2], R. VEH[3], C. NICHOLS[4], S. SKATCHKOV[2];
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Abstract: The polyamines (PAs) spermine (SPM) and spermidine (SPD) are omnipresent molecules stored predominantly in astrocytes, but not in neurons of the adult rat brain. Because we found no synthesis for SPM and SPD in glial cells, we speculate that PAs are taken up by a yet unknown mechanism. Using RT-PCR we found organic cation transporters (OCT1, OCT2 and OCT3) in rodent brain and using immunocytochemistry we observed OCT3 localization in astrocytes in rat hippocampus and cortex. For the visualization of the uptake of SPM in rodent brain slices, we used covalently tagged SPM with fluorescent 5(6)-carboxytetramethylrhodamine (TAMRA). We perfused rat and mouse hippocampal slices with an extracellular solution containing 10 μM SPM-TAMRA and observed rapid and extensive uptake of fluorescent SPM into astrocytes, but not into neurons or other parenchymal cells. When 10 μM of unconjugated TAMRA was perfused, there was no specific staining of any cell type in the hippocampus, suggesting the presence of a specific mechanism for SPM uptake. Similar experiments performed in isolated retina revealed strong staining of retinal Müller cells, while in corpus callosum, characterized by presence of fibrous astrocytes, only weak uptake of SPM-TAMRA was observed. These findings suggest there is SPM-specific transport mechanism present in protoplasmic astrocytes of the grey matter, but not in fibrous astrocytes. When 10 μM SPM-TAMRA was injected to a single astrocyte via the patch pipette, it spread from one into other astrocytes in the stratum radiatum region of CA1 hippocampus. SPM-TAMRA rapidly diffused through the astroglial syncytium and eventually stained hundreds of astrocytes in a 10 min time interval. The same experiments were performed with 10 μM unconjugated TAMRA as control and this resulted in a low level of dye propagation (33 cells on average). In both cases, pre-application of gap-junction blocker carbenoxolone (200 μM) effectively abolished the propagation of the SPM-TAMRA, implicating a gap-junction mediated mechanism. Altogether, our data suggest that SPM-TAMRA is specifically taken up by hippocampal protoplasmic astrocytes and rapidly propagated through their syncitium.

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Poster

129. Brain Wellness

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Title: Differences in resting state functional connectivity between aerobic athletes and sedentary young adults

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Abstract: Recent studies have shown that aerobic exercise can positively impact brain structure and cognitive function across the lifespan. In older adults, physical activity generates the greatest improvements in executive functions, which some have linked to increased functional connectivity in the fronto-parietal resting state network (FPN). Fewer studies have examined the effects of physical activity on young adult brains, a key sample since exercise at young ages may impact the trajectory of brain aging. Here, we compared resting-state functional connectivity in a sample of adult male collegiate cross-country runners (n=12; mean±SD age = 21.3±2.5) and a sample of healthy, sedentary age-matched male controls (n=11; mean±SD age = 20.6±1.1) to test the hypothesis that aerobic activity affects resting state networks associated with executive control functions in young adults. While subjects did not differ in body mass index ($p=0.60$), the athletes had higher estimated $VO_{2,max}$ ($p<0.000001$) and self-reported physical activity levels ($p<0.000001$) than controls. T1 volumetric and resting-state functional connectivity magnetic resonance imaging (MRI) scans were acquired on a 3.0T GE Signa Excite scanner at rest with eyes open. The subject maps of the correlations between the average of two seed regions in the

FPN (left and right anterior prefrontal cortex) and all voxels of the brain were computed using the CONN functional connectivity toolbox (Whitfield-Gabrieli et al., 2012) with functional images registered to the structural scans and significance for cluster extent taken with FDR, $p < 0.05$. The athletes had greater average connectivity from the two seed regions of the FPN to a region in the left superior frontal gyrus (SFG) compared to controls, who showed an anti-correlation in this area. The controls also showed a region of anti-correlation in the right precentral gyrus (PCG) that was not observed in the athletes. There were significant positive associations between the correlations at these two regions and self-reported physical activity (left SFG $p=0.003$; right PCG $p<0.00004$) and estimates of maximum aerobic capacity (left SFG $p<0.0002$; right PCG $p<0.00002$). Since the SFG has been implicated in working memory and task-switching, and the PCG is associated with motor tasks, our results suggest that differences in aerobic activity observed between endurance athletes and sedentary adults may differentially impact the functional connectivity that link aspects of executive and motor functions. These findings suggest that high intensity physical activity, such as running, stresses cognitive domains in ways that lead to altered functional brain connectivity.

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Title: The role of pericytes in cerebrovascular metabolism

Authors: *A. RAMANATHAN, K. KISLER, A. M. NIKOLAKOPOULOU, A. P. SAGARE, B. V. ZLOKOVIC;
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Abstract: At the site of the blood-brain barrier (BBB), pericytes surrounding the cerebral microvessels are known to regulate BBB formation and maintenance, angiogenesis and vascular

architecture. Recently, the key physiological role of pericytes was uncovered; the contractile pericytes modulate cerebral blood flow by regulating capillary diameter in the adult brain. Thus, pericytes may functionally affect the metabolic supply to the brain by acting as the regulatory floodgates surrounding the endothelia. Here, using a mouse model with pericyte deficiency (Pdgfrβ+/-), we examine how the loss of pericyte coverage affects the underlying cerebrovascular metabolism. Using *in vivo* microdialysis, we have observed that in the somatosensory cortex of Pdgfrβ+/- mice, there is a 55% elevation in interstitial fluid (ISF) levels of lactate at baseline, while ISF glucose and serum metabolite levels remain unchanged. Additionally, upon neuronal stimulation via high [K+] aCSF retrodialysis, there was a significant 28% reduction in the ability of the cortical milieu to recruit lactate to maintain the metabolic demands during increased neuronal activity. These early indications of metabolic stress were accompanied by changes in glycolytic capacity in Pdgfrβ+/- microvessels. Using quantitative autoradiography, we observed reduced blood-to-brain uptake of 14C 2-deoxyglucose, suggesting irregular metabolic supply to the brain. Furthermore, electron microscopy studies demonstrate an altered morphology in the endothelial mitochondria of Pdgfrβ+/- mice. Alzheimer's disease (AD) is separately associated with both, a degeneration of pericytes and a hypometabolic brain. These data suggests that pericytes may contribute to the bioenergetic changes observed in AD.

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Departamento de Ciencias de la Salud, Lerma UAM

Title: Effects of high-sucrose diet on cognitive function in a rat model of metabolic syndrome

Authors: *S. HERNANDEZ RAMIREZ[1], K. R. GUZMÁN-RAMOS[3], M. VELASCO[1], D. OSORIO-GÓMEZ[2], L. F. RODRÍGUEZ-DURÁN[3], M. L. ESCOBAR[4], F. BERMUDEZ-

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Abstract: In the last two decades several studies have reported different mechanisms that could link type two diabetes mellitus (T2DM) and dementia. Most of the Alzheimer's disease patients show alterations in the glucose metabolism and insulin signal transduction. It is thought that people develop clinical manifestations of dementia many years after the onset of brain deterioration, hindering the establishment of the disease temporality. Due to this fact, we decided to evaluate the cognitive performance in an earlier stage of metabolic dysfunction, i.e. metabolic syndrome (MS). Since MS is a multifactorial complex of signs that increases the probability to develop several types of health problems, cardiovascular processes, diabetes, certain types of cancer and even cognitive deficit among them. We treated young adult Wistar rats during a period of six months with sucrose 20% in drinking water. After the treatment, we performed three different memory tasks (Morris-Water Maze, Object Recognition Memory and Object Location Memory) and several metabolic parameters were monitored (blood glucose levels, insulin levels, body mass index and oral glucose tolerance test). We observed that MS rats showed a selective hippocampus-dependent cognitive impairment, since cortical function seems to be spared at this stage. In addition, we observed decreased levels in the synaptophysin vesicle protein as well as an impaired hippocampal long-term potentiation and increased levels of the Glial Fibrillary Acidic Protein (GFAP). We concluded that the MS rats present a mild hippocampal dysfunction due to the decreased levels of the synapses and the cognitive function performance in the memory tasks.

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Poster

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Topic: C.01. Brain Wellness

Title: Transcriptional profiling of deep brain stimulation in an animal model of antidepressant treatment resistance

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Abstract: Deep brain stimulation (DBS) is an emerging therapy being evaluated for treatment resistant depression. The current study aimed to identify antidepressant mechanisms of DBS in an animal model of treatment resistant depression. We have investigated the cellular mechanisms mediating antidepressant actions of DBS in an animal model of antidepressant treatment resistance induced via chronic administration of adrenocorticotrophic hormone (ACTH) in male Sprague-Dawley rats. Animals were either stress naïve or were administered the forced swim test (FST) at the end of the treatment. A subset of animals also received DBS of the IL as an anti-depressant treatment. Our goal was to take an unbiased approach to identify the mechanisms through which activity in IL is disrupted in antidepressant resistant state and how it is modified by DBS. The IL was dissected and global gene expression profiles obtained (Agilent). Gene set enrichment analysis was performed (DAVID) following Bonferroni correction and KEGG pathways identified (Fisher exact score $p<0.05$). Pivotal genes were validated in independent groups (n=4-5) by RT-PCR and/or immunoblotting. Significant alterations were observed in key sensors of energy demand, cell division/growth, apoptosis, protein synthesis and glucose/glycogen regulation. Phospho-AMPK and phospho-AKT levels were decreased in ACTH/FST animals compared with ACTH/naïve by 45 and 40% ($p<0.05$), respectively, and not reversed with DBS. Moreover, DBS of IL was shown to effectively reduce immobility time in antidepressant resistant animals and reversing the effects of the genes involved in the control of cellular stressors rather than those involved in energy/glucose regulation.

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Poster

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Title: Anesthesia-induced changes in mouse brain glucose uptake are region-dependent

Authors: *P. BASCUÑANA, J. T. THACKERAY, F. M. BENGEL, J. P. BANKSTAHL; Nuclear Med., Hannover Med. Sch., Hannover, Germany

Abstract: F-18-Fluoro-deoxyglucose (FDG) has been widely used for imaging brain metabolism in both, clinical and animal studies. Due to the fact that preconditioning as well as anesthesia might influence brain FDG levels, this study evaluated how this affects both FDG uptake and kinetics in mice brain. Sixty-minute dynamic PET scans were performed in mice anesthetized with isoflurane [control (n=4), insulin pre-treatment (n=5) and 18h fastening (n=5)], ketamine/xylazine (n=4), sevoflurane (n=6) and chloral hydrate (n=6). These studies were analyzed by comparing standardized uptake values (SUV), FDG influx rate (Slope) and metabolic rate of glucose (MRGlu) calculated by Patlak plot. Ketamine/xylazine and chloral hydrate induced lower uptake of FDG (2.97±0.34, p=0.0017; 4.43±0.11, p=0.022; respectively), whereas, in sevoflurane anesthetized animals uptake was increased (5.58±0.14; p=0.044) compared to the isoflurane group (5.04±0.19). In addition, blood glucose levels showed an inverse correlation to whole brain FDG uptake independently of preconditioning (R2=0.722; p<0.0001). Ketamine/xylazine and chloral hydrate showed a higher uptake in cortex and a lower uptake in cerebellum than isoflurane and sevoflurane animals when normalized to the whole brain uptake. Patlak analysis indicated that use of ketamine/xylazine as anesthetic reduced FDG influx rate in mice brain (0.0135±0.0009 ml/min/g; p<0.005) compared to the isoflurane control group (0.0247±0.0014 ml/min/g). Insulin-treated animals showed a tendency to a higher influx rate than control animals (0.0477±0.0101 ml/min/g; p=0,088). In addition, chloral hydrate animals showed a higher MRGlu (66.72±3.75 mmol/min/100ml; p=0.0014) while pre-treatment with insulin induced a lower MRGlu (21.93±3.12 mmol/min/100ml; p=0.0041) compared to the isoflurane control group (41.55±3.06 mmol/min/100ml). Choice of anesthesia affects not only FDG uptake but also kinetics in mice brain, while an often proposed pre-conditioning, i.e. fastening, did not change any parameter. Total brain uptake is strongly influenced by peripheral blood glucose levels. Interestingly, FDG brain uptake is region-dependently influenced by various anesthesia protocols.

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Poster

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Support: VIEP-BUAP 2015

Title: The combination of alcohol and energy drinks contributes to metabolic and neurodegenerative impairment in rats

Authors: *B. VENEGAS MENESES[1], S. TREVIÑO[1], A. HANDAL[1], J. MORAN[1], P. AGUILAR-ALONSO[1], G. FLORES[1], J. GUEVARA[2], A. DÍAZ[1];
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Abstract: Energy drinks (ED), possess high concentration of caffeine and taurine, which often consumed in combination with ethanol, due to the popular belief that caffeine and taurine may counteract some of the intoxicating effects of ethanol. However, scientific research suggests that chronic use of these psychoactive substances in combination with alcohol trigger an inflammatory response mediated by pro-inflammatory cytokines (IL-1β and TNF-α) and iNOS both in the central and peripheral system modifying the behaviour of individuals and causing cell death (apoptosis). Currently, the mechanism of toxicity caused by the ED-ethanol interaction in the brain, are not well known. In the present study we evaluated the effects of chronic alcohol consumption in combination with energy drinks on the inflammatory response and neurodegeneration in rat. We used male Long Evans rats (200-250g). Were formed, 4 experimental groups (n=10 per group): 1) control (water); 2) Ethanol (Vodka, 40%); 3) ED (Red-bull) and 4) Ethanol + ED. Animals were orally administered for 2 months, in the course of treatment was evaluated, motor activity in closed field (day 0, 30 and 60). After treatment, the animals were sacrificed; the brains were removed for immunohistochemistry and fluorescence immunoassay (ELISA) to identify markers of inflammation (GFAP, IL-1β, TNF-α, iNOS, nitric oxide) and neurodegeneration (caspase-3 and -9, synaptophysin) in the temporal cortex and hippocampus, respectively. The results showed a progressive deterioration in motor activity during the administration of ethanol + ED. Biochemical and histological studies revealed that chronic ethanol + ED caused an increase in reactive gliosis, IL-1β, TNF-α, iNOS and nitric oxide, in the temporal cortex and hippocampus, respectively, also show immunoreactivity positive for caspase-3 and 9, and a decreases synaptophysin in temporal cortex and hippocampus. The results suggest that chronic consumption of alcohol in combination, cause an inflammatory response, which induces neurodegeneration in hippocampus and cerebral cortex, which affects the motor behaviour of the rats. Therefore, it is necessary to show caution in the consumption of these drinks combined with ethanol, so it is necessary to report urgently on the risks it is exposed to the health of consumers of these substances, especially young people.

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Poster

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Title: Strain-related modulation of Cu(I)-ATPase in prion protein-deficient mice

Authors: *J. A. NOVAES[1], R. R. H. F. VALVERDE[2], A. VIEYRA[2], J. LOWE[2], R. LINDEN[2]; [1]Neurobio., [2]Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: The prion protein (PrPC) binds copper at multiple sites and is involved in copper metabolism, but mechanisms of such functional interaction are still poorly understood. Much of the data on functional roles of PrPC were obtained in mice deficient of the PrPC-coding gene Prnp, in mixed B6.129Sv genetic background, and potential pitfalls of such an approach are currently a matter of concern. It has been reported that Prnp-null mice have a decreased content of the copper ATPase ATP7A. The aim of this study was to investigate the effect of knocking out Prnp upon functional properties of copper ATPases in differing mouse strains. In both brain and liver, we found diminished activity, and increased catalytic phosphorylation of Cu(I)-ATPase in Prnp-null mice of mixed B6.129Sv background. However, no difference in either parameter was found between Prnp-null and wildtype mice of B10.129Ola background. Activity of Cu(I)-ATPase was reduced and catalytic phosphorylation was selectively increased in brain tissue from mice of the 129/Sv strain, when compared with wildtype of B6, B6.129Sv, or B10.129Ola strains. The results show a strain-dependent effect of the 129/Sv genotype, rather than a Prnp-dependent regulation of the Cu(I)-ATPase catalytic cycle in Prnp-null mice.

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Title: Regulatory aspects of exercise in mitochondrial remodeling in t2d diabetes

Authors: A. KALANI[1], P. K. KAMAT[1], P. CHATURVEDI[2], L. W. WINCHESTER[2], S. C. TYAGI[2], *N. TYAGI[3];
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Abstract: Abstract: The intense impairment of mitochondrial regulatory functions was suggested to mediate neurodegenerative and cognitive disorders in type-2 diabetes (T2D). Exercise (polypill) has been recommended by clinicians as a secondary protective therapy that exerts myriad beneficial effects against T2D-induced brain pathophysiology; however, exercise effects on diabetic mitochondrial pathology are not well understood. Using mitochondria RT^2 profiler PCR array with exercised and non-exercised db/db (T2D) mice brains, we found that exercise benefit mitochondrial transport, dynamics and regulatory genes. Therefore, we hypothesize that exercise mitigates T2D-induced neurodegeneration and cognitive functions by improving mitochondrial heath. To test this hypothesis, we used 1) db/db mice, 2) db/+ mice as controls, with and without exercise. The mice were exercised at a constant speed of 7 meters/min for 5 days in a week and for the total period of 8 weeks. After that, we investigated different mitochondria-related parameters; ATP production, oxygen consumption, mitochondrial ROS, mitochondrial membrane permeability, mitochondria copy number and mitochondrial dynamics. Interestingly, our results suggest that exercise mitigated almost all mitochondria anomalies in db/db diabetic brains. The intact mitochondria isolated from exercised diabetic db/db brains showed significantly reduced TUNEL positive reactivity as compared to diabetic db/db brains. Furthermore, the loss of neurons was rescued in exercised db/db brains when compared to non-exercised db/db brains, as determined by staining brain coronal sections with Fluoro-Jade C. In addition, exercise significantly mitigated total brain blood flow and memory function, as measured by laser doppler flow and novel object recognition tests, respectively. Taken together after determining gross mitochondrial parameters, these results indicate positive promising results of exercise over mitochondrial pathology in diabetes. Furthermore, our findings suggest regular exercise mitigate neurodegeneration and cognitive disorders thereby improving total mitochondrial health. This work was supported by NIH grant HL107640-NT.

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Title: Chocolate and the brain: Cocoa modulates various levels of sensory awareness and increases power spectral density (μV2) of EEG gamma wave band frequency 31-40Hz associated with brain, mental and physiological benefits

Authors: *L. S. BERK[1,2], E. LOHMAN[1], G. BAINS[1], N. DESAI[3], B. MADANE[3], D. MALI[3], J. BRADBURN[3], S. SHAH[3], S. IYER[3], S. JUNEJA[3], R. MOHITE[3], J. LEE[3];
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Abstract: Cocoa or dark chocolate are a major source of flavonoids. They are potent antioxidant and anti-inflammatory components, with known mechanisms beneficial to cardiovascular health. However, the correlates of neuroelectric activities that initiate the mechanisms of cocoa's beneficial effects on brain neurocognition, synchronization, memory, recall, mood and behavior are unknown. Studies have shown that absorbed cocoa flavonoids penetrate and accumulate in brain hippocampal regions involved in learning and memory. Neurobiological correlates of cocoa flavonoids cascade the expression of neuroprotective and neuromodulatory proteins that promote neurogenesis, neuronal function, brain connectivity, blood-flow improvement and angiogenesis in brain and sensory systems. However, neuroelectric activity initiation and modulatory control of acute action from cocoa flavanoids on brain state has remained unknown. Purpose: Provide evidence for cocoa initiation of brain state frequency modulation and differentiation (0-40Hz) via electroencephalography (EEG). Methods: Assessment of EEG Power Spectral Density (PSD) of 20 subjects during a sequence of cocoa sensory awareness tasks ranging from: cognition of past experience; imagination of eating; visualization; olfaction; taste; and satiation of cocoa consumption (70% cocoa bar), Parliament Chocolates, Redlands, CA). EEG wave band activity was recorded from 9 cerebral cortical scalp locations F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4 using the EEG B-Alert 10X System™ , Carlsbad, CA. Second by second bandwidth for each subject was recorded and summarized. The PSD band width values (BW) were Z-scored, referenced to the eyes closed baseline task. Results: Z-scores, which represent the distance of raw score from baseline mean in units of standard deviation, were graphed and analyzed for each task and (BW) across 0-40 Hz. The overall respective (BW) were collapsed across all 9 EEG channels. Gamma Band Activity (γBA) (31-40HZ) for PSD was greatest in all 9 brain regions for all 6 sensory tasks ($p<0.01$). The most profound observation was that the composite of sensory tasks (PSD) for (γBA) was greater than any other of the six frequencies ($p<0.01$). Conclusion: This study provides objective evidence that (EEG) (γBA) is initiated by different cocoa sensory awareness tasks ranging from prior conditioned experience to

acute cocoa consumption, with subsequent modulation for brain, behavioral and physiological benefits. We propose this protocol as an assessment tool in determining the efficacy of cocoa dose response modulation of PSD (γBA) 31-40 Hz for optimization of brain health benefits.

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Poster

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Title: A high Calorie diet causes metabolic syndrome and oxidative stress into hippocampus and temporal cortex of rats with impairment memory

Authors: ***A. D. DIAZ**[1], P. AGUILAR-ALONSO[2], J. FLORES-HERNANDEZ[1], V. TOXQUI[1], J. GUEVARA[3], G. FLORES[4], G. LOPEZ-LOPEZ[1], G. MUÑOZ-ARENAS[1], E. BRAMBILA[1], S. TREVIÑO[1];
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Abstract: A high calorie intake can induce the appearance of the metabolic syndrome (MS), which is a serious public health problem because it affects glucose levels and triglycerides in the blood. Recently, it has been suggested that MS can cause complications in the brain, since chronic hyperglycemia and insulin resistance are risk factors for triggering neuronal death by inducing a state of oxidative stress and inflammatory response that affect cognitive processes. This process, however, is not clear. In this study, we evaluated the effect of the consumption of a high-calorie diet (HCD) on neurodegeneration and on spatial memory impairment in rats. Our results demonstrate that HCD (90 day consumption) induces an alteration of the main energy metabolism markers, indicating the development of MS in rats. Moreover, an impairment of spatial memory were observed. Subsequently, the brains of these animals showed activation of an inflammatory response (increase in reactive astrocytes and interleukin1-β as well as tumor necrosis factor-α) and oxidative stress (reactive oxygen species and lipid peroxidation), causing a

reduction in the number of neurons in the temporal cortex and hippocampus. Altogether, these results suggest that a high calorie diet promotes the development of MS and contributes to the development of a neurodegenerative process and cognitive failure. In this regard, it is important to understand the relationship between MS and neuronal damage in order to help prevent the onset of neurodegenerative disorders. Furthermore, it will open up the possibility of finding possible treatments to counteract or prevent the occurrence of brain damage caused by metabolic disorders provoked by an excessive calorie intake.

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Topic: C.01. Brain Wellness

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Title: Examining the effects of kale, arugula, and dandelion greens on memory in obese pre-diabetic C57BL/6 mice

Authors: *B. TENG, D. FOSTER, L. BANNER;
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Abstract: The United States is facing a diabetes epidemic. As the 7th leading cause of death and afflicting nearly 10% of the population, diabetes is well studied and its complications are conspicuous, including a battery of neurodegenerative diseases such as Alzheimer's and other cognitive degradation. Diet-induced obesity is an increasingly common occurrence that predisposes individuals to type-2 diabetes and contributes to complications including those of the nervous system and the immune system. Increased body mass is associated with an elevated risk for neurodegeneration and dementia and changes in hippocampal plasticity and spatial learning among others have been documented. While the exact mechanism has yet to be fully understood, neuro-inflammation is thought to be an important factor in impaired cognitive function. Cytokines involved in the inflammatory response are elevated in brains of animals fed a high fat diet (HFD) and a variety of anti-inflammatory/anti-oxidant treatments can reduce this expression and alleviate cognitive changes. What is not well understood is how the progressive nature of

cognitive degradation, as measured by memory loss, manifests itself early in the pre-diabetic stage. Cognitive degradation may be ascribed to neuro-inflammation and differences in diet may either aggrandize or temper its severity. Specifically, we are interested in seeing if adding kale, arugula, or dandelion to an existing obesity inducing diet may taper neuro-inflammation and thus stymie cognitive decline. To address this issue, C57BL/6 mice were fed either a control (10% fat) or high-fat diet (HFD)(60% fat) for 16 weeks until the high-fat group reached a pre-diabetic stage. After 16 weeks, mice on a HFD weighed significantly more than the control mice, displayed elevated blood glucose levels, and showed deficits in spatial learning. During weeks 17 to 25, the diets of all the mice were supplemented daily with 1.0 gram of kale, arugula, or dandelion. Consumption of the greens had no effect on the weights of either groups. During the 8-weeks when the mice are fed their supplemental kale, arugula, or dandelion diets, the mice will be subjected to multiple repetitions of memory tests, such as the Morris Water Maze, Barnes Maze, and Novel Object Recognition, to test for changes in their memory abilities. Furthermore, the brains of the subject animals will also be analyzed for inflammatory and neuronal markers.

Disclosures: **B. Teng:** None. **D. Foster:** None. **L. Banner:** None.

Poster

129. Brain Wellness

Location: Hall A

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Topic: C.01. Brain Wellness

Support: VR Grant 2014-3224

ALF Grant ALFGBG-450961

Hjärnfonden FO2014-0184

Title: Physical fitness in early adulthood: a modifiable risk factor for neurological and psychiatric disorders

Authors: ***G. KUHN**[1,2], J. NYBERG[2], N. D. ABERG[3], M. WAERN[2], A. WALLIN[2], K. TORÉN[3], M. NILSSON[4], M. A. I. ABERG[2,3];
[2]Inst. of Neurosci. and Physiol., [3]Inst. of Med., [1]Univ. of Gothenburg, Gothenburg, Sweden; [4]Hunter Med. Res. Inst., Univ. of Newcastle, Newcastle, Australia

Abstract: Low cardiovascular fitness in mid- and late life represents a risk factor for several neurological diseases. However, the respective effect of aerobic cardiovascular and muscle

fitness in early adulthood is largely unknown. Therefore, we analyzed these in young men with regard to long-term risk depression, bipolar disorders, stroke, and dementia. We performed population-based longitudinal cohort studies of Swedish male conscripts registered in 1968-2005 for mandatory military service (approx. 1,800,000 subjects), representing about 95 % of all Swedish males at age 18 during this time period. Data on cardiovascular fitness (determined by the cycle ergometric test) and muscle strength (isometric contraction) were subdivided into low, medium, high fitness groups. Over a 40-year follow-up, risk of depression, bipolar disorder, stroke and dementia were calculated with Cox proportional hazards models controlling for potential confounders. To identify cases, we used the International Classification of Diseases (ICD) 8-10 in the Hospital Discharge Register. Depression. Adjusted hazard ratios (HRs) with confidence interval (CI) show significant relationships between fitness and severe depression for low compared to high fitness: HR (CI) 1.80 (1.64-1.99) for cardiovascular fitness and 1.43 (1.32-1.55) for muscle strength. Bipolar disorders exhibited a lower, yet significant association with cardiovascular fitness and muscle strength (adjusted hazard ratios (HRs) with confidence interval (CI): 1.35 (1.12-1.63) and 1.22 (1.05-1.42), respectively. Stroke. Adjusted HRs show inverse relationships between fitness and stroke for low compared to high fitness HR (CI) 1.70 (1.50-1.93) for cardiovascular fitness and 1.39 (1.27-1.53) for muscle strength. All three stroke types (subarachnoidal hemorrhage, intracerebral hemorrhage and ischemic stroke displayed similar associations. There were stronger associations for fatal stroke. Dementia. Low compared to high cardiovascular fitness was significantly associated with an increased risk for diagnosis of dementia before age 60: HR (CI) 2.49 (1.87-3.32). Mild cognitive impairment (MCI) showed an even greater association: HR (CI) 3.57 (2.23-5.74). The combination of low cardiovascular fitness and poor cognitive performance in early adulthood was associated with a >8-fold increase in the risk of early-onset MCI and a >7-fold increased risk of early-onset dementia. Overall, this study sheds light on physical fitness as an early-life risk factor for psychiatric and neurological conditions, and underlines the potential for lifestyle modification to reduce the risk of late-life CNS impairments.

Disclosures: **G. Kuhn:** None. **J. Nyberg:** None. **N.D. Aberg:** None. **M. Waern:** None. **A. Wallin:** None. **K. Torén:** None. **M. Nilsson:** None. **M.A.I. Aberg:** None.

Poster

129. Brain Wellness

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Topic: C.01. Brain Wellness

Support: NIH NS050465

NIH NS056413

Title: The role of epigenetic on mediating the action of diet on brain and body metabolism

Authors: *Z. YING[1], E. TYAGI[1], F. GOMEZ-PINILLA[1,2];
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Abstract: An increasing body of evidence suggests that a large number of neurological disorders are the result of complex interactions between genetic factors and the environment. In particular, diet is one of the most crucial factors for species survival and adaptation, and seems to have the power to program the epigenome. The strong dependence of the brain on energy implies that metabolic stimuli such as dietary factors have the intrinsic ability to influence brain plasticity (Agrawal, BBA, 2014) and epigenetic variability (Tyagi, Neurob. Dis. 2015). We have examined the effects of a western diet for 7-weeks on central and peripheral markers of metabolism, and the potential role of epigenetic in mediating the effects of the western diet. We investigated the role of DNA methylation on the regulation of molecular systems associated with metabolic control in the brain and peripheral by using the capacity of the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine to block the effects of the methylation. In the brain, the western diet reduced the mRNA and protein levels of BDNF, and this effect was dependent on the methylation status of the bdnf gene, and could be reversed by the application of 5-aza-2'-deoxycytidine. There also was a reduction in levels of metabolic regulators such as PGC-1a, Sirt1, and NAD/NADH. The exposure to the western diet increased anxiety-like behavior, and these effects were abolished by the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine. We found that exposure to the western diet increased several metabolic parameters associated with the establishment of the metabolic syndrome such as elevated insulin levels (commensurable to insulin resistance according to the HOMA test), elevated triglyceride levels, cholesterol, LDH, UA, and these effects were abrogated by 5-aza-2'-deoxycytidine. The results indicate that exposure to the western diet concerts a sequel of metabolic disorders in the brain and body, in which methylation plays a role. Our results also indicate that epigenetic regulation via DNA methylation can act in the brain and peripheral organs that regulate glucose metabolism and other features involved with development of metabolic syndrome.

Disclosures: Z. Ying: None. **E. Tyagi:** None. **F. Gomez-Pinilla:** None.

Poster

129. Brain Wellness

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Topic: C.01. Brain Wellness

Support: NIH Intramural Research Training Award

Title: Energetic cost of a running wheel: Implications for exercise-based weight loss interventions

Authors: *T. J. O'NEAL, D. M. FRIEND, A. V. KRAVITZ;
NIDDK/NIH, Bethesda, MD

Abstract: Weight loss interventions often stress the need for increased physical activity to facilitate weight loss, based largely on correlative links between obesity and exercise rates. However, other aspects of energy expenditure can change to compensate for energy expended during exercise, diminishing the efficacy of exercise-based weight loss interventions. Here, we used running wheels to investigate whether increases in physical activity can lead to significant and enduring changes in energy expenditure. In our first experiment, C57BL/6 mice (n = 7) were individually housed and given access to a running wheel for 3 weeks, during which time energy expenditure was measured using an energy balance method (Ravussin et al., 2013). Whereas wheel running significantly increased over the 3 weeks (5000/day in week 1 vs. 20000/day in week 3), both food intake and average daily energy expenditure did not significantly change over time. In our second experiment, C57BL/6 mice (n = 6) were individually housed in indirect calorimeters and given access to a running wheel for 72 hours, during which time energy expenditure was constantly measured. Despite progressive increases in wheel running over 72 hours (2400 in day 1 vs 4100 in day 3), both food intake and energy expenditure again remained unchanged over time. These findings suggest that either (1) the energetic cost of a running wheel is extremely low and negligible in terms of overall energy expenditure or (2) mice can compensate for the slight energetic demand due to wheel running by decreasing other aspects of energy expenditure. If translated to weight loss interventions in humans, these findings allude to the inability of exercise alone to facilitate long-term weight loss. Nevertheless, it is important to consider the robust metabolic, cardiovascular, and neurochemical benefits of exercise which, when paired with dietary changes, can lead to better overall health, rather than simply weight loss.

Disclosures: **T.J. O'Neal:** None. **D.M. Friend:** None. **A.V. Kravitz:** None.

Poster

129. Brain Wellness

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FONDECYT REGULAR N°1150677 to VE

FONDECYT REGULAR N°1120156 to NCI

BASAL Grant CONICYT-PFB12/2007 to NCI

Title: The Wnt signaling pathways modulates mitochondrial fusion in hippocampal neurons

Authors: *C. SILVA-ALVAREZ[1,2], V. EISNER[1], N. C. INESTROSA[1,2,3];
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Abstract: Mitochondria are highly dynamic organelles which can be found as interconnected tubular network or fragmented mitochondria. The control of this dynamic equilibrium is essential for proper neuronal function; its deregulation has become in a hallmark of several pathological conditions such as neurodegenerative diseases. The Wnt signaling pathway is essential for several neuronal functions such as synaptic development and plasticity and its dysfunction is associated to diseases such as Alzheimer´s. The mechanism of regulation of mitochondrial dynamics is not completely understood. We recently demonstrated that the non-canonical Wnt5a ligand has a neuroprotective role against Amyloid-β oligomers exposure, through modulation of mitochondrial dynamics and also was able to modulate the activation state of the principal regulator of mitochondrial fission, the dynamin like protein 1 (Drp1), through activation of Wnt/Ca pathway. However, the effect of Wnt signaling in dynamics processes such as mitochondrial fusion or motility are not clear. To analyze this, in this work neurons were transfected with mitochondrial photoactivable-GFP (mtPA-GFP) and mitoDsRed. The cells were incubated with Wnt5a in the presence and absence of specific Wnt scavengers, and inhibitors of the enzymes activated by the non canonical Wnt pathways. The neurons were studied by photoactivation assays of mtPA-GFP and live-cell imaging, and biochemical assays in order to analyze the mitochondrial fusion and motility proteins Opa1 and Mfns, and Milton, respectively. Our results indicate that Wnt5a is able to modulate mitochondrial fusion frequency rates, and the mitochondrial motility in response to Wnt5a concentration, supporting the idea that this ligand could be an important regulator of mitochondrial function and dynamic under physiological conditions in hippocampal neurons.

Disclosures: C. Silva-Alvarez: None. **V. Eisner:** None. **N.C. Inestrosa:** None.

Poster

129. Brain Wellness

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Topic: C.01. Brain Wellness

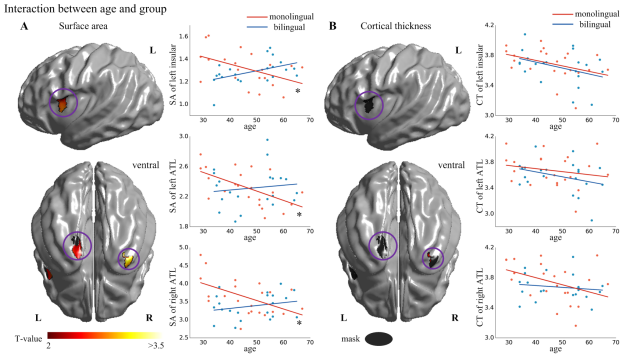
Support: National Key Basic Research Program of China (2014CB846102)

NSFC: 31170969, 81171016

Title: Bilingualism prevents cortical surface area reduction during brain aging

Authors: *L. LI[1], J. ABUTALEBI[2], G. GONG[1], L. ZOU[3], X. YAN[1], G. DING[1];
[1]Beijing Normal Univ., Beijing, China; [2]Dept. of Clin. Neurosciences, Univ. Vita Salute San Raffaele, Milan, Italy; [3]Col. of Psychology and Educ., Zaozhuang Univ., Zaozhuang, China

Abstract: Long-term bilingual experience can delay cognitive decline and neurodegeneration with aging. Monolinguals were reported to show decreased gray matter volume (GMV) in several brain regions during aging as compared to age-matched bilinguals, suggesting a neuroprotective effect of bilingualism. However, it is unclear whether this effect reported for unimodal bilinguals also applies to bimodal bilinguals. Furthermore, GMV is a product of surface area and cortical thickness which have distinct neurobiological basis. It is unknown whether the protective effect on GMV for bilinguals can be ascribed to variation in surface area, cortical thickness or both. Here we compared aging effects on GMV, surface area and cortical thickness of proficient bimodal bilinguals to age-matched monolinguals. GMV analysis revealed a significant interaction effect of age × group, in which bilinguals did not show aging effects in the left insula and bilateral anterior temporal lobes while monolinguals did. The analyses on cortical surface-based measures in these regions further revealed surface area showed the same pattern of group difference as GMV did while cortical thickness did not. These novel findings enhance our understanding of the neuroprotective effects of bilingualism on brain aging by showing that the effects are modality-independent and detectable at the surface area level.



Disclosures: **L. Li:** None. **J. Abutalebi:** None. **G. Gong:** None. **L. Zou:** None. **X. Yan:** None. **G. Ding:** None.

Poster

129. Brain Wellness

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KSEF 2268-RDE-014

Mizutani Foundation Grant

Title: A personalized, molecular diagnosis of Lafora disease patient mutations via structural insights

Authors: ***M. S. GENTRY**[1], M. RATHTHAGALA[2], M. K. BREWER[2], C. W. VANDER KOOI[2];
[1]Biochem., [2]Univ. of Kentucky, Lexington, KY

Abstract: Of all the severe and intractable epilepsies, Lafora disease (LD) is among the most severe, and is inevitably fatal. Mutations in two genes have been identified that cause LD, *EPM2A* (laforin) and *EPM2B* (malin). *Identification of the genetic basis for LD has opened up a new era in our understanding of the cause of LD, leading to rapid progress in the field.* Mutations in either of these genes cause glycogen to transform into malformed (starch-like), aggregated inclusions called Lafora bodies (LBs). LBs overtake the cytoplasm of dendrites, and drive the progressive refractory seizure disorder. The human *EPM2A* gene encodes the phosphatase laforin and recessive mutations in *EPM2A* result in Lafora disease (LD). In the absence of laforin activity, glycogen transforms into hyper-phosphorylated, water-insoluble, starch-like Lafora bodies that drive neuronal apoptosis, neurodegeneration, and eventual death of LD patients. We previously defined that the physiological function of laforin is to dephosphorylate glycogen, yet the mechanism of glycogen dephosphorylation by laforin was unknown. Additionally, LD patient missense mutations are dispersed throughout laforin,

bringing to question the structural mechanism(s) of disease. We recently determined the crystal structure of human laforin at 2.4 Å bound to oligosaccharides with a phospho-glucan product at the active site. The structure reveals an integrated tertiary structure of the carbohydrate binding module and dual specificity phosphatase domains as well as an antiparallel dimer mediated by the phosphatase domain that results in a tetramodular architecture, positioning the two active sites ~31 Å from each other. We utilized the crystal structure and three solution-based, biophysical techniques along with biochemical analyses of LD patient mutations and structured guided mutations to probe this unique tertiary and quaternary structure. We define a cooperative mechanism of action for laforin as well as establish the effect of LD disease mutations, thereby providing atomic level insights that connect basic glycogen metabolism to human neurodegenerative disease. Cumulatively, this work allows us to provide a patient specific, molecular diagnosis of each LD laforin mutation. This personalized, molecular diagnosis will prove invaluable as our groups and others move towards a LD therapeutic and/or cure.

Disclosures: **M.S. Gentry:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder of OptiMol Enzymes, LLC.. **M. Raththagala:** None. **M.K. Brewer:** None. **C.W. Vander Kooi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder of OptiMol Enzymes, LLC..

Poster

129. Brain Wellness

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 129.18/C79

Topic: C.01. Brain Wellness

Support: PrQF Grant

Title: Saving Football: using the repulsive force of magnets to reduce impact forces during helmet collisions

Authors: *R. J. COLELLO;
Anat. and Neurobio., Virginia Commonwealth Univ., Richmond, VA

Abstract: The prevalence of concussions in American football, which is largely the result of helmet-to-helmet collisions, has become a serious health concern for players at all ages. As the average collegiate football player will take over a 1000 hits to the head during one season, and as repeated concussions can lead to severe brain disease, it is imperative to develop better means to

protect the head from impact trauma. Modern football helmets consist of an outer polycarbonate shell that distributes force away from the point of impact and an inner padded liner that can absorb some of that impact energy. Although this general design is acknowledged for reducing mortality on the playing field, the continued prevalence of concussions at all levels of play suggest that further helmet design modifications are required. With this in mind, we have begun to investigate the extent to which repulsive forces, as those generated by the like poles (NN or SS) of neodymium magnets, can be used to reduce impact force generated during helmet-to-helmet collisions. Using a pressure assay we first established that a four ounce N52 magnet can generate 130lbs of repulsive force, which represents a 500:1 repulsive force to weight ratio. We next tested if these magnets can reduce impact energies associated with a wide range of impacts seen on the playing field. Using a weight drop assay, a moving and stationary 10lbs weight were fixed with a pair of four ounce magnets with like poles facing each other and the moving weight was dropped from 6in, 1ft, 2ft, 3ft, 4ft, or 5ft. An accelerometer mounted to the top of the moving weight recorded an 80% reduction in impact forces at the lower drop heights, a 40% reduction at the medium drop heights and a 15-20% reduction at the highest drop heights. Finally, football helmets were custom fitted with N52 arch magnets (magnetized or non-magnetized) that were permanently mounted to the inside polycarbonate shell. These helmets were then tested using a pendulum assay where a helmet is secured to both the stationary and moving component of the pendulum and impacted from varying heights (.6in, 1ft, 2ft, 3ft, 4ft, 5ft). Similar to the weight drop assay, impact forces were reduced by >80% at the lower drop heights, 40% at the medium drop heights and 15-20% at the highest drop heights. Collectively, these studies demonstrate the significant repulsive force of neodymium magnets and suggest that the addition of magnets to the standard football helmet design would complement existing helmet pad lining to dramatically reduce impact forces during helmet-to-helmet collisions. The likely consequence of this novel helmet modification is a reduction in the number of concussions on the playing field.

Disclosures: R.J. Colello: None.

Poster

129. Brain Wellness

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 129.19/C80

Topic: C.01. Brain Wellness

Title: Appetitive resistance training protocol has an anxiolytic effect on male, Long-Evans rats

Authors: *R. A. COUNTRYMAN, R. C. CROUCH, N. O. V. CUNNINGHAM;
Psychology, Austin Col., Sherman, TX

Abstract: The benefits of aerobic exercise on a variety of issues from metabolism to improvements in learning & memory is well established; however, substantially less research has examined the efficacy of resistance training for modifying behavior and the neuronal changes that result from resistance training. This study created a novel appetitive protocol for resistance exercise modified from previous ladder climbing paradigms (Cassilhas et al., 2012; Hornberger & Farrar, 2004). Male, Long-Evans rats were divided into three treatment groups: Cardiovascular Exercise (voluntary wheel running), Resistance Training (weighted ladder climbs), and Sedentary Controls. After 5 weeks of exercise, rats performed the elevated plus-maze task and novel object discrimination task. Rat were transcardially perfused, and brains were prepared for immunohistochemistry. c-Fos and BDNF were measured in the amygdala and hippocampus while BAX and BCL-2 were only measured in the hippocampus. Additionally, vastus lateralis mass was measured for each rat to verify changes in muscle mass due to exercise. Although we did not see any differences in memory as measured by the object discrimination task, exercise caused an anxiolytic effect as measured by the elevated plus maze. Both the cardiovascular exercise and resistance training rats spent significantly more time in the open arms of the elevated plus-maze and made more overall arms entries in comparisons with sedentary control rats (all ps < .05). In addition, the vastus lateralis of rats in both exercise conditions weighed more than the sedentary control rats. Resistance training rats had increased c-Fos expression in the CA3 region of the hippocampus compared to both cardiovascular exercise and sedentary controls while the cardiovascular exercise rats had significantly fewer Fos-positive cells than those in the resistance training or sedentary control conditions in the basolateral amygdala. BDNF, BAX, and BCL-2 analyses are ongoing. Of particular importance, modifying the resistance training protocol from aversive to appetitive was successful. The current study opens up the opportunity to study resistance exercise training using a protocol that is neither aversive nor forced.

Disclosures: R.A. Countryman: None. **R.C. Crouch:** None. **N.O.V. Cunningham:** None.

Poster

129. Brain Wellness

Location: Hall A

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Program#/Poster#: 129.20/C81

Topic: B.08. Synaptic Plasticity

Support: FAPESP

CAPES

CNPQ

UNICID

Title: Different types of exercise induce distinct changes in the expression of synaptic proteins in the motor cortex and prefrontal cortex of adult and aged rats

Authors: R. M. S. GUTIERREZ[1], C. R. SCARANSI[2], C. C. REAL[4], S. R. M. ORTIZ[3], L. R. G. BRITTO[4], *R. S. PIRES[5];
[1]Master's and Doctoral Program in Physical Therapy, [2]Graduation in physical therapy, [3]Doctoral Program in health sciences, Univ. Cidade de São Paulo, São Paulo, Brazil; [4]Lab. of Cell. Neurobiology, Dept. of Physiol. and Biophysics, Univ. of São Paulo, São Paulo, Brazil; [5]Univ. Cidade De São Paulo, Sao Paulo, Brazil

Abstract: The central nervous system of humans and animals most often respond positively to exercise, inducing an increase in neurogenesis and neuronal activation, an increase of BDNF and dendritic branching. However, little is known of exercise effects on synaptic proteins that mediate transmission synaptic. The objective was to investigate the effect of acrobatic exercise (AC) and treadmill exercise (TE) on the expression of synaptic proteins synapsin I (SYS) and synaptophysin (SYP) in the areas involved in planning and motor learning of adult and aged rats submitted to two months training. Twenty four male Wistar rats, being 12 aged (18 months old) and 12 adults (7 months) were used which were divided into 6 groups: sedentary adult (SED A, n=4), exercise treadmill in adult (TE A, n=4), adult acrobatic exercise (AC A, n=4), sedentary elderly (SED E, n=4), exercise treadmill elderly (TE E, n=4) and acrobatic elderly exercise (AC E, n=4). The rats were trained 3 days/week for 8 weeks. The AC moved through a circuit of obstacles 5 times/day, and TE E speed at 5m/min and the TE A at 8m/min for 40 min.Two months after the training, their brains were removed for Western blotting assay to quantify the SYS and SYP. Statistical analyses were performed using two-way ANOVA with the Tukey post hoc test. Our data revealed that the AC promoted distinct changes in the expression of synaptic proteins in different ages and regions analyzed. In the motor cortex, the AC A induced an increase of SYP (ca. 31%, p=0.005), and SYS relative to the SED A and SED E (ca. 31%, p=0.01). The AC A and AC E promoted an increase in relation to TE E (ca.59%, p=0.0009; ca.51%, p=0.02, respectively). The AC A had a SYS increase in relation to the EE E (ca.57%, p=0.006). In the prefrontal cortex, AC E induced increases in the SYP expression compared to ACA (ac.32%, p=0.01). The SYS in the prefrontal cortex showed no significant difference between groups. We suggest that the AE was able to increase expression of synaptic proteins in the motor cortex of adults and elderly rats, but the prefrontal cortex changes happen only in older animals, suggesting that the learning of a new motor skill can generate neuronal plasticity, but the activated areas can be different in adult and elderly rats.

Disclosures: **R.M.S. Gutierrez:** None. **C.R. Scaransi:** None. **C.C. Real:** None. **S.R.M. Ortiz:** None. **L.R.G. Britto:** None. **R.S. Pires:** None.

Poster

129. Brain Wellness

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 129.21/C82

Topic: F.04. Neuroethology

Support: UCLA Division of Life Sciences Recruitment and Retention Fund (Izquierdo)

Title: Reward experience determines the selection of behavioral strategies to maximize foraging efficiency

Authors: ***A. STOLYAROVA**, A. B. THOMPSON, A. IZQUIERDO;
Brain Res. Institute, Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: The adaptive solution to exploration vs. exploitation depends on the current state of the world: stable environments favor exploitation and uncertain environments favor exploration. Any deviations from these behavioral constraints have been interpreted as irrational or maladaptive. However, most investigations are done in primitive environments of the laboratory without much consideration that behavioral adjustments are driven by ancestral evolutionary history in different habitats that are characterized by spatial and temporal heterogeneity and patchy reward sources. In the present investigation, we extend previous computational work (McNamara et al., 2013; Berge-Tal and Avgar, 2012) and develop a model of animal foraging in a naturalistic environment to examine the effects of structured variability on the expression of behavioral strategies. Our model is derived from reports simulating foragers' behavior in a novel landscape of discrete, heterogeneous, and renewable resource patches based on optimal foraging theory, but different in several critical aspects, most importantly the presence of fluctuations in overall environmental quality, closely approximating spatial and seasonal variability in natural environments. We show that variability in the environment encountered early on leads to long-lasting changes in behavioral strategies that determine how an animal samples its environment and produces prolonged misrepresentations of environmental state. Specifically, experience with resource reductions early (when an agent has not yet established a trace of favorable patches to which it can return) favored development of an explorative phenotype, whereas experience with positive resource shifts led to establishment of a narrow preference set, decreasing exploration even when an explorative strategy was dictated by environmental conditions. The present simulation demonstrates that acting according to optimal foraging theory is favorable in

spatially, but even more so temporally, heterogeneous environments as it maximized reward procurement. This solution to temporally structured environmental fluctuations generates a unique behavioral phenotype which is frequently observed in laboratory studies, namely a tendency to persevere rather than adopt an explorative strategy when conditions return to baseline following a rewarding experience. Despite the common interpretation of such responses as maladaptive, we demonstrate that they are on the contrary emergent properties of an evolved endophenotype that likely developed under constraints imposed by a neural mechanism that subserves adaptive foraging behavior.

Disclosures: **A. Stolyarova:** None. **A.B. Thompson:** None. **A. Izquierdo:** None.

Poster

129. Brain Wellness

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 129.22/C83

Topic: A.01. Neurogenesis and Gliogenesis

Support: NICHD Grant RO1HD069238 (Goetzl, PI)

Title: Maternal obesity alters fetal brain development

Authors: *N. MERABOVA[1], N. DARBINIAN[1], G. TATEVOSIAN[1], R. A. SIMMONS[3], L. GOETZL[2];
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Abstract: Maternal obesity is a growing global public health concern. Epidemiologic studies suggest that high maternal BMI is associated with an increased risk of neurodevelopmental and metabolic morbidities in children. Molecular mechanisms underlying this possible link are poorly understood. We hypothesized that fetal exposure to maternal obesity alters gene expression of key neurodevelopmental and metabolic proteins. Study Design: An IRB-approved, matched case-control study was performed in women with singleton fetuses without structural anomalies undergoing elective pregnancy termination in the second trimester (GA 17 - 21 wks). Twelve cases (OBESE) were compared to 12 controls (LEAN). Cases had a BMI >30 (mean 36.7; range 30.2-43.9); controls had a BMI <25 (mean 21.7; range 17.7-24.8). Subjects were matched for gestational age, race and fetal gender. We analyzed CNS-specific genes such as Apolipoprotein D (APOD) and Presynaptic cytomatrix protein (PCLO), as well as Adiponectin (ADIPOQ), a gene regulating metabolism. Total RNAs and samples for FACS were prepared

from snap-frozen fetal brain tissue. Quantitative RT-PCRs were performed, utilizing specific primers and fold change was calculated relative to controls. For FACS analysis samples were incubated with neuronal and astrocyte specific markers (NeuN and GFAP respectively) with subsequent assessment of apoptosis by Guava Nexin assay. Results: Gene expression in the fetal brain was differentially regulated following exposure to maternal obesity. PCLO was significantly down-regulated 3.6 folds (p= 0.0005), and ADIPOQ was significantly upregulated 6.7 folds (p=0.03). FACS analysis suggested a significantly decreased level of apoptosis in the astrocytes of fetuses exposed to maternal obesity *in utero* (8.9% vs 36.8%, p= 0.0004). Assessment of proteins is ongoing. Conclusion: We present novel human data identifying potential molecular mechanisms by which maternal obesity predisposes offspring to neurodevelopmental abnormalities and metabolic complications. Dysregulation of cell death in fetal brain may adversely influence fetal neurodevelopment. Our data suggest that molecular changes may be detected as early as the second trimester (17-21 weeks GA).

Disclosures: N. Merabova: None. **N. Darbinian:** None. **G. Tatevosian:** None. **R.A. Simmons:** None. **L. Goetzl:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.01/C84

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Exploring the biological and behavioral effects of methylene blue on a triple transgenic mouse model of Alzheimer’s disease

Authors: *D. A. MITRANO[1], K. JACKSON[1], S. FINK[1], Q. PACE[1], B. GENOVESE[1], N. KHAN[2], H. GRAU[1], L. SMITH WEBB[1];
[1]Mol. Biol. & Chem., [2]Neurosci., Christopher Newport Univ., Newport News, VA

Abstract: Alzheimer’s disease (AD) is a debilitating neurodegenerative disease that is characterized by dementia that increases in severity over time. There are neurological and biological changes that have been shown to accompany, or in some cases precede, the cognitive decline. These changes include the development of amyloid-B plaques and neurofibrillary tangles that lead to cognitive decline, changes in certain markers in the blood, and deficits in olfaction. Currently there are very few diagnostic tools and effective treatments for AD, and no known means of preventing it. Methylene blue (MB) is an FDA approved compound that has been shown to reduce the formation of protein aggregates in AD and other diseases. MB is

currently in clinical trials to determine if it can slow the progression of AD; however, no longitudinal studies have been done using the 3xTg-AD mouse model to determine if MB can effectively prevent, or delay the onset of, the disease in this model. In this study, we use the 3xTg-AD mouse to further determine the validity of the model by examining the effect of MB on the onset and progression of AD; specifically, can MB prevent or delay the accumulation of the amyloid plaques, neurofibrillary tangles, and cognitive and olfactory deficits seen in AD. MB (10mg/kg) or 0.9% saline has been administered intraperitoneally, weekly, to both male and female mice starting at 4 weeks of age. Behavioral and biological tests will be performed on the mice at several time points, starting at 3 months, and continuing to 4.5, 6, 7.5, 9, 12, 15 and 18 months of age. Once mice have reached their set time points we will examine: (1) brain pathology, for the accumulation of plaques and tangles using immunohistochemistry; (2) lipid markers in the blood that have been shown to be associated with, or predictive of AD, through the use of GC-MS; (3) behavioral tests to assess cognitive ability, using the Morris Water Maze; and (4) olfactory deficits through the use of a peanut butter test. This will allow us to determine the extent and progression of AD over the normal life span of these mice and provide further insight into the efficacy of MB as a possible treatment or neuroprotective drug for AD.

Disclosures: **D.A. Mitrano:** None. **K. Jackson:** None. **S. Fink:** None. **Q. Pace:** None. **B. Genovese:** None. **N. Khan:** None. **H. Grau:** None. **L. Smith Webb:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.02/C85

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Soy flavonoids prevent beta-amyloid-induced degenerative changes of the enteric nervous system of the mouse: relevance to the treatment of Alzheimer’s disease

Authors: ***Y. LIU**[1], G. LIN[2], M. FANG[3], J. A. RUDD[2,4];
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Abstract: Late-onset Alzheimer’s disease (AD) accounts for more than 80% of AD cases. It is not known why amyloidogenisis occurs during the aging process in these patients. It is possible that aggregation of soluble beta-amyloid (Aβ) peptide first occurs in the periphery to initiate a cascade of events, which ultimately affects cognition. In this regard, the gastrointestinal (GI)

tract interfaces with the environment and is exposed to chemical and biological stress. In previous studies, we found Aβ and plaques in the GI tract of a transgenic mouse model of AD. We also found that Aβ injected directly into the serosal side of the GI tract of normal ICR mice caused a significant reduction of enteric neurons. Hormonal replacement therapy (HRT) may have beneficial actions to reduce the incidence of AD. Therefore, we investigated the expression of estrogen receptor alpha and beta (ERα/ERβ), and the novel G-protein coupled estrogen receptor, GPR30, in the GI tract of the mouse. We found a high expression of ERβ in the cytoplasm within neuronal cell bodies. GPR30 was also found in neurites, while ERα was expressed in both the nucleus and cytoplasm. Estrogen receptors were only found on enteric neurons, but not enteric glial cells. We also found that Aβ could induce loss of $PGP9.5^{+}$ neurons from a longitudinal muscle and myenteric plexus tissue culture. The soy flavonoids, daidzein, genistein, glycitein, and luteolin, which are known to activate estrogen receptors, antagonized the Aβ-induced toxicity; these protective effects extended to both cholinergic $ChAT^{+}$ and nitrergic $nNOS^{+}$ neurons. Our studies provide evidence that soy flavonoids have neuroprotective effects on the ENS. Further studies are being conducted to identify the estrogen receptor subtypes involved, and if the protective effect on the GI tract can translate to a protective effect on the brain.

Disclosures: **Y. Liu:** None. **G. Lin:** None. **M. Fang:** None. **J.A. Rudd:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.03/C86

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R03AG046216

Serbian Ministry of Education, Science and Technological Development Grant ON173056

Title: Short term fish oil dietary supplementation reduces plaque load in 5xFAD female mice

Authors: ***M. BRKIC**, D. MILANOVIC, M. PEROVIC, V. TESIC, S. RUZDIJIC, S. IVKOVIC, S. KANAZIR;
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder, characterized by formation of neurofibrilary tangles in neurons and amyloid beta plaques formation. Overabundance of amyloid beta 42, promotes neuroinflammation, triggering cascade of detrimental effects, which further aggravate the consequences of the disease. Docohexaenoic acid (DHA) plays an important role in the central nervous system, promoting proper synapse function and neuroprotection. Recent findings revealed that its deficiency is related to cognitive decline in aging and neurodegenerative diseases. Furthermore, it has been shown that treatment with DHA contributes to significant improvement in cognitive abilities and protects from synaptic loss in transgenic mouse AD models. Present study was done on 3-month-old female 5xFAD mice, treated for 3 weeks with fish oil as an abundant natural source of DHA. Immunohistochemical analysis of brain tissue showed decrease in amyloid beta plaque load, accompanied with changes in lipid status in the brain and serum of treated animals. However, recently revealed major blood to brain DHA transporter, mfsd2a, showed no changes, on both RNA and protein levels in the brain cortex, while it significantly decreased in the liver after the treatment. This study reveals a new beneficial aspect of short-term fish oil dietary supplementation on mitigation of Alzheimer's disease pathology.

Disclosures: M. Brkic: None. **D. Milanovic:** None. **M. Perovic:** None. **V. Tesic:** None. **S. Ruzdijic:** None. **S. Ivkovic:** None. **S. Kanazir:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.04/C87

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A western diet exacerbates plaque load and plaque-associated TREM2+ cells in a mouse model of Alzheimer's disease

Authors: *L. C. GRAHAM, J. M. HARDER, I. SOTO, W. N. DE VRIES, S. W. M. JOHN, G. R. HOWELL;
The Jackson Lab., Bar Harbor, ME

Abstract: Alzheimer's disease (AD) is caused by a combination of genetic and environmental risk factors such as diet. In particular, components of food commonly consumed in the westernized world, including high levels of fat and simple carbohydrates that are sourced from animal-based rather than plant-based products, are considered to contribute to an increased risk for AD. However, the mechanisms by which these dietary components influence AD

susceptibility and progression are not clear. A western diet was developed that mimicked the average fat, carbohydrate, protein, vitamin and mineral levels of diets in western societies. The western diet, along with a standard mouse chow was fed to APP/PS1 mice, a widely used mouse model of AD, and C57BL/6J control mice, for eight months. After long-term consumption of the western diet, mice were assessed for metabolic and AD-relevant phenotypes. The western diet caused both AD and control mice to become obese but not diabetic. Astrocytosis and microglia/monocyte activation were dramatically increased in multiple brain regions in both control and AD mice fed the western diet, compared to mice fed the standard chow. Consumption of the western diet also resulted in a small but significant loss of cortical neurons in both AD and control mice. Amyloid plaques significantly increased in the hippocampus, but not the cortex, of AD mice fed the western diet suggesting region-specific outcomes in response to the western diet. AD mice fed the western diet also showed a significant increase in the number of TREM2+ microglia/monocytes surrounding plaques compared to AD mice fed the control diet. These data suggest that a western diet induces neuroinflammation, possibly mediated by TREM2+ microglia/monocytes, that increases severity of AD. These findings further support modulating diet or specific neuroinflammatory processes to reduce risk for AD.

Disclosures: L.C. Graham: None. **J.M. Harder:** None. **I. Soto:** None. **W.N. de Vries:** None. **S.W.M. John:** None. **G.R. Howell:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.05/C88

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R01AG042890 (GT)

NIEHS T32ES007254 (MC)

Title: Near infrared light treatment reduces amyloid beta oligomer binding to synapses in a transgenic mouse model of Alzheimer’s disease

Authors: *M. M. COMEROTA[1], G. TAGLIALATELA[2];
[2]Dept. of Neurol., [1]Univ. of Texas Med. Br., Galveston, TX

Abstract: The neuropathological characterization of Alzheimer’s disease, the most common neurodegenerative dementia, includes deposits of aggregated amyloid beta (Aβ) and hyper-

phosphorylated tau protein neurofibrillary tangles. The cognitive decline that is associated with AD is believed to be driven by the dysfunction of synapses due to the binding of small toxic Aβ oligomers on the post-synaptic density (PSD). Despite the growing prevalence of AD, current treatment options do not address the underlying causes of the disease, providing limited alleviation of symptoms. Near infrared (NIR, 600-1000 nm) light therapy has previously been used in pain management and to accelerate wound healing. NIR light is believed to target mitochondria through photostimulation of cytochrome c oxidase that results in an increase in ATP. Recent studies have suggested that the application of NIR light treatment can be expanded to include neurodegenerative disorders such as AD and Parkinson's disease (PD). Notably, it has been reported that NIR light treatment on APP/PS-1 transgenic mice induced a reduction of amyloid β plaque load and improved memory function; however, the mechanism contributing to this effect remains unresolved. In the present study, we focused on mechanistic changes that occur at the synaptosome after NIR light treatment that may lead to the restoration of cognitive integrity. Specifically, we investigated the susceptibility of amyloid β binding to the synaptosome (an important event linked to amyloid beta-driven synaptic disruption and memory deficits) and found that after a treatment with NIR at 670 nm (90 sec a day for 4 weeks), the amount of amyloid β was significantly reduced at synapses from various brain areas of 6 month old APP transgenic mice (Tg2576). This study provides evidence that NIR can effectively reduce the amount of damaging amyloid beta at synapses, thus furthering light therapy as a viable treatment for AD.

Disclosures: **M.M. Comerota:** None. **G. Taglialatela:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.06/C89

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Franco-Cuban FSP program 29967RG (CampusFrance)

Title: Neuro-EPO, an intranasally deliverable low sialic acid formulation of erythropoietin, is neuroprotective in the APPSwe mouse model of Alzheimer's disease

Authors: ***T. MAURICE**[1], Y. RODRIGUEZ CRUZ[2], M. STREHAIANO[1], J. C. GARCIA RODRIGUEZ[3];
[1]INSERM U 1198, Montpellier, France; [2]Histology Dept., Preclinical and Basic Sci. Institute; Havana Med. Univ., Havana, Cuba; [3]Havan Med. School, Fac. Victoria de Giron, Havana, Cuba

Abstract: Besides its role in erythropoiesis, the cytokine erythopoietin (EPO) is a potent cell protectant and it showed a strong potential in protecting brain cells from ischemic injury or from toxicity observed in neurodegenerative pathologies, including Alzheimer's disease (AD). Different types of EPO without erythropoiesis-stimulating activity have been developed, including carbamylated-EPO, erythro-EPO and Neuro-EPO with low sialic acid content (Garcia Rodriguez & Sosa Teste, WorldScientificJournal 2009). This latter form allows efficient intranasal (IN) delivery, thus avoiding systemic administration related potential side effects, and showed effective neuroprotective activity in the nontransgenic AD model induced in mice by intracerebroventricular injection of Aß25-35 oligomers (Maurice et al., J Psychopharmacol 2013). In the present study, we analyzed the neuroprotective effect of Neuro-EPO in APPSwe mice. Twelve-month old animals were treated IN, 3 times a week during two months with Neuro-EPO (0, 125, 250 µg/kg). Animal weight, attrition, motor impairments and general activity of the animals (open-field) were checked after treatment. Animals were submitted to memory procedures (spontaneous alternation, place learning in the water-maze and novel object recognition) and we observed that Neuro-EPO, at the lowest or both doses, alleviated the learning impairments measured in APPSwe animals. After the behavioral observations, animals were sacrificed and biochemical and histological analyses were performed. Oxidative stress (increase in lipid peroxidation), neuroinflammation (increases in GFAP and Iba-1 expression), induction of apoptosis markers (increases in Bax expression, Bax/Bcl2 ratio, TNFalpha expression...) measured in hippocampus extracts of APPSwe mice were significantly restored by Neuro-EPO. The cytokine had however marginal effects on decreased BDNF levels and synaptophysin expression in APPSwe. Finally, brain insoluble Aß1-42 content was significantly decreased with Neuro-EPO (125 µg/kg). These data are the first evidence for a neuroprotective efficacy of Neuro-EPO in a transgenic mouse model of AD and confirmed the benefits of the IN route in this disease.

Disclosures: T. Maurice: None. **Y. Rodriguez Cruz:** None. **M. Strehaiano:** None. **J.C. Garcia Rodriguez:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.07/C90

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Translational Research Network (JST)

Title: Inhibition of Amyloid beta accumulation by SAK3 as an Alzheimer's disease drug candidate

Authors: *H. IZUMI, Y. SHINODA, Y. YABUKI, K. FUKUNAGA;
pharmacology, Tohoku Univ. Grad Sch. Pharm Sci., Sendai, Japan

Abstract: As Alzheimer disease therapeutics candidate, we introduced SAK3 (Ethyl 2',3'-dyhydro-8-methyl-2',4-dioxo-2-peperidinospiro[2-cyclopentene-1,3'-imidazo[1,2-a]-pyridine]-3-carboxylates) which stimulates T-type voltage-gated Ca2+ channels (T-VGCC) in mouse cortical slices and neuro2A cells over-expressed T-type calcium channel, Cav3.1 (Moriguchi et al., J Neurochem 2012;121:44-53) (PCT/JP2013/051388). We also reported that SAK3 stimulates acetylcholine release and promotes long-term potentiation in mouse hippocampus (Neuroscience 2014 abstract 265.21). We here tested whether SAK3 reduced amyliod beta (1-42) accumulation in Alzheimer model mice. Female mice aged 6-8 months were treated for two month with SAK3 (0.5mg/kg, p.o.) and measured amyliod beta (1-42) levels in both soluble and insoluble fraction from the cortex. The chronic administration significantly reduced amyliod beta (1-42) levels in both soluble and insoluble fractions from the cortex. Consistent with reduced amyliod beta (1-42) levels, the numbers of amyloid plaques assessed by thioflavin staining were significantly reduced by the chronic SAK3 treatment. Furthermore, the cognition assessed by novel object recognition task was improved by the chronic administration. Taken together, novel T-type calcium channel stimulator SAK3 possibly elicits the improvement of cognitive impairment in Alzheimer disease model mice and reduction of amyliod beta (1-42) accumulation/aggregation. However, further extensive studies are required to elucidate the mechanism underlying inhibition of amyliod beta (1-42) accumulation/aggregation. This work is supported by Translational Research Network Program (JST).

Disclosures: H. Izumi: None. **Y. Shinoda:** None. **Y. Yabuki:** None. **K. Fukunaga:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.08/C91

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Neuroprotection of medial septal cholinergic neurons by memantine after intralateral septal injection of aβ1-40

Authors: *M. T. CASTANEDA[1,2], E. LOPEZ[2], H. RODRIGUEZ[2], A. TOUHAMI[3], J. RODRIGUEZ[2], M. ORTEGA[2], R. TOVAR[2];
[1]Univ. of Tamaulipas, Matamoros Tamaulipas, Mexico; [2]Biomedicine, [3]Univ. of Texas at Brownsville, Brownsville, TX

Abstract: Alzheimer's Disease (AD) is a progressive disorder of the brain that leads to memory loss, dementia and death. Several lines of evidence suggest that the accumulation of amyloid beta peptides (Aβ) may trigger the dysfunction and degeneration observed in the AD brain. The basal forebrain, including the septal region which regulates the excitability of the hippocampus and neocortex, is early affected in AD because its neurons are vulnerable to the Aβ peptides. In addition, the connections between lateral and medial septal region (medial septum and diagonal band of Broca, MS/DB) have been demonstrated in previous studies. In order to demonstrate the presence of excitotoxicity in Aβ-induced septal damage, we compared rats injected with Aβ1-40 into the lateral septal region structure with rats treated with memantine (a non-competitive NMDA receptor antagonist), prior, during, and after Aβ1-40 injections. Medial septal cholinergic neurons were immunochemically identified and their numbers were estimated using Image J cell count. Our results show that Aβ1-40 treated animals have significantly a low number of MS/DB cholinergic neurons compared to Aβ/memantine treated group.

Disclosures: M.T. Castaneda: None. **E. Lopez:** None. **H. Rodriguez:** None. **A. Touhami:** None. **J. Rodriguez:** None. **M. Ortega:** None. **R. Tovar:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.09/C92

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 2015 AACP New Investigator Award

Title: Inhibition of phosphodiesterase 2 reverses β-amyloid-induced memory impairment: involvement of anti-inflammatory and anti-apoptotic responses

Authors: *Y. XU[1], J. PAN[2], Y. YU[3], X. XU[2], Z. CHEN[4], W. HUANG[4], L. LIAN[2], H. ZHANG[5], J. M. O'DONNELL[1];
[1]Dept. of Pharmaceut. Sci., State Univ. of New York at Buffalo, Buffalo, NY; [2]Brain Institute, Wenzhou Med. Univ., Wenzhou, China; [3]Wenzhou Third People's Hospital, Wenzhou Med. Univ., Wenzhou, China; [4]Nanjing Med. Univ. Affiliated Changzhou Second People's Hosp.,

Changzhou, China; [5]Dept. of Behavioral Med. & Psychiatry, West Virginia Univ., Morgantown, WV

Abstract: Emerging evidence suggests that β-amyloid (Abeta) has a detrimental effect on short-term and long-term memory that is the initial and major symptom of Alzheimer's disease (AD) via inhibition of both cAMP/cGMP-dependent neuroprotective pathway. Microinfusion of Abeta in animal models results in Abeta deposition, neuroinflammation, apoptosis and cognitive impairment. However, whether the neuroinflammation and apoptosis are required for the Abeta-induced learning deficits and whether phosphodiesterase 2 related cAMP and/or cGMP pathway is involved in the progression of AD is unclear. In the studies reported here we investigated that intraventricular infusion of Abeta1-42 into bilateral CA1 subregions led to deficits in learning and memory, as evidenced by an increased latency to find the platform and decreased number of platform crossing in the probe trial of the water maze task and decreased 24-h retention in the step-down passive avoidance test. These effects were blocked by treatment with increasing doses of phosphodiesterase 2 (PDE2) inhibitor Bay 60-7550 (0.5, 1 and 3 mg/kg every day for 21 days, i.p.). Abeta-treated mice were also found to increase in the expression of IL-6, IL-10 and Bax; while decrease in the expression of BCl-2, PKA, PKG and brain-derived neurotrophic factor (BDNF) in the hippocampus. These effects were also reversed by treatment with Bay 60-7550 in a dose-dependent manner. The findings suggest that inhibition of PDE2, such as Bay 60-7550, would reverse Abeta-induced learning and memory impairment at least in part by its regulation of neuroinflammatory and apoptotic processes, which are involved in cAMP/cGMP-BDNF signaling.

Disclosures: **Y. Xu:** None. **J. Pan:** None. **Y. Yu:** None. **X. Xu:** None. **Z. Chen:** None. **W. Huang:** None. **L. Lian:** None. **H. Zhang:** None. **J.M. O'Donnell:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.10/C93

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC 30800312

NSFC 31271134

Title: Sodium butyrate ameliorates memory deficit,increases neurogenesis and reduces inflammation in presenilins-deficient mice

Authors: *C. LI[1], X. ZHOU[2], T. CAO[2], Y. CUI[2], H. WANG[2];
[1]Sch. of Psychology and Cognitive Science, East China Normal Univ., Inst. of Cognitive Neurosci., Shanghai, China; [2]Sch. of Psychology and Cognitive Neurosicence, East China Normal Univ., Shanghai, China

Abstract: Histone acetylation has been shown to play a crucial role in memory formation and histone deacetylase inhibitor sodium butyrate (NaB) can improve memory performance as well as rescue the neurodegeneration of several Alzheimer's disease (AD) mouse models. Presenilin-1 and presenilin-2 double knockout (cDKO) mice showed memory decline, forebrain degeneration, tau hyperphosphorylation and inflammation that closely mimics phenotypes in AD patients. Here we have investigated the effects of systemic administration of NaB on different phenotypes in cDKO mice. We found that chronic NaB treatment (3weeks) significantly restored the deficit of hippocampus- dependent contextual memory, but didn't alter amygdala- dependent memory in cDKO mice. Biochemical analysis showed that tau hyperphosphorylation and inflammation related protein GFAP level were decreased in the forebrain of cDKO mice after NaB treatment. In addition, NaB treatment increased neurogenesis in SGZ of cDKO mice. Although we found synaptic numbers were dramatically decreased in cDKO mice,NaB treatment didn't increase synaptic numbers, nor did it attenuate forebrain neurodegeneration. Furthermore, mRNA-Seq analysis demonstrated that not only did NaB decrease the expression of inflammation related genes, but also increased the expression of genes involved in homeostasis and neural activity in the forebrain of cDKO mice. These results may shed some lights on the possible molecular mechanisms of HDAC inhibitor in alleviating the neurodegenerative phenotypes of cDKO mice.

Disclosures: **C. Li:** None. **X. Zhou:** None. **T. Cao:** None. **Y. Cui:** None. **H. Wang:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH P01 NS080675

NSF DGE-1143954

NIH F32 NS080320

New Vision Award through Donors Cure Foundation

Title: Treatment with the diabetic medication, glibenclamide, improves disease pathology in the APPswe/PSEN1dE9 mouse model of Alzheimer's disease

Authors: E. E. CAESAR[1], *S. L. MACAULEY[2], M. STANLEY[1], T. E. MAHAN[1], D. M. HOLTZMAN[1];
[2]Neurol., [1]Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Epidemiological studies demonstrate that type-2-diabetes mellitus (T2DM) patients have a 2-4 fold increased risk for developing Alzheimer's disease (AD). Although studies suggest that pathological changes associated with T2DM exacerbate AD pathology, it is unclear how diabetic therapies affect AD pathogenesis. A recent study from our laboratory indicated that systemic hyperglycemia increased amyloid-beta (Aβ) levels within the hippocampal interstitial fluid (ISF) in conjunction with increased neuronal activity. We identified inward rectifying, ATP-sensitive potassium (KATP) channels within the brain as a mechanistic link between elevated glucose levels, neuronal excitability, and Aβ metabolism. By altering the open probability of brain KATP channels, changes in cellular metabolism result in altered neuronal activity and Aβ production. Therefore, KATP channels are one mechanism by which neurons respond to hyperglycemia and couple cellular metabolism with neuronal firing. Therefore, investigating the role of KATP channels in both the pathogenesis and treatment of AD warrants further study. Recent studies suggest T2DM medications differentially alter risk for developing AD. Monotherapy with sulfonylureas, a class of drugs that modulate KATP channels, may reduce AD risk; while insulin therapy increased risk. These data suggest that T2DM medications might be a novel therapeutic approach for the treatment of some aspects of AD. To investigate the effects of the KATP channel antagonist, glibenclamide (i.e. glyburide), on AD pathology, 4-month-old female APP/PS1 mice were treated systemically with glibenclamide or placebo for 3 months via the implantation of a slow release, subcutaneous pellet. Initially, glibenclamide-treated APP/PS1 mice experienced decreased blood glucose levels that rebounded within 2 weeks of implantation. At 7 months, glibenclamide-treated mice weighed more than placebo and had an observable increase in visceral fat. However, both blood glucose levels and serum insulin levels were comparable in treated and untreated mice at 7 months. There was a significant reduction in Aβ deposition in the brains of glibenclamide-treated mice. Interestingly, there was no difference between the levels of microglial or astrocyte activation, suggesting neuroinflammation was unaffected in treated mice. These data suggest that KATP channel antagonists can reduce Aβ deposition in APP/PS1 mice and the pathway it is affecting may be a potential therapeutic area for further investigation. Funding: NIH P01 NS080675, NSF DGE-1143954, NIH F32 NS080320, New Vision Award through Donors Cure Foundation

Disclosures: E.E. Caesar: None. **S.L. Macauley:** None. **M. Stanley:** None. **T.E. Mahan:** None. **D.M. Holtzman:** 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.; Holtzman lab receives research grants from, AstraZeneca, Eli Lilly, and C2N Diagnostics. C. Other Research

Support (receipt of drugs, supplies, equipment or other in-kind support); Holtzman lab receives research grants from Cure Alzheimer's Fund, JPB Foundation, and the Tau Consortium. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder, C2N Diagnostics LLC and ownership interests. F. Consulting Fees (e.g., advisory boards); Scientific advisory boards/consulting: AstraZeneca, Genentech, Eli Lilly, Neurophage, C2N Diagnostics.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.12/C95

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT 169023

Title: C-terminal fragment of tetanus toxin protects against cholinotoxicity by intraseptal injection of β-amyloid peptide (25-35) in rats

Authors: *A. PATRICIO[1], L. MENDIETA[2], I. MARTÍNEZ[3], O. REYES CASTRO[1], V. ALEMÁN ALEMÁN[4], J. AGUILERA[5], I. LIMÓN[1];
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Abstract: The C-terminal domain of tetanus toxin (Hc-TeTx) is new nontoxic peptide of the tetanus toxin that has a protective action against glutamate excitotoxicity. Moreover its efficacy as a neuroprotective agent has demonstrated in several animal models of neurodegeneration. The Aβ(25-35) fraction mimics the toxic effects of the full complete peptide Aβ. This fraction modify the cholinergic system in medial septum (MS) leading alterations of septo-hippocampal pathway, and results in cognitive impairments. The aim of this study was examined the neuroprotective effects of Hc-TeTx fragment against neurotoxicity of Aβ(25-35) fraction into MS in rats. Rats received an injection of Hc-TeTx fragment 2μL at 2μM, and later with Aβ(25-35) (2μg/2 μL) into MS. Animals were tested for spatial learning and memory in the eight-arm radial maze. The

brains were obtained to assess cholinergic markers such as choline acetyltransferase (ChAT) and acetylcholinesterase (AChE), even to explore the neurodegeneration in MS and hippocampus, by the amino-cupric silver and H&E staining. We found that Hc-TeTx protects against cholinergic markers (ChAT and AChE) loss, and neurodegeneration by Aβ(25-35) in MS, CA1 and CA3 subfields of hippocampus. All these improvements could be reflected on spatial learning and memory performances. In summary, our findings suggest that Hc-TeTx fragment protects the septo-hippocampal pathway, since Hc-TeTx reduces the neurodegeneration by Aβ(25-35) and improves learning and memory process.

Disclosures: A. Patricio: None. **L. Mendieta:** None. **I. Martínez:** None. **O. Reyes Castro:** None. **V. Alemán Alemán:** None. **J. Aguilera:** None. **I. Limón:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.13/C96

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CSIR(09/475(0194)/2014-EMR-I)

ICMR(55/44/CFP/GER/11-NCD-11)

Title: Amyloid beta toxicity induces autophagic flux via sequestosome-1 mediated alpha-7 nicotinic acetylcholine receptor activation; Role of resveratrol as homeostatic regulator

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Abstract: Objective: Abnormal accumulation of aggregated protein amyloid beta (Aβ) in selectively vulnerable region is a key event in Alzheimer's disease (AD) pathology, while resveratrol (RSV) is very promising against Aβ induced neurodegenerative disorders. However, the more precise mechanism of how RSV renders neuroprotection remains elusive. More highlights demonstrate autophagy defect and deterioration of the hippocampal cholinergic system as a leading neurochemical feature of Alzheimer's disease. Loss of homeostatic regulators is suspected to alter autophagic response by disturbing the expression of autophagosome enhancing factors. Our previous studies showed that RSV could effectively

normalize the expression of nicotinic acetylcholine receptor alpha-7 (α7nAchR) in Ibotenic acid induced AD models. The current study thus aim at understanding whether the neuroprotective effect of reseravatrol in α7nAchR activation is facilitated via autophagy regulatory factors in Aβ (1-42) induced AD male Rattus novergicus. Methodology: Current study utilizes Aβ (1-42) induced experimental animals with or without trans-resveratrol, studying the spatial learning memory and expression profile of various factors like Atg5, Atg6, Atg7, Atg12, USP10/13 and SQSTM1 regulating autophagic response using RT-qPCR analysis. Results: A stereotactic intrahippocampal infusion of 500nmol/µl of aggregated Aβ (1-42) revealed working memory and reference memory errors during 8-arm Radial Arm Maze (RAM) and spontaneous alterations percentage within Y-maze task. RSV (20mg/kg bodyweight for 21 days) administration significantly ($p<0.05$) improved RAM task performance and spatial working memory in Aβ (1-42) induced experimental animals. mRNA expression profile for Atg5, Atg6, Atg7, Atg12, USP10/13 and SQSTM1 in control and experimental animals demonstrated that RSV mediated autophagic activation is regulated by upregulating α7nAchR protein expression via SQSTM1 in Aβ induced Alzheimer's disease. Conclusion: Resveratrol could protect neurons by enhancing autophagic response via SQSTM1 upregulation thereby maintaining homeostasis invivo favoring clearance of beta-amyloid and reducing the stress in neurons.

Disclosures: C. Karthick: None. **S. Periyasamy:** None. **K. Jayachandran:** None. **M. Anusuyadevi:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.14/D1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG037637

Title: Reducing S6K1 levels improves cognitive and synaptic deficits in a mouse model of Alzheimer's disease

Authors: *A. CACCAMO[1,2], C. BRANCA[1,3], J. TALBOOM[1], D. SHAW[1], D. TURNER[4], L. MA[4], A. MESSINA[2], Z. HUANG[4], J. WU[4,3], S. ODDO[1,3];
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Abstract: Aging is the major risk factor associated with Alzheimer's disease (AD); however, the molecular mechanisms linking aging to AD remain unclear. Suppression of the ribosomal protein S6 kinase 1 (S6K1) increases healthspan and lifespan in several organisms, from nematodes to mammals. Here we show that S6K1 expression is upregulated in the brains of AD patients. Using a mouse model of AD, we found that genetic reduction of S6K1 improved synaptic plasticity and spatial memory deficits, and reduced the accumulation of amyloid-β and tau, the two neuropathological hallmarks of AD. Mechanistically, these changes were linked to reduced translation of tau and the *beta-site APP cleaving enzyme 1, a key enzyme in the generation of amyloid-β. Our results* implicate S6K1 dysregulation as a previously unidentified molecular mechanism underlying synaptic and memory deficits in AD. These findings further suggest that therapeutic manipulation of S6K1 could be a valid approach to mitigate AD pathology.

Disclosures: A. Caccamo: None. **C. Branca:** None. **J. Talboom:** None. **D. Shaw:** None. **D. Turner:** None. **L. Ma:** None. **A. Messina:** None. **Z. Huang:** None. **J. Wu:** None. **S. Oddo:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.15/D2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG037637

Alzheimer's Drug Discovery Foundation

Title: PRAS40 as a novel therapeutic target for Alzheimer's disease

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Abstract: Clinically, Alzheimer's disease (AD) is characterized by impairments of memory and cognitive functions. Amyloid-β (Aβ), neurofibrillary tangles and synaptic loss are the prominent neuropatholgies in patients with AD. Strong evidence indicates that an imbalance between production and degradation of key proteins contributes to the pathogenesis of AD. The mammalian target of rapamycin (mTOR) plays a key role in maintaining protein homeostasis as it regulates both protein synthesis and degradation. In human AD brains, mTOR signaling is

hyperactive and negatively correlates with cognitive function. A key regulator of mTOR activity is the proline-rich AKT substrate 40 kDa (PRAS40), which directly binds to mTOR and reduces its activity. Phosphorylation of PRAS40 reduces its ability to bind to mTOR thereby removing the inhibitory constrain on mTOR activity. Our preliminary data of human AD brains show a significant increase in the phosphorylated levels of PRAS40 confirming hyperactive mTOR activity. Physiologically, PRAS40 phosphorylation is regulated by Pim-1, a protein kinases of the protoconcogene family. We have identified a Pim-1 inhibitor (Pim-1i) that crosses the blood brain barrier, reduces PRAS40 phosphorylation, and thereby reduces mTOR signaling. The main objective of the present experiment was to test the effects of the selective Pim-1i on learning and memory in 3xTg-AD mice, a widely used mouse model of AD. We found that daily intraperitoneal injections of the Pim-1i for 4 weeks dramatically improved spatial and working memory deficits in 3xTg-AD mice. Specifically, Pim-1i-treated 3xTg-AD mice performed significantly better than their vehicle treated counterparts, and as well as non-transgenic mice. These findings suggest that reductions of mTOR by decreasing phosphorylated PRAS40 is sufficient to ameliorate the cognitive deficits in ~8 month old 3xTg-AD mice. These results set the stage for the development of clinical trials aimed at using Pim-1 inhibitors for AD. Given that Pim-1 inhibitors are already being tested in ongoing human clinical trials for cancer, these findings may open a new venue of drug discovery for AD by developing more Pim-1 inhibitors. Furthermore, given the role of mTOR/PRAS40 in aging, our results may unveil new mechanisms by which age-associated risk factors contribute to the development of AD.

Disclosures: **R. Velazquez:** None. **D.M. Shaw:** None. **J.S. Talboom:** None. **S. Oddo:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.16/D3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effects of NPT088, a novel fusion protein bivalent for a General Amyloid Interaction Motif (GAIM), on behavior, neuropathology and CSF biomarkers in transgenic mice

Authors: *J. C. CARROLL[1], J. M. LEVENSON[1], S. SCHROETER[1], E. ASP[1], M. PROSCHITSKY[1], V. CULLEN[1], C. CHUNG[1], H. TSUBERY[1], R. KRISHNAN[1], B. SOLOMON[2], R. FISHER[1], K. GANNON[1];
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Abstract: Alzheimer's disease (AD) is characterized by both amyloid-β (Aβ) plaque deposition and intracellular neurofibrillary tau tangles accompanied by progressive cognitive decline. We isolated a protein motif from bacteriophage M13 that binds and disaggregates multiple amyloids including those formed by Aβ, tau, prion protein, huntingtin and α-synuclein (Krishnan et al., 2014). We call this motif the General Amyloid Interaction Motif (GAIM). The present studies were conducted to evaluate whether systemic administration of NPT088, a Hu-IgG_1-Fc fusion protein bivalent for GAIM, could alter disease-relevant CSF biomarkers, neuropathology and functional endpoints in transgenic AD mouse models. NPT088 (2 or 20 mg/kg) or PBS was administered weekly via intraperitoneal injection for ~14 weeks. Transgenic mice burdened with either Aβ (Tg2576; ~22 mo at last dose) or tau (rTg4510; ~9 mo at last dose) were used. Behavior (locomotor activity and object recognition) was assessed at various times throughout experiments. In Tg2576 mice, Aβ in CSF was measured by MSD ELISA for $Aβ_{38}$, $Aβ_{40}$, $Aβ_{42}$. Brain levels of $Aβ_{42}$ were measured by Innotest ELISA in RIPA soluble and formic acid soluble fractions of frontal cortex and hippocampus. In rTg4510 mice, CSF tau was measured with MSD ELISA. Levels of pTau in brain were measured by ELISA and western blot. Administration of NPT088 to Tg2576 mice reduced levels of Aβ in both CSF and brain. The reduction in levels of Aβ was associated with significant reductions in hyperactivity, and modest improvements in novel object recognition. In rTg4510 mice, 14 weeks of NPT088 treatment resulted in less brain weight loss relative to rTg4510 mice dosed with PBS. In addition, reduced levels of tau in CSF and brain, and a significant reduction of hyperactivity and a significant improvement in novel object recognition were present. Collectively, these results indicate that NPT088 reduces Aβ and tau neuropathology, improves functional endpoints and affects disease-relevant biomarkers in both Aβ and Tau transgenic mouse models. These data support clinical studies with NPT088 as a unique therapeutic approach to AD that targets multiple misfolded proteins. An IND is planned for 4Q2015 with initiation of clinical trials in 1Q2016.

Disclosures: **J.C. Carroll:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **J.M. Levenson:** A. Employment/Salary (full or part-time):; NeuroPhage. **S. Schroeter:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **E. Asp:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **M. Proschitsky:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **V. Cullen:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **C. Chung:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **H. Tsubery:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **R. Krishnan:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **B. Solomon:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **R. Fisher:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. **K. Gannon:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.17/D4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institute of Health Research

Fonds de recherche Québec-Santé

Institute of nutrition and functional foods

Title: Docosahexaenoic acid intake improve visual memory and decreases the lipolysis-lipoprotein stimulated receptor (LSR) in mice knock-in for human APOE

Authors: *A. G. PINÇON[1], J.-D. COULOMBE[2], R. CHOUINARD-WATKINS[2], M. PLOURDE[2];
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Abstract: Dyslipidemias are significant risk factors for Alzheimer's disease (AD) and remain an important health concern. Individuals with elevated levels of plasma cholesterol in mid-life have an increased susceptibility to AD. ApoE is one of the major apolipoproteins in the plasma and the principal cholesterol carrier protein in the brain. The 4 allele of APOE is the most important genetic risk factor for AD. A number of epidemiological and *in vivo* studies suggest that a higher intake of fatty fish containing docosahexaenoic acid (DHA) is associated with a reduced risk of AD and might improve cognitive functions. Whether DHA intake has a role in maintaining cholesterol homeostasis is yet unknown. Hence, we explored the potential mechanisms focusing on DHA intake on the regulation of cholesterol homeostasis in model of mice expressing human APOE isoforms. Four months old APOE3 and APOE4 mice were fed an isocaloric control diet or a diet containing DHA (n = between 8 and 10 /genotype/diet) for 8 months. At 12 months, animals were tested for visual memory using the object recognition (OR) test. A recognition index (RI) was calculated in the OR test. There were independent significant diet and genotype effects. Mice fed the DHA diet recognized the new object compared to the control diet ($P < 0.0001$). The lipolysis-lipoprotein stimulated receptor (LSR) is involved in the clearance of triglyceride-rich lipoproteins and low-density lipoproteins, and also acts as an apolipoprotein B/E-containing lipoprotein receptor. DHA supplementation decreased by two fold LSR protein levels in the liver of APOE3 and APOE4 mice ($p < 0.01$). The low density lipoprotein receptor-related protein-1 (LRP1), a chylomicron remnant receptor in the liver was not modified by dietary DHA intake neither by APOE genotype. Therefore, a diet rich in DHA seems to modify

LSR and this in independent of APOE genotype. It is however premature to conclude that modifications in the expression of lipoprotein receptor are involved in cognition in this mouse model.

Disclosures: A.G. Pinçon: None. **J. Coulombe:** None. **R. Chouinard-Watkins:** None. **M. Plourde:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.18/D5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Combined secretase modulation therapy provides cognitive benefit and ameliorates cerebral amyloid pathology in transgenic mice

Authors: *T. MORI[1], N. KOYAMA[1], J. TAN[2], T. TOWN[3];
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Abstract: Alzheimer disease (AD) is the most common progressive age-related neurodegenerative disorder. While pharmacological targets have been identified, to date there has not been a successful disease-modifying therapy. We screened a class of naturally-occurring dietary substances (‘nutraceuticals’) and have focused on two promising compounds [ferulic acid (FA), a β-secretase inhibitor and octyl gallate (OG), an enhancer of α-secretase activity] that inhibit amyloidogenesis. Here, we examined whether FA plus OG combination therapy might synergistically improve cognitive impairment and mitigate amyloid-β (Aβ)/β-amyloid pathology in the PSAPP transgenic mouse model of cerebral amyloidosis. Beginning at 12 months of age, PSAPP mice were orally administered (via gavage) FA plus OG, FA, OG (all at 30 mg/kg), or vehicle once daily for 3 months. At 15 months of age, each treatment improved PSAPP transgene-associated behavioral impairment of activity, novel object recognition, working memory, and spatial reference memory, but left non-transgenic mouse behavior unaltered. Notably, combination therapy synergistically improved most behavioral outcome measures. Moreover, brain parenchymal and cerebral vascular β-amyloid deposits as well as levels of various Aβ species including oligomers were synergistically decreased in FA plus OG-treated versus singly-treated PSAPP mice. These effects were due to a shift toward non-amyloidogenic amyloid precursor protein (APP) cleavage via β-secretase inhibition and α-secretase

enhancement. Notably, β-carboxyl-terminal APP fragment and β-site APP cleaving enzyme 1 (BACE1) protein expressions were decreased, whereas soluble APP-α and tumor necrosis factor α-converting enzyme (TACE; a primary candidate α-secretase protein) expression were increased. Together, these data provide pre-clinical proof-of-concept that secretase modulation via combination therapy is a promising avenue for AD treatment.

Disclosures: T. Mori: None. **N. Koyama:** None. **J. Tan:** None. **T. Town:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.19/D6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Comission for technology Innovation, Switzerland

Title: Repeated intravenous administration of human adult ischemia-tolerant mesenchymal stem cells reduces Abeta amyloid pathology in a mouse model of Alzheimer's disease

Authors: *T. BOLMONT[1], T. HARACH[1], T. LASSER[1], A. LUKASHEV[2];
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Abstract: The impact of a repeated intravenous administration of adult ischemia-tolerant mesenchymal stem cells (hMSC) on cerebral Abeta amyloid pathology was investigated in a transgenic mouse model of Alzheimer's disease. To this end, hMSC were administered to aged and young transgenic mice that co-express human mutated amyloid precursor protein (APP) with presenilin 1 (PS1) and develop age-dependent cerebral Abeta amyloidosis. Ten weeks of intravenous hMSC treatment (one injection / week) safely reduced cerebral amyloid plaques in both aged and young amyloid-depositing transgenic mice (-44% and -35%, respectively), while significantly reducing neuroinflammation (-35% and -39%, respectively) without increasing cerebral amyloid angiopathy or microhemorhages. Quantitative RT-PCR biodistribution analysis demonstrated that intravenously-delivered hMSC were readily detected in transgenic brains at 1 hour post-delivery. We next investigated if hMSC migration to the brain was necessary for reducing cerebral Abeta amyloidosis, or whether one or several soluble factors released by hMSC in the periphery would subsequently alter cerebral Abeta amyloidosis. Daily intranasal application for a total of three weeks of soluble hMSC factors, which are secreted by hMSC in culture, reduced cerebral Abeta amyloid plaques (-25%) in the absence of intravenous hMSC delivery. Altogether, our pre-clinical results demonstrate that intravenously-delivered hMSC

lower Abeta amyloid pathology in a mouse model of Alzheimer's disease. They also suggest that intranasal application of hMSC factors may be used as a maintenance therapy to accompany intravenous delivery of hMSC, paving the way for a next clinical trial in Alzheimer patients.

Disclosures: **T. Bolmont:** A. Employment/Salary (full or part-time):; Stemedica International. **T. Harach:** None. **T. lasser:** None. **A. Lukashev:** A. Employment/Salary (full or part-time):; Stemedica International.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.20/D7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant P30NS47466

Title: APPSweDI mice have severe olfactory deficits and olfactory bulb pathology, treatment with Dpeptide improves outcome

Authors: *T. VAN GROEN[1], I. KADISH[1], A. KUMAR[1], D. WILLBOLD[2];
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Abstract: The transgenic AD model mice we use express three AD-related mutations in APP, i.e., with the APP_{SDI} (Swedish, Dutch and Iowa) mutations, they develop plaques at about five months of age, and we have described that three types of deposits are present, i.e., plaques (with a thioflavine S positive core), and, especially, cerebral amyloid angiopathy (CAA). We tested the olfactory capabilities of these mice at 9 months of age, since we hypothesized that Aβ pathology would result in decreased olfaction. Thus, in this study we investigated the effects on amyloid deposition on olfaction. Further we tested whether treatment with an Aβ42-binding D-peptide (i.e., consisting of D-amino acids), RD2RD2 would improve outcome. We used groups of C57BL/6J mice and APP_{SDI} mice to test olfactory sensitivity, and a group of female, Tg AD model mice for treatment; these animals received ip infusions using Alzet minipumps with either saline, or the RD2RD2 peptide for one month, i.e., from 9 to 10 months of age. Following behavioral analysis, the treated animals were perfused transcardially with a saline solution followed by a 4% paraformaldehyde solution. The brain was cut in 6 series of 35 µm sections, and these were stained with 1) amyloid β, 2) GFAP, 3) CD11b, series 4-6 were stored for later analysis. The density of labeling in the stained sections was quantificated with densitometric

analysis. Whereas C57 mice can smell urine at dilutions of 1:1000, but not at 1:2000, in contrast the Tg AD model mice clearly distinguish urine only at 1:100. The treatment with the amyloidβ42-binding peptides significantly improves olfactory functioning, mice could distinguish urine at 1:250. Further the treatment significantly reduces amyloid β pathology in the olfactory bulb in this mouse model of AD compared to the saline control. Together this suggests that this mouse model of AD has significant olfactory deficits (and olfactory bulb pathology), and that treatment with amyloid-binding peptides decreases the amyloid β pathology, likely by improvement of clearance of amyloid β, leading to improved olfactory cognition.

Disclosures: **T. Van Groen:** None. **I. Kadish:** None. **A. Kumar:** None. **D. Willbold:** None.

Poster

130. Alzheimer's Disease: *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 130.21/D8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BioTherapeutix LLC

Title: Potential memory restorative effects of a neurotrophic factor mimetic in an aged, transgenic mouse model of Alzheimer’s disease

Authors: *L. S. HONICAN[1], J. BURRILL[2], A. E. CASLER[1], J. E. GEORGAKAS[1], D. S. ADAMS[2], M. D. SPRITZER[1];
[1]Dept. of Biology, Program in Neurosci., Middlebury Col., Middlebury, VT; [2]Dept. of Biol. and Biotech., Worcester Polytechnic Inst., Worcester, MA

Abstract: Alzheimer’s disease (AD) is associated with an increased accumulation of amyloid beta (Aβ) peptide in the brain, which is believed to lead to cognitive impairment. Neurotrophic factors, which aid neuronal growth and survival, have shown promise for treating AD symptoms. We tested the effectiveness of a growth factor mimetic (BTX-1039) in the treatment of spatial memory deficits. The transgenic mice used in this study have three mutations that lead to over-production of the 770 isoform of the human amyloid beta-precursor protein and associated Aβ in a C57BL/6J background strain. We conducted separate experiments with 6-month-old and 9-month-old mice. Our four treatment groups were: AD mouse/drug, AD mouse/vehicle, wild type mouse/drug, and wild type mouse/vehicle. Mice received daily i.p. injections of 0.20 ml saline or BTX-1039 (60mg/kg) in saline for 14 consecutive days prior to starting behavioral testing, with the researcher blind to treatment. We used a Morris water maze protocol that consisted of 6 days

of place learning, 1 day of probe trials, and 3 days of cued learning. For both experiments, the transgenic mice given saline had significantly longer paths to the target platform during place learning than did all other groups, indicating that the drug restored some memory function. The groups showed no differences in learning during the cued trials, indicating no effect of the transgenes or the drug on stimulus-response learning for the ages tested. For the probe trials, we observed significant impairment in memory retention in the transgenic mice relative to the wild type mice at 6 months of age, but we observed no differences between the strains at 9 months of age and no effects of the drug in either age class. This indicates that mice of this strain under 9 months of age should be used to test memory retention, but also suggests that our drug mainly impacts spatial learning rather than memory retention. In a pilot study, we tested mice of an intermediate age (8 months) using a higher dose of the drug (100 mg/kg), and these results suggest even stronger effectiveness for the drug in restoring spatial learning. We also quantified cell proliferation (Ki67 expression) within the dentate gyrus of the 9-month-old mice, as some studies indicate AD causes dysregulation of the cell cycle. Preliminary results indicate that the transgenes cause a decrease in cell proliferation within the granule cell layer and a significant increase in cell proliferation in the hilus. The drug had no effect on cell proliferation. Together, the results indicate some memory restorative effects of a neurotrophic factor mimetic that were not associated with changes in hippocampal cell proliferation.

Disclosures: **L.S. Honican:** None. **J. Burrill:** None. **A.E. Casler:** None. **J.E. Georgakas:** None. **D.S. Adams:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BioTherapeutix LLC. F. Consulting Fees (e.g., advisory boards); BioTherapeutix LLC. **M.D. Spritzer:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BioTherapeutix LLC.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 131.01/D9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FRM postdoctoral fellowship - SPF20130526736

Senior Innovative Grant 2010 - French National Foundation for Alzheimer's disease and related disorders

ATIP/AVENIR program - CNRS

Title: Increased APP intracellular domain (AICD) production perturbs synaptic signal integration via increased NMDAR function

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that begins as mild short-term memory deficits and culminates in total loss of cognition and executive functions. The main culprit of the disease, resulting from Amyloid-Precursor Protein (APP) processing, has been thought to be amyloid-b peptide (Ab). However, despite the genetic and cell biological evidence that supports the amyloid cascade hypothesis, it is becoming clear that AD etiology is complex and that Ab alone is unable to account for all aspects of AD [Pimplikar et al. J Neurosci.30: 14946. 2010]. Gamma-secretase not only liberates Ab, but also its C-terminal intracellular counterpart called APP intracellular domain (AICD) [Passer. et al. JAlzheimers Dis.2: 289-301. 2000], which is known to also accumulate in AD patient's brain [Ghosal et al. PNAS.106:18367. 2009], but surprisingly little is known about its functions in the hippocampus. To address this crucial issue, we increased AICD production *in vivo* in adult CA1 pyramidal neurons, mimicking the human pathological condition. Different ex-vivo electrophysiological and pharmacological approaches, including double- patch of neighbor neurons were used. We clearly demonstrate that *in vivo* AICD production increases synaptic NMDA receptor currents. This causes a frequency-dependent disruption of synaptic signal integration, leading to impaired long-term potentiation, which we were able to rescue by different pharmacological approaches. Our results provide convincing and entirely novel evidence that increased *in vivo* production of AICD is enough, per se, to cause synaptic dysfunction in CA1 hippocampal neurons.

Disclosures: **P.A. Pousinha:** None. **E. Raymond:** None. **X. Mouska:** None. **M. Willem:** None. **H. Marie:** None.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

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FIRB Merit RBNE08HWLZ

Title: CREB is a target for the physiological signaling pathway activated by beta-amyloid monomers

Authors: M. GIUFFRIDA[1], G. PANDINI[1], *A. G. COPANI[3,2], E. RIZZARELLI[1];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with synaptic dysfunction, pathological accumulation of ß-amyloid (Aß) in plaques, and neuronal loss. The self-association of Aß monomers into soluble oligomers seems to be crucial for the development of neurotoxicity (Walsh and Selkoe, J Neurochem 2007). Different from oligomers, Aß monomers activate IGF-1-like surviving and metabolic pathways. Specifically, Aß monomers act at type I IGF-1 receptors and stimulate PI-3-K/AKT-dependent responses (this presentation and Giuffrida et al, J. Neurosci 2009). We now provide the evidence that, in cultured neurons, Aß monomers induce the activation of the transcription factor CREB in a ser/thr kinase-dependent manner. The results of our study will be presented and discussed in light of the evidence that CREB dysfunctions occur in the brain of transgenic AD mice (Gong B et al., J. Clin Invest., 2004) and in the human AD brain (Yamamoto-Sasaki M et al , Brain Res., 1999; Pugazhenthi S. et al., Mol Neurod., 2011)

Disclosures: M. Giuffrida: None. **G. Pandini:** None. **A.G. Copani:** None. **E. Rizzarelli:** None.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The effect of the mitochondria-targeted antioxidant MitoQ on memory retention and neuropathology in aged 3xTg-AD mice

Authors: M. L. YOUNG[1,2], M. PATEL[1,2], *J. L. FRANKLIN[2];
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Abstract: Considerable evidence suggests that oxidative stress is a major mediator of Alzheimer's disease (AD) pathology. Malfunctioning mitochondria, the likely source of reactive oxygen species (ROS) responsible for the oxidative stress, are among the earliest pathologies of

the disease preceding β-Amyloid plaque formation, neurofibrillary tangles, and memory loss. To further understand the role of oxidative stress originating from the mitochondria, we took advantage of a novel mitochondria-targeted antioxidant mitoquinone mesylate (MitoQ). Our previous studies show that treatment of the 3xTg-AD mouse model of AD with MitoQ prevents development of pathologies that occur from 2-7 months after birth including cognitive decline, β-Amyloid deposition, synaptic loss, astrogliosis, and increased caspase-3 activity. In this continuation study, we explore the effects of MitoQ with mice that were well into disease progression. Twelve-month-old female 3xTg-AD mice were administered MitoQ (100 μM) continuously in their drinking water until seventeen months after birth. Following the treatment period, behavioral assessments and biochemical assays were conducted to evaluate the effect of MitoQ on known Alzheimer's disease-like pathologies present in 3xTg-AD mice. Morris water maze training, a measure of spatial memory retention, showed that mice treated with MitoQ were able to learn spatial cues 3 days before littermate controls and were also able to retain memory better in both short-term and long-term probe trials. Sensorimotor deficiencies and escape motivation from the water maze were evaluated and did not prove to be significantly different between treatment groups. These findings suggest that MitoQ improved cognitive abilities rather than physical abilities such as swim speed or eyesight. In support of our behavioral studies, synaptophysin, a marker of synapse loss, was significantly increased after treatment. Glial fibrillary acidic protein, a measure of reactive astrocytes that are normally increased in aged 3xTg-AD mice, was reduced by 50%. β-Amyloid (1-42) deposition measured by ELISA was not significantly different from littermate controls. However, caspase-3 activity, implicated in tau pathology, was significantly reduced by treatment. Our behavioral studies suggest that MitoQ may prevent further cognitive decline when administered after disease progression has begun. Interestingly, MitoQ did not have an effect β-Amyloid deposition. We are currently exploring the effects of MitoQ on tau pathology.

Disclosures: **M.L. Young:** None. **M. Patel:** None. **J.L. Franklin:** None.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Mitochondrial dysfunction and cellular toxicity by mitochondria-targeted amyloid β

Authors: *D. KIM, M.-Y. CHA, H. SONG, I. MOOK-JUNG;
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Abstract: Amyloid β (Aβ) peptide is the key molecule in Alzheimer's disease (AD) pathogenesis and also implicated in mitochondrial abnormality during disease progress. Several studies reported that cellular toxicity, mitochondrial dysfunction, and mitochondrial accumulation of Aβ have been shown in the brain of patients with AD and AD model mice. However, the direct correlations among those triangular phenomena remain unclear. Here, we constructed mitochondria-targeted amyloid β sequence, containing a mitochondrial targeting sequence. We demonstrated that mitochondrial dysfunction and cellular toxicity was induced by mitochondria-targeted amyloid β in transfected mouse hippocampal HT22 cells. Furthermore, we constructed the lentiviral system which contain mitochondria-targeted amyloid β sequence. We infected mouse hippocampus with this system to investigate whether accumulation of Aβ only in mitochondria causes memory impairment. In addition, proteomic analysis of the hippocampus indicated that pathological events appeared in the hippocampus matched to partial AD database. These results indicated that mitochondrial Aβ accumulation is sufficient to cause memory impairment *in vivo* system.

Disclosures: D. Kim: None. **M. Cha:** None. **H. Song:** None. **I. Mook-Jung:** None.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: O-GlcNAcylation of amyloid-b precursor protein at T576 regulates trafficking and processing

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Abstract: Deposition of amyloid-b (Ab) in the brain is the main culprit of Alzheimer's disease (AD). Ab is derived from sequential proteolytic cleavage of amyloid-b precursor protein (AbPP). AbPP undergoes post-translational modification including N- and O-glycosylation. O-GlcNAcylation is a novel type of O-glycosylation, mediated by O-GlcNAc transferase (OGT) attaching O-β-N-acetylglucosamine (O-GlcNAc) to serine/threonine residues of the target proteins. O-GlcNAc is removed by O-GlcNAcase. We have previously reported that increasing

O-GlcNAcylated AbPP using the O-GlcNAcase inhibitor, PUGNAc, increases its trafficking rate to the plasma membrane and decreases its endocytosis rate, resulting in decreased Aβ production. However, O-GlcNAc modification sites in AβPP are unknown. In this study, we mutated three predicted O-GlcNAc modification threonine residues of AbPP695 into alanines (T291A, T292A, and T576A) and expressed them in HeLa cells. These AbPP mutants showed reduced O-GlcNAcylation levels, indicating that these sites were endogenously O-GlcNAcylated. Thr 576 was the major O-GlcNAcylation site when AbPP was treated with PUGNAc. Surface levels and trafficking to the plasma membrane were decreased in the T576A mutant. Consistent with these observations, T576A mutant accumulated in the early endosomes. In the T576A mutant, the level of secreted Ab was increased and the inhibitory effect of PUGNAc on Ab production was prevented. These results implicate Thr 576 as the major O-GlcNAcylation site in AβPP and indicate that O-GlcNAcylation of this residue regulates its trafficking and processing. Thus, specific O-GlcNAcylation of AbPP at Thr 576 may be a novel and promising drug target for AD therapeutics.

Disclosures: **O. Kwon:** None. **Y. Chun:** None. **Y. Park:** None. **H. Oh:** None. **S. Chung:** None.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Program#/Poster#: 131.06/D14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DFG Postdoctoral Award

Title: Direct *in vivo* assessment of glutamate dysfunction in APPPS1 transgenic mice

Authors: ***J. K. HEFENDEHL**[1], J. LEDUE[1], R. KO[1], T. MURPHY[2], B. A. MACVICAR[1]; [1]Djavad Mowafaghian Ctr. For Brain Health, UBC, Vancouver, BC, Canada; [2]Dept. of Psychiatry, UBC, Vancouver, BC, Canada

Abstract: Extracellular deposition of the amyloid-β peptide (Aβ) are a hallmark lesion of Alzheimer's disease (AD) and are surrounded by regions of neuronal and glial hyperactivity. Used as a predictive marker for the progression of preclinical to symptomatic AD we examined the close surroundings of Aβ deposits for pathological alterations of glutamate dynamics in APPPS1 transgenic mice. Using *in vivo* two-photon imaging of the glutamate sensor iGluSnFR we examined evoked and spontaneous glutamate dynamics in awake and anaesthetized mice in two cortical brain areas. Timing characteristics characteristics of glutamate changes in an Aβ-

plaque dependent manner. Decay rates of glutamate increase when analysed in close proximity to amyloid deposits and a chronic state of high glutamate fluctuation was found in the direct vicinity of Aβ-plaques. The upregulation of GLT-1 by ceftriaxone was able to partially restore the pathologically altered glutamate dynamics. Together, our results identify a new component of the toxic microenvironment in the close surrounding of Aβ-plaques and a potential new target for early intervention strategies.

Disclosures: J.K. Hefendehl: None. **J. LeDue:** None. **R. Ko:** None. **T. Murphy:** None. **B.A. MacVicar:** None.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Support: JSPS KAKENHI 26870209

JSPS KAKENHI 15H04839

Title: Neuronal activity enhances APP processing in cultured neurons

Authors: K. SAITO, K. KASUGA, *T. IKEUCHI;
Niigata University, Brain Res. Inst., Niigata, Japan

Abstract: Alzheimer’s disease (AD) is the most common cause of dementia, which is pathologically characterized by extracellular deposits of β-amyloid (Aβ) and intracellular accumulation of hyperphosphorylated tau. Aβ is derived from amyloid precursor protein (APP) by sequential cleavages. It has been reported that Aβ production is enhanced by neuronal activation. Early in the disease course, hippocampal neurons showing hyperactivity send those axons to the default-mode network where Aβ accumulates remarkably in the AD brain. It has been suggested that Aβis released from an axonal terminal of the hippocampal neuron, subsequently accumulated in the default-mode network. However, it is unclear whether neuronal activity dependent Aβ release affects phosphorylation of tau within the network. Here we performed *in vitro* assay with rat cortical neuron primary cultures. After glutamatergic stimulation, we found that full length APP was reduced and this reduction was preceded by Egr-1 activation. In addition, C-terminal fragments of APP were increased after the stimulation, suggesting that the stimulation enhanced β-cleavage. We previously showed Aβ-dependent tau

phosphorylation by using the coculture system. Taken together, neuronal activity may affect APP processing and tau phosphorylation in neuronal network.

Disclosures: **K. Saito:** None. **K. Kasuga:** None. **T. Ikeuchi:** None.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Title: The analysis of endosomal proteins, which specifically interact with APP-βCTF

Authors: *N. TAKASUGI, T. SAKURAI;
Juntendo Univ. Sch. of Med., Tokyo, Japan

Abstract: Alzheimer disease (AD) is a progressive neurodegenerative disorder neuropathologically characterized by senile plaques (SP) and neurofibrillary tangles. Amyloid β peptide (Aβ) is the major component of SP, and produced from sequential cleavage of Aβ precursor proteins (APP) by β-site APP cleaving enzyme 1 (BACE1) and γ-secretase. Numerous studies support the hypothesis that AD pathology is associated with aggregation of Aβ in patient brain. However, the growing evidence suggests Aβ independent abnormality in AD brain precedes Aβ deposition. Intriguingly, abnormally enlarged neuronal endosomes, which is considered to be the reminiscent of the impairment of endosomal-lysosomal systems, have been observed in the early phase of AD. Further study suggests the accumulation of β-carboxy terminal fragment of APP (βCTF), which is the biproduct of Aβ, is a causative molecule for endosomal dysfunction. However, the mechanisms of βCTF accumulation and the effect on endosomal-lysosomal systems are largely unknown.To gain an insight into the molecular

mechanisms, we screened endosomal proteins that interact with βCTF. We will report the analysis of the interaction between these candidates and βCTF, and the impact of the interaction on endosomal-lysosomal systems.

Disclosures: **N. Takasugi:** None. **T. Sakurai:** None.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant AG047812

Title: Reduced dynamin-related protein 1 protects against phosphorylated tau, mitochondrial dysfunction and synaptic damage in Alzheimer's disease

Authors: ***R. KANDIMALLA**[1,2], M. MANCZAK[2], A. PANDEY[3,2], C.-S. KURUVA[2], D. FRY[2], X. YIN[2], C. OSBORN[2], S. YEGUVAPALLI[2], P.-H. REDDY[2,4,3,5];
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Abstract: BACKGROUND Increasing evidence suggests that phosphorylated tau (pTau) and mitochondrial (mt.) abnormalities are involved in the loss of synapses, defective axonal transport and cognitive decline, in patients with Alzheimer's disease (AD). Molecular, biochemical studies revealed pTau is capable of reducing anterograde transport of vesicles and subcellular organelles such as mt. by blocking microtubule tracks in AD neurons, suggesting that dysregulation of tau causes starvation of synapses, oxidative stress and mt. dysfunction in AD. Further, our co-IP and IHC analysis revealed mt. fission Drp1 interacted with pTau, and this interaction increased as AD progressed. Based on these, we hypothesize that a partial deficiency of Drp1 inhibits Drp1-pTau interactions and protects neurons from pTau induced mt. and synaptic toxicities, and maintains mt. and neuronal functions in AD progression. METHODS We crossed dynamin-related protein heterozygote knockout (Drp1+/-) mice with tau transgenic mice (P301L line) and created double mutant mice - Drp1+/-xTau (DT). Using real time RT-PCR and immunoblotting analyses, we measured mRNA expressions and protein levels of genes related to the mt.

dynamics - Drp1 and Fis1 (fission), Mfn1, Mfn2 and Opa1 (fusion), CypD (Matrix), biogenesis - Nrf1, Nrf2, PGC1α and TFAM and synaptic - synaptophysin, PSD95, synapsin 1, synaptobrevin 1, neurogranin, GAP43 and synaptopodin in brain tissues from 6-mo. Drp1+/-, Tau, DT and WT mice. Using biochemical assay, we also studied mt. function and measured soluble Tau & pTau in brain tissues from all lines of mice in this study. RESULTS Decreased mRNA expressions and protein levels of Drp1 and Fis1, and CypD, and increased levels of Mfn1, Mfn2 and Opa1, Nrf1, Nrf2, PGC1α, TFAM, and synaptophysin, PSD95, synapsin 1, synaptobrevin 1, neurogranin, GAP43 and synaptopodin genes were found in 6 mo. old DT mice relative to AβPP mice. Mitochondrial functional assays revealed that mt. dysfunction is reduced in Drp1+/-xTau mice relative to tau mice, suggesting that reduced Drp1 enhances mitochondrial function in AD neurons. Measurement of pTau studies revealed that soluble and pTau levels were significantly reduced in DT mice relative to Tau mice, indicating that reduced Drp1 decreases soluble and pTau production in AD progression. CONCLUSIONS These findings suggest that partial reduction of Drp1 decreases soluble tau and pTau, reduces mt. dysfunction, maintains mt. dynamics, and enhances mt. biogenesis and synaptic activity in tau mice. These findings may have implications for the development of Drp1 based therapeutics for patients with AD and tauopathies.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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AG047812

Title: Synaptic gene expression analysis of autopsy brains from garrison institute on aging brain bank: eva-green biochemistry based real-time rt-pcr detects mrna levels better than sybr-green

Authors: ***A. K. PANDEY**[1,2], M. MANCZAK[2], R. KANDIMALLA[2], X. YIN[2], C.-S. KURUVA[2], P.-H. REDDY[1,2,3,4];
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Biol. & Biochem., [4]Neurosciences & Pharmacol., TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER, Lubbock, TX

Abstract: BACKGROUND The Garrison Institute on Aging (GIA) Brain Bank was created in 2007 and brains have been primarily donated in the State of Texas, and neighboring states in the United States. The objectives of GIA Brain Bank are provide a free brain autopsy and to confirm clinical diagnosis of dementia and to provide brain tissues to qualified researchers studying diseases related to Alzheimer's disease (AD), related dementias and other neurodegenerative diseases. The measurement of synaptic and mitochondrial gene expressions in autopsy brains with a long-term postmortem interval is a challenging and major area of brain research. The objective of our research was to compare messenger RNA (mRNA) expressions of synaptic genes using Eva-Green and Sybr-Green biochemistry based quantitative real-time RT-PCR in autopsy AD brains with a long postmortem interval. METHOD Using Eva-Green and Sybr-Green biochemistry based quantitative real-time RT-PCR, we measured mRNA expressions of a large number of postmortem brains from AD patients and non-demented control subjects. We measured mRNA levels of several synaptic genes, including - synaptophysin, PSD95, GAP43 and others after normalizing cycle threshold values with commonly used reference genes - beta-actin and GAPDH. In addition, we also assessed whether post-mortem interval influences mRNA expressions using Eva-Green and Sybr-Green chemicals. RESULTS Our real-time PCR analysis results indicate that mRNA expression can be detected in all brain specimens for beta-actin and GAPDH. The cycle threshold values of reference gene GAPDH is similar in AD patients and control specimens. Further, the cycle threshold values are lower in Eva-Green compared to Sybr-Green in AD patients with a long postmortem interval, indicating that Eva-Green is a better chemical to measure mRNA levels in brain specimens with a long postmortem interval. CONCLUSIONS These findings suggest that Eva-Green can be used as a better chemical to detect mRNA expressions using real-time RT-PCR in AD postmortem brains with a long postmortem interval. ACKNOWLEDGEMENTS The present study was supported by NIH grants AG042178 and AG047812 and the Garrison Family Foundation.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Program#/Poster#: 131.11/D19

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: *In vivo* electrophysiological profile of tg-2576 mice

Authors: *H. ROBB[1], P. VESELCIC[2], K. WICKE[2], L. RUETER[1], K. KOHLHAAS[1];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease in humans that leads to dementia. The disease process is associated with Aβ plaques and pTau neurofibrillary tangles in the brain. Electroencephalographic (EEG) changes have been reported in AD patients, some of which include slowing of delta waves, increased theta power, seizure activity and evoked potential deficits. TG-2576 mice (129.Cg-Tg(APPSWE)2576), a pre-clinical model of Alzheimer's disease, over express the amyloid precursor protein (APP) with the Swedish mutation, the precursor to beta amyloid found in amyloid plaques. Increased beta amyloid levels in the TG-2576 mice have been reported in literature beginning at approximately 6 months of age. Transgenic AD mouse models are reported to have EEG changes that can include changes in frequency bands and sleep, hippocampal hyper-synchrony, and seizures. Here we longitudinally profile EEG in theTG-2576 mouse model of AD across 5 months. Spontaneous EEG recordings from frontal and parietal cortex over CA1 in freely-moving male TG-2576 and wildtype (WT) mice began at 4 months of age. Analysis of the EEG spectrum up to 30 Hz demonstrated an increase in power across theta/alpha frequencies in TG-2576 mice compared to wildtypes for both frontal and parietal cortex. When compared to frontal cortex, there was prominent theta activity recorded from the parietal cortex from both TG-2576 and WT mice. An increase in theta power in the parietal cortex from TG-2576 mice demonstrated a hippocampal hyper-synchrony. The spectral analysis findings were maintained for the duration of the longitudinal experiment. Also, there were no signs of spontaneous seizure or spike wave discharge (SWD) activity noted at any time during the course of the investigation. These data indicate that there are marked changes in the spontaneous EEG profile in APP-overexpressing TG-2576 mice compared to WT mice, and that these changes remain consistent across time. Future studies include assessing the potential impact of progression of pathology, i.e., Abeta plaque deposition, on spontaneous EEG in TG2576 mice. HR, PV, KW, LR, and KK are employees of AbbVie, and may own AbbVie Stock. This study was sponsored by AbbVie. Abbvie contributed to the study design, research, and interpretation of data, writing, reviewing, and approving the publication.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Synaptic hyperactivity and BDNF induction are early neuronal responses to sub-lethal beta-amyloid concentrations in organotypic hippocampal cultures

Authors: S. MERLO[1], S. F. SPAMPINATO[1], *M.-A. SORTINO[2];
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Abstract: The β-amyloid peptide (Aβ) has been identified as a key molecular factor in the etiology of AD. Aβ overproduction leads to protein build-up and abnormal aggregation into toxic oligomers which progressively assemble into fibrils. This process is slow and the culminating events are neuronal death and formation of extensive extracellular brain deposits. Aβ toxicity is directed to synaptic function with significant synaptic loss when neuronal degeneration is ongoing. Little is yet known on the exact mechanisms preceding synaptic dysfunction coinciding with pre-symptomatic stages of AD. As these events likely represent a critical time span for preventive intervention, further studies are needed. To this end we used an *in vitro* model of organotypic hippocampal slice cultures (OHC) exposed to low, sub-lethal concentrations of Aβ in order to re-create a condition of slow maturation of neuronal damage in the hippocampus. Treatment of OHC with Aβ leads to early and transient (16-72 h for 2 μM A Aβ(25-35) and 7 d for 500 nM Aβ(1-42)) increase of synaptic proteins synaptophysin, synapsin and PSD95, followed by their decrease coincident with neuronal death (7d or 14d). At 72 h of Aβ(25-35) (2 μM) exposure, higher levels of released glutamate and increased loading and unloading of FM 1-43-labeled synaptic vesicles point to increased synaptic activity. All these effects are prevented when OHC are pre-exposed to neuroprotective agent 17-β-estradiol (10 nM). BDNF is an important neurotrophic factor expressed at high levels by neuronal and glial cells in the brain. Recently, a biphasic pattern of expression similar to that we observed for synaptic proteins has been shown for BDNF in AD, with an increase in early stages and reduction in advanced stages of disease. Also, BDNF up-regulation has been linked to sustained microglial activation and release of TNFalfa. In order to test the involvement of BDNF in early synaptic changes induced by Aβ, BDNF levels were evaluated by Western blot. A significant increase of BDNF was detected in OHC exposed to Aβ(25-35) for 72 h or Aβ(1-42) for 7 d. To assess the contribution of each cell type to BDNF increase, its levels were also assessed in primary neurons, astrocytes and microglia treated with Aβ(25-35) 25 μM for 18 h. Early increase of synaptic components and activity, likely mediated by BDNF, may represent an initial step that precedes synaptic loss and neuronal death and may be interpreted as an attempted neuronal response to Aβ toxicity appearing as a new potential target for intervention in AD.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Title: Reduced dynamin-related protein 1 protects against amyloid beta, mitochondrial toxicities and synaptic damage in Alzheimer's disease

Authors: *M. MANCZAK[1,2], R. KANDIMALLA[2], A. PANDEY[2,3], X. YIN[2], C.-S. KURUVA[2], S. YEGUVAPALLI[2], D. FRY[2], C. OSBORN[2], P.-H. REDDY[2,4,3,5];
[1]Garrisson Inst. on Aging, [2]The Reddy Laboratory, Garrison Inst. on Aging, [3]Neurol., [4]Cell Biol. and Biochem., [5]Neurosci. and Pharmacol., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: BACKGROUND Mounting evidence suggests that amyloid beta (Aβ) and mitochondrial (mt.) structural and functional abnormalities are critically involved in the loss of synapses and cognitive decline, in patients with Alzheimer's disease (AD).Molecular and biochemical studies revealed that impaired mt. dynamics - increased mt. fragmentation and decreased fusion - in neurons from autopsy brains of AD patients, and from transgenic AD mice and neurons expressing Aβ, suggesting that Aβ causes mitochondrial fragmentation in AD. Further, our co-IP and IHC analysis revealed that the mt. fission protein Drp1 interacted with Aβ, and this interaction increased as AD progressed. Based on these, we hypothesize that a partial deficiency of Drp1 inhibits Drp1-Aβ interactions and protects Aβ-induced mitochondrial and synaptic toxicities, and maintains mt. dynamics and neuronal function in AD neurons. METHODS We crossed dynamin-related protein heterozygote knockout (Drp1+/-) mice with AβPP transgenic mice (Tg2576 line) and created double mutant mice - Drp1+/-xAβPP (DAβ). Using real-time RT-PCR and immunoblotting analyses, we measured mRNA expressions and protein levels of genes related to the mt. dynamics - Drp1 and Fis1 (fission), Mfn1, Mfn2 and Opa1 (fusion), CypD (Matrix), mt. biogenesis - Nrf1, Nrf2, PGC1α and TFAM and synaptic - synaptophysin, PSD95, synapsin 1, synaptobrevin 1, neurogranin, GAP43 and synaptopodin in brain tissues from 6-month-old Drp1+/-, AβPP, Drp1+/-xAβPP and wild-type mice. Using biochemical methods, we also studied mitochondrial function and measured soluble Aβ in brain tissues from all lines of mice in our study. RESULTS Decreased mRNA expressions and protein levels of Drp1 and Fis and CypD genes, and increased levels of Mfn1, Mfn2 and Opa1, Nrf1,

Nrf2, PGC1α, TFAM and synaptophysin, PSD95, synapsin 1, synaptobrevin 1, neurogranin, GAP43 and synaptopodin were found in 6-month-old DAβ mice relative to AβPP mice. Mitochondrial functional assays revealed that mt. dysfunction is reduced in DAβ mice relative to AβPP mice, suggesting that reduced Drp1 enhances mt. function in AD neurons. Sandwich ELISA assay revealed that soluble Aβ levels were significantly reduced in DAβ mice relative to AβPP mice, indicating that reduced Drp1 decreases soluble Aβ production in AD progression CONCLUSIONS These findings suggest that partial reduction of Drp1 reduces Aβ production, reduces mt. dysfunction, and maintains mt.dynamics, enhances mt. biogenesis and synaptic activity in AβPP mice. These findings may have implications for the development of Drp1 based therapeutics for AD patients.

Disclosures: M. Manczak: None. **R. Kandimalla:** None. **A. Pandey:** None. **X. Yin:** None. **C. Kuruva:** None. **S. Yeguvapalli:** None. **D. Fry:** None. **C. Osborn:** None. **P. Reddy:** None.

Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

Location: Hall A

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Title: Induction of phosphorylated tau in synapse from AD model mice

Authors: *T. KAWARABAYASHI[1], T. NAKAMURA[2], T. NAKATA[2], Y. WAKASAYA[2], N. NAKAHATA[2], M. SHOJI[2];
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Abstract: Oligomers of amyloid β protein (Aβ) are supposed to exert neurotoxicity in synapse in Alzheimer's disease (AD). Tau is recently considered an executor of neuronal damage and cognitive dysfunction in AD. Aβ oligomers are suggested to induce phosphorylated tau, however, the relationship between Aβ oligomers and tau and the mechanism of neurotoxicity are not obvious. Recently presynaptic localization of tau and increased release of tau from presynaptic terminals from AD are shown. We prepared synaptosomes from brains of AD model, Tg2576 mice (Tg n=33, NonTg n=33). Aβ monomer and oligomers were localized in synaptosomes. Phosphorylated tau was increased in synaptosomes from Tg2576 mice compared to NonTg mice. Tau fragments were also increased in synaptosomes from Tg2576 mice. These findings suggest that Aβ oligomers may induce phosphorylated tau in presynaptic compartment.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

Location: Hall A

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Program#/Poster#: 131.15/D23

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: NMDA receptor regulation by endogenous ceramide in the hippocampus

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Abstract: The lysosomal acid ceramidase, an enzyme known to limit intracellular ceramide accumulation, has been reported to be defective in neurodegenerative disorders associated with high ceramide levels. We show here that rat hippocampal slices preincubated with an acid ceramidase inhibitor (ACI) exhibit increased NMDA receptor-mediated field excitatory postsynaptic potentials (fEPSPs) in CA1 synapses. The ACI by itself did not interfere with either paired pulse facilitation or AMPA receptor-mediated fEPSPs; indicating that its influence on synaptic transmission is postsynaptic in origin and specific to the NMDA subtype of glutamate receptors. From a biochemical perspective, we observed that Tau phosphorylation at the Ser262

epitope was highly increased in hippocampal slices preincubated with the ACI, an effect totally prevented by the global NMDA receptor antagonist AP-5, the calcium chelator BAPTA and the GluN2B receptor antagonist RO25-6981. Preincubation of hippocampal slices with the compound KN-62, an inhibitor known to interfere with calcium/calmodulin-dependent protein kinase II (CaMKII), also totally abolished the effect of ACI on Tau phosphorylation at Ser262 epitopes. Collectively, these results provide experimental evidence that production of endogenous ceramide, resulting from acid ceramidase inhibition, play an important role in regulating Tau phosphorylation via a pathway dependent on GluN2B receptors and CaMKII.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Natural Sciences and Engineering Research Council

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Title: Mitochondrial dysfunction in 3xTg mouse brain is accompanied by deficits in Complex I

Authors: *J. DJORDJEVIC[1], S. ROY CHOWDHURY[2], W. M. SNOW[2,3], D. MCALLISTER[2], C. CADONIC[2,4], E. THOMSON[2], P. FERNYHOUGH[2,3], B. C. ALBENSI[2,3,4];
[2]Div. of Neurodegenerative Disorders, [1]St. Boniface Hosp. Res., Winnipeg, MB, Canada;
[3]Pharmacol. & Therapeut., [4]Biomed. Engin., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Objective: Alzheimer’s disease (AD), the most common late onset neurodegenerative dementing disorder, affects almost 800 000 elderly in Canada. AD disproportionately affects women in both prevalence and severity; 72% of AD sufferers in Canada are women. AD is characterized by progressive neuronal loss, especially in the hippocampus and cortex. While plaques and tangles are hallmarks of the disease, emerging evidence strongly support the idea that mitochondrial dysfunction is an early event in the onset and progression of AD. Although mitochondrial dysfunction was reported in AD cases, the origin(s) of the mitochondrial dysfunction, its causal relationship to oxidative stress and the mechanisms of downstream effects

to yield synaptic dysfunction and neuronal death are not clear. These experiments characterize dysfunctional processes in brain mitochondria that contribute to AD at different points in disease progression in 3xTg AD mice. Methods: Hippocampus and cortex of 3xTg and control male and female mice (2, 6 and 14 month-old) were analyzed for mitochondrial function by measuring oxygen consumption rates (OCR) on the XF24 Analyzer, (Seahorse Bioscience) and Oxygraph 2K (Oroboros) instruments. Western blot experiments were used to determine protein levels of selected Complex I-V subunits in cortical and hippocampal mitochondria. Results: Our data showed significantly decreased OCR, maximal respiration, spare respiratory capacity, basal and coupled respiration in cortical mitochondria from both male and female 3xTg mice ($*p < 0.05$, n=4) compared to their age-matched controls (14 month old). However, hippocampal mitochondria showed decreased maximal respiration and spare respiratory capacity only in 3xTg females. Western blot results of Complex I-V subunits revealed significant decrease in Complex I protein level (almost 50%) in both brain structures in female 3xTg ($*p < 0.05$). Experiments to determine mitochondrial function and Complex I-V protein levels at younger ages are ongoing. Conclusion: Our preliminary data confirm compromised mitochondrial function in hippocampus and cortex of 14 month-old 3xTg mice and suggest that deficits in Complex I are mitigating factors. Acknowledgement: Funding from Natural Sciences and Engineering Research Council (NSERC), the St. Boniface Research, and the Everett Endowment Fund.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Title: CRTC1 activates a memory-related transcriptional program deregulated at early Alzheimer's disease pathological stages

Authors: ***A. J. PARRA-DAMAS**, J. VALERO, M. CHEN, J. ESPAÑA, E. MARTIN, J. RODRÍGUEZ-ALVAREZ, C. A. SAURA;
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Abstract: Cognitive decline is associated with gene expression changes in the brain, but the transcriptional mechanisms underlying memory impairments in cognitive disorders, such as Alzheimer's disease (AD), remain largely unknown. In this study, we aimed to elucidate relevant mechanisms responsible for transcriptional changes underlying early memory loss in AD by examining pathological, behavioral, and transcriptome changes in control and mutant β-amyloid precursor protein (APP Sw,Ind) transgenic mice during aging. Genome-wide transcriptome analysis using mouse microarrays revealed deregulation of a gene network related with neurotransmission, synaptic plasticity, and learning/memory in the hippocampus of APP Sw,Ind mice after spatial memory training. APP Sw,Ind mice show changes on a transcriptional program dependent on the CREB-regulated transcription coactivator-1 (CRTC1). Importantly, we found that synaptic activity and spatial memory induces CRTC1 dephosphorylation and activation leading to CRTC1-dependent transcription in the hippocampus, and these events are impaired in APP Sw,Ind mice at early pathological and memory decline stages. Furthermore, CRTC1 overexpression in the hippocampus of APP Sw,Ind mice efficiently reverses Aβ -induced spatial learning and memory deficits by restoring a specific subset of CREB/CRTC1 target genes. Our results reveal a critical role for CRTC1-dependent transcription on spatial memory and provide evidence that targeting CRTC1 can ameliorate memory deficits in AD.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PRIN Grant, IN-BDNF

Title: Visual system dysfunction in a murine model of Alzheimer's disease

Authors: *L. DOMENICI[1,2], C. CRISCUOLO[1,2], S. CAPSONI[3,4], E. CERRI[1], A. CATTANEO[3,4];
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Abstract: In addition to progressive cognitive disorder, there is increasing evidence of visual involvement in Alzheimer's disease (AD). Indeed, a wide range of visual disturbances is reported in AD patients, including a decrease in visual acuity. As well, there is evidence of amyloid beta (Aβ) deposits and cell death in the retinas of AD patients and these, together with an Aβ deposition found in the lens fiber cells, may be responsible for some of the visual deficits seen in AD. Moreover, these visual deficits are strongly correlated with cognitive ability. In particular, visual spatial impairment and thinning of the retinal ganglion fiber layer may be one of the earliest symptoms of AD-type neurodegenerative change. The opportunity to study microscopic functional cellular changes in the eye non-invasively may be used as a window to facilitate early diagnosis of AD and monitor treatment efficacy. We used a familial AD mouse model, 5xFAD, which most closely approximates the human Aβ40 or Aβ42 peptide load in the retina, besides showing the highest concentration of Aβ peptides in the brain compared with other murine models. These characteristics make 5xFAD mouse a suitable in-vivo model for studying the mechanism of amyloidogenic neurodegeneration in the retina and the pathogenic signals that may progressively spread across the antero-posterior axis of the visual circuitry. In our work, visual retinal function was monitored using Pattern Electroretinogram (P-ERG), whose source is in the inner retina at the level of retinal ganglion cells, and visual cortical evoked potential (VEP). We found P-ERG significant alteration in 6 months old 5xFAD, when cognitive impairment is well shown; preliminary data showed VEP lower amplitude. These data highlight a first involvement of the retinal ganglion cells in the AD progression. In ongoing experiments we are monitoring the visual function at earlier stages of neurodegeneration, in order to describe the time course of the visual dysfunction in relationship with the cognitive impairment.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NINDS R01 NS086965

Title: Aberrant dynamics of hippocampal neural stem cells in Alzheimer's disease transgenic mice

Authors: *C. H. FU, D. IASCONE, A. HAZRA, M. PYFER, X. ZHANG, J. CHIN; Neurosci., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Alzheimer's disease (AD) is associated with impaired hippocampal function in both human patients as well as in transgenic mouse models of disease. Because adult neurogenesis in the dentate gyrus is critical to normal hippocampal function, alterations in neurogenesis may contribute to hippocampal impairments in AD patients and mice. Hippocampal neural stem cells appear to undergo a finite number of divisions, which could limit the stem cell pool's capacity for neurogenesis with aging. Seizure activity, exhibited by both AD patients and mice, aberrantly accelerates neurogenesis and may drive premature depletion of the hippocampal neural stem cell pool, thus contributing to functional deficits. We found that alterations in the rates and levels of neurogenesis in AD mice throughout disease progression are associated with seizure activity, correspond to the development of impairments in dentate function, and are normalized by treatment with an antiepileptic drug. Current studies in the lab investigate the dynamics of depletion of the hippocampal neural stem cell pool in AD mice. Our findings are consistent with the hypothesis that seizures induce aberrant neurogenesis and drive premature depletion of the hippocampal neural stem cell pool, which may contribute to cognitive deficits in AD mice.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG039452, AG023084 and NS034467 to B.V.Z.

U01 HL087947 and R01DK092065 to E.D.A.

Title: GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration

Authors: *A. P. SAGARE[1], E. A. WINKLER[2], Y. NISHIDA[3], S. V. REGE[4], R. D. BELL[3], D. PERLMUTTER[3], P. KONG[1], A. R. NELSON[1], Z. ZHAO[1], J. SOTO[5], E. DALE ABEL[5], J. MAKSHANOFF[1], D. C. DE VIVO[6], B. V. ZLOKOVIC[1];
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Abstract: The glucose transporter GLUT1, encoded by *SLC2A1*, mediates glucose transport into the brain. GLUT1 is expressed at the blood-brain barrier (BBB), but not in neurons. Brain glucose uptake correlates with GLUT1 levels in cerebral microvessels. Alzheimer's disease (AD) is characterized by early reductions in glucose transport associated with diminished GLUT1 expression at the BBB. Whether GLUT1 reductions can lead to cerebrovascular damage contributing to and/or accelerating AD-like neurodegeneration remains, however, elusive. To address this question, we crossed transgenic mice overexpressing human amyloid β-peptide (Aβ) precursor protein ($APP^{Sw/0}$) with GLUT1-deficient $Slc2a1^{+/-}$ mice. We also utilized conditional $Slc2a1^{lox/lox}$ mice to determine the effects of cell-specific GLUT1 deletions from endothelium and astrocytes on the BBB phenotype. We show that GLUT1 deficiency in $APP^{Sw/0}$ mice leads to early cerebral microvascular degeneration, cerebral blood flow reductions, and BBB breakdown, and to reductions in Aβ clearance, via reduced expression of LRP1 in the microvasculature, accelerated Aβ pathology, diminished neuronal activity, behavioural deficits, and progressive neuronal loss and neurodegeneration that develop after initial cerebrovascular degenerative changes. Our data suggest that GLUT1 reductions at the BBB play an early pathogenic role in neuronal demise in an AD-like neurodegenerative process and may represent a novel therapeutic target for Alzheimer's disease vasculo-neuronal dysfunction and degeneration.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FONDECYT 1109091

FONDECYT 1130747

Title: P2X2R-FE65 interaction induces synaptic failure and neuronal dyshomeostasis after treatments with soluble oligomers of amyloid beta peptide

Authors: *J. FUENTEALBA[1,3], P. A. GODOY[2], T. SILVA-GRECCHI[2], K. M. BARRA[2], T. CELIS[2], J. D. PANES[2];
[2]Physiol., [1]Univ. de Concepcion, Concepcion, Chile; [3]Physiol., Ctr. for Advanced Res. on Biomedicine (CIAB-UdeC), Concepcion, Chile

Abstract: Alzheimer´s Disease (AD) is mainly caused by soluble oligomers of amyloid β peptide (SO-Aβ) toxicity. Aβ is generated from APP proteolytic processing, given by β and γ-secretases cleavage, releasing as well the Amyloid Intracellular domain of APP (AICD) to the cytoplasm. On the other hand, Fe65 a multidomain adaptor protein which is able to interact with different proteins from the intracellular milieu and membrane, one if the most important interactions is with AICD, these proteins form a transcriptional complex which accomplishes many fundamental roles in the cell normal function; furthermore, impairment in the formation of this complex could generate neuronal dyshomeostasis, mitochondrial dysfunction, and synaptic failure. Besides, it has been reported that Fe65 can interact with the purinergic receptor P2X2 (P2X2R), whose family has been described to have an important role in neurodegenerative diseases. The aim of this work is to study the effects of the treatment with the SOAβ1-40 on the P2X2R, Fe65 and APP expression on two different models to evaluate the role of these proteins on the mechanism underlying the onset of AD. We have demonstrated that treatments with SOAβ1-40 (0,5 μM) enhance the P2X2R expression (control: 100±8%, Aβ: 143±16%) in PC12 cells without significant changes on Fe65 levels (control: 100±10%, Aβ: 134±18); additionally, our results reinforce the idea of an active interaction between P2X2R and Fe65 which has been demonstrated by coimmunoprecipitation. The increase of P2X2R expression could suggest a possible sequestration of Fe65 which may induce deterioration in other key functions of Fe65, like interaction with AICD. Searching for another approach, we used mice hippocampal slices treated with SO-Aβ1-40 (0,5 uM), where we were able to see a fast and acute increase on Fe65 protein immunoreactivity on the dentate gyrus and also an increase of APP immunoreactivity in CA3 region after subchronic treatments. On slices treated with SOAβ1-40 (1 uM) it was possible to see changes in the APP signal localization, from cytosol to the nucleus on dentate gyrus and CA1 areas. Concerning Fe65, we were able to see a fast and significant increase of the immunoreactivity in the mossy fibers region near the CA3 area of the hippocampus. Moreover we evaluated P2X2R after said treatments, and we observed a fast and significant increase of its immunoreactivity in all hippocampal areas that were studied. In conclusion, Aβ toxicity promote

acute and chronic changes on key protein redistributions an expression, enhancing P2X2R-FE65 interaction, inducing alterations on mitochondrial dynamics.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH GRANT R01AG046275-01A1

Title: Amyloid β-protein from aqueous extracts of Alzheimer's disease brain disrupts excitatory-inhibitory tone and impairs synaptic plasticity

Authors: *Z. WANG[1,2], T. T. O'MALLEY[1], W. HONG[2], W. LIU[2], S. LI[2], D. M. WALSH[2]; [1]Brigham and Women's Hosp., Boston, MA; [2]Ann Romney Ctr. for Neurologic Diseases, Harvard Inst. of Med., Boston, MA

Abstract: Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disease that mostly affects persons over the age of 65 years. Genetic studies on familial AD indicate that APP and its metabolites, including the amyloid β-protein (Aβ), play a central role in AD. Recent studies found that Aβ can exist in multiple different forms that differ both in terms of primary structure and aggregation state. This heterogeneity makes modeling Aβ *in vitro* very complex. Given that significant data suggest that Aβ plays an initiating in role in AD, we focus on brain tissue of individuals who died with very mild forms of disease, studying a region of temporal cortex implicated in the early stages of the disease. So far we have prepared extracts from entire temporal cortices of 3 mild AD cases. In each case tissue was homogenized in artificial cerebrospinal fluid and a portion of these treated with a pan anti-Aβ polyclonal antibody to remove all detectable forms of Aβ. The effect of AD brain extracts (+/- immunodepletion) on excitatory-inhibitory (E/I) tone and long-term potentiation (LTP) were then investigated. Extracts from all 3 brains suppressed LTP in the hippocampal CA1 area of mouse brain slices in an Aβ-dependent manner, i.e. samples immunodepleted of Aβ did not disrupt synaptic plasticity. However, the extent of LTP inhibition varied between extracts. This variation was not attributable to a simple difference in the total amount of Aβ since the sample which contained the highest levels of Aβ as measured by Western blotting and 3 different ELISAs actually produced

the weakest impairment of synaptic plasticity. We are currently investigating the basis of the apparent differences in potency of extracts. Simultaneously, we are also seeking to understand the underlying mechanism of inhibition of Aβ on LTP. We have used whole cell voltage clamp recording and measured E/I balance on individual pyramidal neurons of the CA1 area. Our results show that extracts of AD brain decrease inhibitory activity without altering excitatory input on pyramidal neuron. These findings suggest that certain (as yet undisclosed) forms of Aβ act by disrupting GABA release and that the subsequent increase in excitability leads to disruption of LTP. Interestingly, conditioned medium (CM) from a cell line (referred to as 7PA2) which contains significant amounts of N-terminal extended Aβ (NTE-Aβ) species also blocks LTP and disrupts E/I balance. However, 7PA2 CM disturbs the E/I balance by increasing excitatory input without altering inhibitory input. These results indicate that human brain extracts (which contain little or no NTE-Aβ) act by a mechanism distinct from the NTE-rich 7PA2 CM.

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Poster

131. Alzheimer's Disease: Synaptic and Neuronal Dysfunction

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5R01AG047589-02

Title: An anatomical correlate of the mouse default mode network

Authors: *J. D. WHITESELL, P. BOHN, M. T. MORTRUD, K. E. HIROKAWA, S. W. OH, S. MIHALAS, H. ZENG, J. A. HARRIS;
Allen Inst. for Brain Sci., Seattle, WA

Abstract: The default mode network (DMN) is a group of brain regions that are active when individuals are in a state of internal focus or "alert rest" and deactivated during goal-directed tasks. The DMN is defined in humans and nonhuman primates on the basis of correlated activity during resting state functional MRI (fMRI), and analogous networks in rodents have recently been observed using fMRI in anesthetized rats and mice. Several neurodegenerative disorders affect the DMN, including Alzheimer's disease (AD). In AD, network activity persists during task-oriented activities and DMN regions show early and extensive deposition of amyloid-beta plaques. We hypothesize that brain regions comprising the DMN are preferentially linked by a

class of cells selectively vulnerable to neurodegeneration in AD, while a separate group of cells links these regions to non-DMN brain areas. To examine the structural connectivity underlying the functionally-defined rodent DMN, we first performed graph-theoretical analysis on our large dataset of stereotaxic anterograde viral tracing experiments (the Allen Mouse Brain Connectivity Atlas) to identify a group of anatomically connected regions which closely resemble the putative mouse DMN. We further probed the connectivity of this network with dual stereotaxic injections of retrograde CAV2-Cre virus and anterograde rAAV expressing Cre-dependent fluorescent protein (e.g. rAAV2/1.pCAG.FLEX.EGFP.WPRE.bGH) in pairs of connected regions inside and outside the DMN. For a given DMN source, retrograde Cre expression was produced by CAV2-Cre injections into both DMN and non-DMN targets. Whole brain projections from these target-defined neuron populations were labeled by injecting Cre-dependent rAAV-eGFP in the source area. Projections to targets within the DMN were compared with projections to outside-DMN targets and with interareal connectivity datasets obtained as part of the Allen Mouse Connectivity Atlas. When completed, this collection of target-defined projection patterns from DMN sources will provide a detailed map of cell type-specific anatomical circuit architecture that may in part explain the functional rodent DMN, and also help identify neuron types selectively vulnerable to AD. Future experiments will test the vulnerability of the DMN to AD pathology in mouse models. These experiments may provide important insight into the spread of pathology through neural networks in AD.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson’s Disease

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Gardner Family Center for Parkinson's Disease and Movement Disorders

NIH T32 DK059803

Title: Effects of minocycline treatment in a neurotoxin rat model of Parkinson’s disease

Authors: *S. N. CASSELLA[1,2], D. SEXTON[1,3], A. M. HEMMERLE[1], T. L. KYSER[1,2], K. B. SEROOGY[1,2];
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Abstract: A growing number of human post-mortem and animal studies indicate that neuroinflammation contributes to the nigrostriatal degeneration observed in Parkinson's disease (PD). Microglial cells, the resident immune cells of the central nervous system, are present in an activated state in the substantia nigra (SN) of PD patients and in animal models. In this state, they release pro-inflammatory cytokines, nitric oxide, and reactive oxygen species, all of which are toxic to the local dopaminergic neurons. Previous work has shown that the catecholamine-specific neurotoxin 6-hydroxydopamine (6-OHDA) can activate microglia. In this study, we sought to determine if systemic administration of an anti-inflammatory agent, concurrent with a 6-OHDA lesion, would abrogate the behavioral dysfunction and degeneration of nigral dopaminergic neurons found in the unilateral 6-OHDA striatal-lesion model of PD. In a flanked paradigm, rats received the broad-spectrum antibiotic minocycline dissolved in drinking water for two weeks before and four weeks following unilateral striatal injection of 6-OHDA. The rats received a minocycline dosage of 30-40 mg/kg/day. Motor deficits were assessed as a measure of forelimb asymmetry (cylinder test) at two- and four-weeks post-lesion. Following the final cylinder test, the rats were sacrificed and the brains were sectioned and processed for Iba-1 and tyrosine hydroxylase (TH) immunohistochemistry for markers of microglia and dopaminergic neurons, respectively, in the ventral midbrain. Morphological analysis of microglia in the SN was performed using ImageJ and unbiased stereology was used to count the number of TH-positive (TH+) neurons and Iba-1-positive (Iba-1+) microglia in the SN. Initial results demonstrate a strong trending interaction effect of minocycline and 6-OHDA in both the cylinder test and the TH+ cell counts in animals receiving both minocycline treatment and 6-OHDA lesion compared to animals receiving regular water and 6-OHDA lesion (P=0.0553 and 0.0832, respectively). Morphological analysis of the microglial perikarya indicated no differences in somal area at the four-week post-lesion time point; counts of Iba-1+ cells in the SN are ongoing. Overall, the results suggest that minocycline treatment may prevent both TH+ cell loss and motor dysfunction in the 6-OHDA rat model of PD, presumably by preventing activation of microglia.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

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Nu Rho Psi

Title: Analysis of sumoylation mutants of alpha-synuclein in yeast models

Authors: *A. N. ROMAN, G. LIPKIN, S. BELLO-ROJAS, R. THOMAS, S. K. DEBBURMAN;
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Abstract: Parkinson's disease (PD) is a hypokinetic neurodegenerative disorder linked to the death of midbrain dopaminergic neurons. Within affected cells are Lewy bodies composed primarily of insoluble aggregates of the protein alpha-synuclein. PD can be genetic or sporadic; genetic forms account for 10% of cases and thus far, seven mutant genes have been identified, including alpha-synuclein. Six mutations on alpha-synuclein cause PD (A30P, E46K, H50Q, G51D, A53E, A53T). Alpha-synuclein is also a highly post-translationally modified protein and it exists in full length and three alternatively spliced isoforms (Syn-126, Syn-112, Syn-98). Unlike regulation by phosphorylation, less is known about alpha-synuclein sumoylation, which is proposed to be neuroprotective based on limited studies. Sumoylation influence on familial mutants and splice variants of alpha-synuclein are also not well understood. The majority of sumoylation takes place on lysine-96 and lysine-102 in alpha-synuclein. Our goal was to test these related hypotheses: 1) sumoylation will protect against wild-type alpha-synuclein toxicity; 2) sumoylation will also protect against toxicity of familial mutant and spice variants; 3) phosphorylation will regulate sumoylation-dependent properties. First, we have created and expressed sumoylation-blocking variants (K96R, K102R, K96R/K102R) on wild-type and familial mutant backgrounds in our well-established budding and fission yeast models through site-directed mutagenesis. We found that reducing alpha-synuclein sumoylation not only increases its aggregation, but also reduces its association with the plasma membrane. We believe this is the first description of sumoylation effects on alpha-synuclein membrane association. We are currently studying the effects of sumoylation mutants on splice variants and phosphorylation-modified variants of alpha-synuclein. Together, these studies will add insight into alpha-synuclein based pathogenesis in both sporadic and familial forms of PD.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

Support: "Construction of System for Spread of Primate Model Animals" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Mutated α-Synuclein(A30P) transgenic marmosets for the pathological research on Parkinson's disease

Authors: *R. KOBAYASHI[1], C. HARA-MIYAUCHI[1,2], J. OKAHARA[4], J. TAKAHASHI-FUJIGASAKI[3], F. OZAWA[1], T. MAEDA[1], H. J. OKANO[1,2], E. SASAKI[1,4], H. OKANO[1]; [1]Keio Univ. Sch. of Med., Tokyo, Japan; [2]Div. of Regenerative Med., [3]Div. of Neuropathology, Jikei Univ. Sch. of Med., Tokyo, Japan; [4]Central Inst. for Exptl. Animals, Kanagawa, Japan

Abstract: α -Synuclein which mainly expressed at presynaptic terminals is implicated in Parkinson's disease (PD), dementia with Lewy body (DLB) and multiple system atrophy (MSA). Actually, mutations in α-Synuclein gene were identified in PD family lines. In neurons of PD patients, α-Synuclein forms aggregations and most of them is modified such as ubiquitination and phosphorylation. This aggregation is thought to cause the Lewy body formation, however, its function is not well understood. To identify the initial pathological change of PD, it is necessary to establish the non-human primate model. We established a novel non-human primate model of PD using mutated α-Synuclein (A30P) to explore the pathology of α-Synucleinopathy. As for the common marmoset (Callithrix jacchus), a small New World primate, its structure of the brain evolves in comparison with rodents. Therefore, we expected that it is possible to link between an exercise or non-exercise dysfunction and a lesion part because the structure of the basal nuclei is clear in marmoset. In the transgenic (Tg) marmoset, the transgene of mutated α-Synuclein (A30P) expression was evident. Moreover some proteins which are component of Lewy body showed higher expression in Tg than in wild-type marmoset. We investigated for pathological changes in tissues of Tg marmoset. In this presentation, we will show the recent results of pathological and exercise functional analysis.

Disclosures: **R. Kobayashi:** None. **C. Hara-Miyauchi:** None. **J. Okahara:** None. **J. Takahashi-Fujigasaki:** None. **F. Ozawa:** None. **T. Maeda:** None. **H.J. Okano:** None. **E. Sasaki:** None. **H. Okano:** None.

Poster

132. Parkinson's Models

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Program#/Poster#: 132.04/D35

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1R01NS069574

Title: The lipase activity of phospholipase D2 is responsible for nigral neurodegeneration in a rat model of Parkinson's disease

Authors: H. MENDEZ-GOMEZ[1,2], J. SINGH[2], O. GORBATYUK[3], C. MEYERS[2], W. CHEN[2], *N. MUZYCZKA[1,2];
[1]Dept Mol Gen & Microbiol, Powell Gene Therapy Ctr., Gainesville, FL; [2]Univ. of Florida, Gainesville, FL; [3]Univ. of Alabama, Birmingham, AL

Abstract: Phospholipase D2 (PLD2), an enzyme involved in vesicle trafficking and membrane signaling, interacts with α-synuclein, a protein that it is involved in Parkinson's disease. We previously reported that PLD2 overexpression in rat substantia nigra pars compacta (SNc) caused a rapid neurodegeneration of dopamine neurons and that α-synuclein suppressed PLD2 neurodegeneration. Here, we show that PLD2 toxicity is due to its lipase activity. Overexpression of a catalytically inactive mutant (K758R) of PLD2 prevents the early loss of dopaminergic neurons in the SNc and does not show signs of toxicity after 10 weeks of overexpression. Further, mutant K758R does not affect dopamine levels in the striatum. In contrast, mutants that prevent PLD2 interaction with dynamin or Grb2 (a tyrosine kinase receptor scaffold protein) but retained lipase activity, continued to show rapid neurodegeneration. Our findings rule out the interaction of PLD2 with dynamin and a potential role in vesicle trafficking, and the interaction with tryrosine kinase complexes and their associated downstream signal transduction as the cause of PLD2 induced neurodegeneration. Instead, the synthesis of phosphatidic acid, the product of PLD2, which is a second messenger in multiple cellular pathways appears to be the key to PLD2 induced neurodegeneration. The fact that α-synuclein is a regulator of PLD2 activity suggests that regulation of PLD2 activity could be important in the progression of Parkinson disease.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

Title: Attempts to measure cognitive function of the common marmoset for the purpose of detecting its impairment in Parkinson's disease model

Authors: *K. ANDO, R. INOUE, C. NISHIME, E. NISHINAKA, H. TSUTSUMI;
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Abstract: Cognitive impairment is one of the main syndromes of Alzheimer's disease and other neuropsychiatric diseases although pathophysiology is not well known. The impairment is also reported in patients of Parkinson's disease (PD) of which primary syndrome is progressive impairment of movement derived from degeneration of dopaminergic neurons at the substantia nigra. Preclinical studies using marmosets treated with 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) as PD models have been reported on movement impairments by us in SfN. The present report briefly reviews on our attempts to establish methods measuring cognitive function for the future and final purpose of evaluating its impairment in PD model marmosets. In our earlier attempts, lever-pressing behavior was established in intact marmosets by reinforcing with sweetened liquid using operant chambers originally designed for the rat. Furthermore, pressing on the lighting side of either of 2 levers was reinforced. This discrimination behavior, however, was not stably established owing to weak motivation due to limited water deprivation and restlessness of the behavioral trait of this monkey species. Apart from operant chambers, mailbox-type equipment consisting of 10 compartments was used. A food pellet was placed in each compartment through an opaque door. By repeated training, marmosets opened up each door with knob and took all pellets within 5 min. Then, MPTP (2, 2 and 1 mg/kg, SC on the 1st, 2nd and 3rd consecutive days, respectively) was given to these marmosets. Door opening behavior was tested in 4 months thereafter. Both of the percent correct opening responses and the response rate significantly decreased compared with the levels before MPTP administration and with those of the intact control marmosets. The results indicated that MPTP-treated marmosets of PD model showed cognitive impairment along with movement impairment. Minimizing movement factor, touch response of marmosets on iPad

screen was measured. Touching either of 9 moving stimuli was reinforced with enlarging the touched stimulus and giving music sound. By training, responses were stably maintained and were sensitively affected by some dopaminergic drugs such as methamphetamine. Sensory reinforced touch behavior may be used for testing impairment of cognitive function in PD model marmosets with less involvement of motor impairment. In conclusion, test system measuring cognitive impairment in the PD model marmosets may be useful and important in preclinical studies as well as evaluation of movement impairment.

Disclosures: K. Ando: None. **R. Inoue:** None. **C. Nishime:** None. **E. Nishinaka:** None. **H. Tsutsumi:** None.

Poster

132. Parkinson's Models

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Topic: C.03. Parkinson’s Disease

Support: Akdeniz University scientific research projects management committe: Proje number :2012.01.0103.002

Title: The effect of sodium metabisulfite on locomotor activity in experimental Parkinson’s model: the role of cyclooxygenase and nuclear factor kappa B

Authors: *A. AGAR[1], A. OZKAN[1], H. PARLAK[1], O. OZSOY[2];
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Abstract: Sulfite commonly used as preservative in food,beverages and pharmaceuticals ,is a very reactive and potentially toxic molecule.The aim of this study was to investigated the possible effects of sulfite on neuron toxicity by measuring locomotor activity in normal and 6-hydroxydopamine (6-OHDA)induced experimental Parkinson model(PA) rats.The changes were determined by levels of activities of cyclooxygenase (COX) ,Prostaglandine E2 (PGE2),Nuclear factor Kappa B(NF-ƙB) ,caspase-3 and locomotor activity. Male Wistar rats were divided into four groups as follows: control (Control), sulfite treated (Sulfite), (6-OHDA) injected (6-OHDA), sulfite treated and 6-OHDA injected (6-OHDA+Sulfite).Sodium metabisulfite was administered at dose 100mg/kg/day for 45 days by gavage in sulfite and 6-OHDA group +sulfite. Experimental PD was created stereotactically via unilateral infusion of 6-OHDA into the medial forebrain bundle (MFB).In sulfit +6-OHDA group experimental PD was created on 38 rd day of sulfite treatment and continued for remaining 7 days. Locomotor performance decreased in 6-

OHDA group compared to kontrol.Additional effect of sulfite treatment did not induced this impairment.COX and PGE2 activities of substantia nigra(SN) were significantly increased in 6-OHDA group compared with control group.COX and PGE-2 activities significantly elevated in 6-OHDA+sulfite group compared with control and sulfite groups. COX and PGE2 activities of SN of 6-OHDA +S group were not changed compared with the 6-OHDA group. Caspase-3 activity and NF ƙ B p 65 of SN were increased in 6-OHDA and 6-OHDA+Sulfite groups compared with Control group. The tyrosine hydroxylase (TH)-positive neuron number in substantia nigra was reduced in the animals treated with 6-OHDA and 6-OHDA+Sulfite compared with control. In conclusion : Sulfite treatment is not potentially effective for activities of COX,PGE2 ,caspase-3, NF ƙ B p 65 in 6-OHDA induced by experimental Parkinson model.
Key word:Sodium metabisulfite,Parkinson model,caspase-3, Nuclear factor Kappa B(NF-ƙB), cyclooxygenase, Prostaglandine E2

Disclosures: **A. Agar:** None. **A. Ozkan:** None. **H. Parlak:** None. **O. Ozsoy:** None.

Poster

132. Parkinson's Models

Location: Hall A

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Program#/Poster#: 132.07/D38

Topic: C.03. Parkinson’s Disease

Title: Differential cortical oscillation activities in hemiParkinsonian rats under apomorphine and L-DOPA treatments

Authors: *B. POUYATOS, R. MAURY, C. BOUYSSIÈRES, C. ROUCARD, Y. ROCHE, V. DUVEAU;
Synapcell, La Tronche, France

Abstract: Motor symptoms observed in Parkinson’s disease result from a dysfunction of the cortico-basal ganglia circuits mainly due to the death of dopaminergic neurons in the substantia nigra pars compacta. A hyper synchronization of beta frequency oscillatory activity in the cortico-basal ganglia circuit has been well characterized in both parkinsonian patients and animal models of the disease. This abnormal beta oscillation is positively correlated to motor symptoms and can be suppressed by dopaminergic treatment. Moreover, a recent study has demonstrated an increase of gamma power oscillation in the motor cortex of both hemiparkinsonian rats (Halje et al. 2012) and patients (Oswal et al. 2013) after chronic injection of L-DOPA. The aim of this work was to further explore oscillatory activities in the motor cortex of hemiparkinsonian rats under normal behaving conditions and after treatment with the dopamine agonist apomorphine

and the dopamine precursor levodopa (L-DOPA) in order to develop a powerful and predictive tool for drug discovery and development. Using the 6-hydroxydopamine-lesioned rat model of PD, we found a significant increase of the beta band power (30Hz) in the cortex compared to controls. Acute injection of apomorphine or L-DOPA alleviated the beta band peak previously observed in the motor cortex. Concomitantly to this reduction, we observed an increase of the gamma power (80-100Hz). Altogether these data suggest that dopaminergic treatments in hemiparkinsonian rats induce a switch of EEG activities from beta to gamma resonant oscillations. One can hypothesize that the abnormal cortical beta oscillation is correlated to the motor symptoms of PD whereas the gamma band would be correlated to the levodopa-induced dyskinesia. We discuss here how these two distinct oscillatory activities could be used as predictive biomarkers for the development of new treatments in Parkinson's disease. ref: Oswal A, Brown P, Litvak V. Synchronized neural oscillations and the pathophysiology of Parkinson's disease. Curr Opin Neurol. 2013 Dec;26(6):662-70. Halje P, Tamtè M, Richter U, Mohammed M, Cenci MA, Petersson P. Levodopa-induced dyskinesia is strongly associated with resonant cortical oscillations. J Neurosci. 2012 Nov 21;32(47).

Disclosures: **B. Pouyatos:** A. Employment/Salary (full or part-time):; SynapCell. **R. Maury:** A. Employment/Salary (full or part-time):; Synapcell. **C. Bouyssières:** A. Employment/Salary (full or part-time):; SynapCell. **C. Roucard:** A. Employment/Salary (full or part-time):; Synapcell. **Y. Roche:** A. Employment/Salary (full or part-time):; Synapcell. **V. Duveau:** A. Employment/Salary (full or part-time):; SynapCell.

Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

Support: Santos Dumont Institute

AASDAP

FINEP

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FAPERN

CAPES

CNPq

Title: Behavioral and histological effects of adeno-associated viral vectors (AAV2 and AAV5) for alpha-synuclein overexpression in rats

Authors: *K. B. COSTA, J. NUNES, M. FREIRE, M. ARAÚJO;
Edmond and Lily Safra Intl. Inst. of Neurosci., Santos Dumont Inst., Macaiba, Brazil

Abstract: Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide, characterized by the progressive loss of dopaminergic neurons in the nigrostriatal pathway. The injection of adeno-associated viral vectors for overexpression of alphasynuclein (AAV-αsyn) in the substantia nigra (SN) is a promising model for studying this pathophysiology since it reproduces the pathologic and neurochemical features found in the human disease. However, many factors such as the serotype of the AAV or the amount injected can affect both the transfection and the alpha-synuclein expression in transfected cells. In this study, we evaluated the behavioral and histological profiles after injection of different volumes of two types of viral vectors in the SN of rats. Twelve Sprague-Dawley rats housed at the Edmond and Lily Safra International Institute of Neuroscience's (ELS-IIN) animal facility were used. All procedures were approved by the institution's Ethics Committee for the Use of Animals (protocol 05/2014) and under the knowledge and agreement of scientific direction of ELS-IIN. AAV-αsyn was injected in the left SN of all animals, which were divided in 4 groups according to the amount and serotype injected (4µl or 2µl of AAV2 and 4µl or 2µl of AAV5). Animals were tested for forepaw asymmetry use in the cylinder test one week before and for 8 weeks after injection. After this period the rats were perfused and their brains were processed for TH immunohistochemical assessment. For each animal, we analyzed 3 sections in which the striatum and 3 sections in which SN could be easily identified. Striatal dopaminergic degeneration was assessed by optical densitometry of TH immunoreactivity in both injected and contralateral sides using ImageJ software. Striatal denervation and neuronal cell loss in the SN was observed in the injected hemisphere of all animals. The denervation was higher in the groups injected with 4µL, regardless of viral serotype. Preliminary analysis suggests that animals in the group injected with 4 µl of AAV2 tended to decrease the use of their right paw in the cylinder test over the weeks. These results contribute to the standardization of an animal model to investigate the histological changes observed in PD and the mechanisms involved in the causes of the human disease

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Poster

132. Parkinson's Models

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Title: Intracranial chronic administration of prolyl oligopeptidase inhibitor, KYP-2047, counters the behavioural effects and alpha-synuclein overexpression after nigral AAV-alpha-synuclein injection

Authors: *R. SVARCBAHS, U. JULKU, T. MYÖHÄNEN;
Univ. of Helsinki, Helsinki, Finland

Abstract: Prolyl oligopeptidase (PREP) is a serine protease able to hydrolyse small (less than 30 amino acid) proline containing neuropeptides. Recently, it has been recognised that PREP non-enzymatic interaction with alpha-synuclein (aSyn) leads to aSyn aggregation. Our previous results have shown that interaction of PREP with aSyn can be modulated with PREP inhibitors, leading to decreased aggregation process. Moreover, our findings showed that PREP inhibitors enhance autophagy. However, the extent of PREP's involvement in aSyn aggregation processes, clearance from neurons, and aSyn mediated neuronal toxicity is still vaguely understood. In this study, we injected AAV2-CBA-aSyn in a stereotaxic surgery into the mouse (C57Bl/6RccHsd) substantia nigra, and AAV2-CBA-GFP served as a control. Intracranial KYP-2047 treatment with Alzet minipump was initiated one month after the virus administration and continued for 2 weeks. We observed that KYP-2047 administration cleared aSyn from the mice brains and partially reversed aSyn caused motor impairment. Immunohistochemical staining showed a decrease in aSyn immunostaining and an increase in tyrosine hydroxylase (TH) positive staining in the KYP-2047 treated animals compared to the vehicle group. Behavioural scores for the cylinder test paw preference showed improvements in KYP-2047 treated animals. Prior to the study, aSyn and GFP virus vectors were evaluated to establish their activity. AAV-aSyn gave rise to the robust aSyn expression and loss in the TH positive staining with moderate behavioural effects while the GFP control vector did not cause observable neurotoxicity. Our results suggest that PREP inhibitors could be of the therapeutic use in the clearance of accumulated aSyn in synucleinopathies.

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Poster

132. Parkinson's Models

Location: Hall A

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Topic: C.03. Parkinson's Disease

Support: MJFF Research Grant

Title: A non-human primate model of Parkinson's disease based on viral vector mediated overexpression of alpha-synuclein

Authors: *J. B. KOPRICH[1], T. H. JOHNSTON[1], J. SEIBYL[2], K. MAREK[2], Y. MA[3], D. EIDELBERG[3], C. ZUO[4], Y. GUAN[4], J. H. KORDOWER[5], S. H. FOX[6], J. M. BROTCHIE[1]; [1]Atuka Inc., Toronto, ON, Canada; [2]Mol. Neuroimaging, New Haven, CT; [3]Feinstein Inst. for Med. Res., Manhasset, NY; [4]PET Ctr., Huashan Hospital, Fudan Univ., Shanghai, China; [5]Neurolog. Sci., Rush Univ. Med. Ctr., Chiacago, IL; [6]Movement Disorders Clin., Toronto Western Hosp., Toronto, ON, Canada

Abstract: Rodent models of Parkinson's disease based on the viral overexpression of alpha synuclein (aSyn) have proven useful in better understanding aSyn function. However, the field currently lacks a robust, well-characterized primate model. In this study, 12 cynomolgus macaques each received monthly baseline assessments on motor activity, a functional rating scale (MPPrs) and were trained to conduct a fine motor task (mMAP test). Baseline PET scans were obtained bimonthly to visualize VMAT-2 and FDG fluorodeoxyglucose (FDG). Animals were injected with either AAV1/2 A53T aSyn or empty vector (EV) (both, 1.7 x 10exp12 gp/ml) bilaterally into the substantia nigra (SN) and killed 8 months post surgery. In an independent study (validation study), another 4 macaques were injected unilaterally with the same vector and pathology was characterized at 4 months. In the first study, postmortem analyses, showed that at 8 months, there was a 42% and a 39% reduction in putaminal DA and DAT, respectively, compared to controls (all, $P<0.05$), along with a reduction in TH positive neurons in the SN. Neurites also showed transgene expression throughout the striatum and had a dystrophic Lewy morphology. Remaining neurons showed overexpression of aSyn, however, pS129 aSyn and thioflavin-S positive inclusions were absent. By 5 months post surgery, A53T aSyn macaques showed 45% less motor activity in the 2-4 hr period of a 4 hr observation, compared to EV controls ($P<0.05$). This deficit persisted through to the final month (53%, 56% and 60%, respectively, to month 8, [all, $P<0.05$]). No significant effects were observed on mMAP performance. PET imaging showed no significant change in striatal VMAT-2 to assess dopaminergic activity or change in FDG uptake evaluated to derive the Parkinson related pattern

(PrP). In the validation study, we found that, at 4 months, aSyn aggregates were positive for p129 aSyn and thioflavin-S. Relative to the contralateral side, there were reductions in the number of remaining TH positive neurons (reduced by 30%), TH optical density in the putamen and caudate nucleus (reduced by 26 and 20%, respectively), and striatal dopamine as measured by HPLC (reduced by 34%). The presence of pathological aSyn (pS129 and thioflavin-S positivity) observed at 4 months and the absence of such, despite continued overexpression of the aSyn transgene, at 8 months, may be evidence of more vulnerable neurons degenerating over the period of 4-8 months. In conclusion, the macaque model of PD alpha-synucleinopathy produced here is in a position to assess therapeutics aimed at reducing or preventing aSyn accumulation and concomitant neurodegeneration in the nigrostriatal system.

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Poster

132. Parkinson's Models

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Crédit Agricole Sud Rhône Alpes

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ANR Carnot Institute

Carnot Nanospectrome

Title: Kinetics of MPTP elimination in non-human primate’s urine intoxicated by MPTP: study by a new nano-interface based Matrix-Assisted Laser Desorption / Ionization Mass Spectrometry approach

Authors: *F. DARLOT[1,2,3], C. YEROMONAHOS[4,3], A. MOMBRUN[2,3], F. REINHART[2,3], D. AGAY[2,3], N. TORRES-MARTINEZ[2,3], V. JOUSSEAUME[4,3], C. MORO[2,3], F. BERGER[2,3], A.-L. BENABID[2,3], A. BOUAMRANI[2,3];

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Abstract: Parkinson's disease is a progressive neurodegenerative disease that leads to dopaminergic neurons death and induces motor disorders such as resting tremor, rigidity, and akinesia. This disease has a significant socioeconomic impact and, currently, only symptomatic treatments exist and there is no curative treatment. In this context, research on this disease is a main health concern. Several experimental models exist, including models by injecting toxins or genetic models. Here we focus on MPTP non-human primate model. The use of MPTP involves the use of health protections for user in order to protect from contamination. Indeed, MPTP is a neurotoxin that crosses the blood-brain barrier and after transformation into MPP+ induces oxidative stress and mitochondrial dysfunction leading to dopaminergic neurons death. In animal experiments, there are many risks of contamination to the user: during MPTP administration, in the case of a bite, and during contact with the excreta of animals (blood, saliva, urine, feces). The major risk highlighted here is the risk of contact with urine. We study the elimination kinetics of MPTP in the urine in order to adapt the means of protection and facilitate experiments. In our laboratory, we have developed new biochips from nanoporous SiOCH thin films that allow the selective detection of low concentrated low molecular weigth molecules by Matrix-Assisted Laser Desorption / Ionization Mass Spectrometry (MALDI MS) in complex biological fluids such as urine. We used this technique to determine when MPTP is completely metabolized, it means when there is no significant detectable MPTP remaining in urine. Monkeys (5-7kg) are injected (intramuscular injection) with 0.3mg MPTP/kg daily for several days, and urine was collected daily for measure processing. Results show that we no longer detect MPTP in urine beyond 48 hours after last MPTP injection. In addition, the number of repetition of daily injection has no cumulative effect on the detection time of MPTP in urine. We detect MPP+ up to 6 days after the last injection of MPTP, but as this molecule does not go through the blood-brain barrier, it induces minor risk to the user. Results of this study can be used as a scientific basis for MPTP protocols on non-human primate to adapt user protection measures.

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Poster

132. Parkinson's Models

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Title: Chronic variable stress increases the neuroimmune response in an experimental Parkinson's model

Authors: *A. M. HEMMERLE[1], S. N. CASSELLA[1], K. H. LUNDGREN[2], J. P. HERMAN[3], K. B. SEROOGY[1];
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Abstract: There is an increasing realization that the non-motor symptoms of Parkinson's disease (PD) affect the quality of life of patients as much, if not more, than the motor symptoms. In particular, there is high comorbidity with the psychiatric disorder of depression, but few good animal models exist with which to investigate the potential convergence of pathologies. To address this issue, we have developed a novel paradigm in which a neurotoxic lesion model of PD is combined with the chronic variable stress (CVS) model of depression. Previously we found that administration of the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) concurrently with chronic unpredictable stress resulted in the worsening of both the motor deficits and nigral cell loss normally present in the 6-OHDA model (Hemmerle et al., Mol. Psychiatry, 2014). In the current study, we sought to examine a potential mechanism underlying the enhanced dysfunction by exploring the neuroinflammatory environment of the substantia nigra (SN). Inflammation is thought to underlie, at least in part, the pathogenesis of both PD and depression. Adult male Sprague Dawley rats were subjected to CVS for two weeks both prior to and after receiving 6-OHDA. Two weeks after the cessation of stressors, animals were sacrificed, processed for immunohistochemistry of the microglia marker Iba-1, and labeled cells counted using unbiased stereological techniques. Cell counts in the SN pars compacta (SNpc) revealed that animals exposed to both the stress regimen and 6-OHDA displayed significantly higher numbers of Iba-1+ cells compared to lesioned-only animals. Determination of immune cell activation by examination of microglial soma size and process length remains ongoing. These results indicate that the exacerbated deficits found in the combined chronic stress/PD model may be a response to increased neuroinflammation within the SNpc and has implications for the optimal therapeutic treatments for comorbid depression and PD.

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Poster

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Support: NIH Grant NS058830

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Title: Neuroinflammatory profile associated with intrastriatal injection of pre-formed alpha-synuclein fibrils in a new rat model of Parkinson's disease

Authors: *M. F. DUFFY[1], K. L. PAUMIER[1], K. C. LUK[2], L. FISCHER[1], N. K. POLINSKI[1], T. J. COLLIER[1], C. J. KEMP[1], J. Q. TROJANOWSKI[2], V. M. LEE[2], C. E. SORTWELL[1]; [1]Translational Sci. and Mol. Med., Michigan State Univ., Grand Rapids, MI; [2]Ctr. for Neurodegenerative Dis. Research, Dept. of Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Our lab has recently characterized the accumulation of phosphorylated alpha-synuclein (a-syn) intraneuronal inclusions and bilateral nigrostriatal degeneration that results from intrastriatal injection of a-syn preformed fibrils (PFFs) into rats, extending previous work done in mice (Luk et al., Science, PMID: 23161999). Recent evidence has implicated a-syn as an initiator of neuroinflammation in Parkinson's disease with neuron-released a-syn activating toll-like receptor 2 (TLR2) on microglia. The present study examined the neuroinflammatory signature resulting from intrastriatal a-syn PFF injections and its temporal relationship to nigrostriatal degeneration. Young adult, male F344 rats received 2 x 2.0 µl unilateral intrastriatal injections of mouse a-syn PFFs (8 µg protein total) or saline. Rats were euthanized 2, 3, 4 or 6 months post-injection. To differentiate the contribution of nigrostriatal degeneration from the accumulation of phosphorylated a-syn on neuroinflammation, rats in which nigrostriatal degeneration was induced by 6-hydroxydopamine (6-OHDA) were used as comparators. This cohort of young-adult, male, F344 rats received 2 x 2.0 µl unilateral, intrastriatal injections of 6-OHDA or vehicle and were euthanized at multiple time points corresponding with increasing nigrostriatal degeneration. Immunohistochemical analysis and stereological quantification is ongoing. The focus of our analysis will be on the magnitude of nigrostriatal degeneration (tyrosine hydroxylase), phosphorylated a-syn and neuroinflammation (ionized calcium binding adaptor molecule 1, major histocompatibility complex-II). We will examine the temporal relationship between a-syn aggregation, microglia-mediated neuroinflammation and nigrostriatal

degeneration. Results from this study will provide new insight into neuroinflammatory dynamics and mechanisms in relation to a-syn aggregation and nigral degeneration and could establish the use of the a-syn PFF rat model as a platform for testing anti-inflammatory therapeutics.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

Title: The light at the end of the axon - a fast, quantitative assay to track *in vivo* alpha-synuclein oligomerization

Authors: ***M. DELENCLOS**[1], T. TRENDAFILOVA[1], D. R. JONES[1], S. MOUSSAUD[1], A.-M. BAINE[1], M. YUE[1], W. D. HIRST[2], P. J. MCLEAN[1,3];
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Abstract: Alpha synuclein (αsyn) aggregates are associated with the pathogenesis of Parkinson's disease and others related disorders. The mechanism of αsyn accumulation is not well understood, but a large body of evidence points to αsyn oligomers as a species contributing to neurodegeneration. Although modulation of αsyn aggregation is an attractive therapeutic target, new powerful methodologies are desperately needed to facilitate *in vivo* screening of novel therapeutics. Here we report a novel *in vivo* rodent model with the unique ability to rapidly track αsyn oligomers using a bioluminescent protein complementation strategy that monitors spatial and temporal αsyn oligomers formation *ex vivo*. We find that αsyn forms oligomers *in vivo* as early as 1 week after stereotactic AAV injection into rat substantia nigra (SN). Strikingly, although abundant αsyn expression is also detected in striatum (STR) at 1 week, no αsyn oligomers are detected at this time point. By 4 weeks, oligomerization of αsyn is detected in both STR and SN homogenates giving insight into the kinetics of oligomers formation *in vivo*. Moreover, the effect of a de novo Hsp90 inhibitor, known to prevent αsyn oligomers formation, dramatically reduced αsyn oligomerization. Taken together, we demonstrate that this de novo viral-vector rodent model, that uses Gaussia princeps luciferase as a surrogate reporter of αsyn oligomerization, can be utilized to track the dynamics of oligomers formation *in vivo*. We also

show that αsyn oligomers formation may not necessarily be concentration-dependent as previously thought. We believe this model will be useful rapid and sensitive animal model for future testing of novel therapeutics.

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Poster

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Topic: C.03. Parkinson's Disease

Support: A*STAR BMRC-TCRP

NMRC-TCR

Title: Identification of PP2A as a novel modulator of LRRK2 G2019S-mediated toxicity

Authors: ***J. P. SIM**[1], S. LIN[1], C. NG[1], K. LIM[1,2,3];
[1]Res., Natl. Neurosci. Inst., Singapore, Singapore; [2]Duke-NUS Grad. Med. Sch., Singapore, Singapore; [3]Physiol., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, affecting millions of people worldwide. Although the etiology of PD remains poorly understood, a subset of PD cases is inheritable and attributable to mutations in specific genes. Among these, mutations in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene is currently recognized to be the most common genetic cause of PD, of which the G2019S substitution accounts up to 4% of familial PD and 1% of sporadic PD. It is widely accepted that G2019S mutation enhances the kinase activity of LRRK2, leading to neurotoxicity. Based on the hypothesis that antagonizing kinase activity will be neuro-protective, we hypothesized that the cognate phosphatase(s) that reverses LRRK2-mediated phosphorylation would also be neuro-protective. Accordingly, we performed a genetic screen using a phosphatase library in *Drosophila* melanogaster LRRK2 model. One of the phosphatases that we have identified to rescue G2019S-induced neurotoxicity is the serine-threonine phosphatase PP2A. We found that over-expression of PP2A ameliorates the climbing defects of G2019S transgenic flies and restores dopaminergic neuronal function. In addition, PP2A over-expression mitigates the mitochondrial pathology induced by LRRK2 G2019S. Similarly, pharmacological activation of PP2A also rescues the parkinsonian

phenotypes of G2019S flies. Collectively, these results suggest that PP2A has an antagonistic role to LRRK2 possibly via the modulation of its downstream substrate phosphorylation. In conclusion, we found that pharmacological or genetic activation of PP2A reduces LRRK2-mediated toxicity, suggesting that the reversal of G2019S kinase activity by PP2A may be a therapeutic strategy for LRRK-related PD.

Disclosures: J.P. Sim: None. **S. Lin:** None. **C. Ng:** None. **K. Lim:** None.

Poster

132. Parkinson's Models

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LABEX BRAIN ANR-10-LABX-43

Title: Octodon degus: a model for the study of age-associated synucleinopathy?

Authors: *S. DOVERO, M. BOURDENX, E. BEZARD, B. DEHAY;
Inst. of Neurodegenerative Dis., Bordeaux, France

Abstract: Animal models are an essential tool for basic pathophysiological research, age-related research as well as drug development and compound testing in neurodegenerative diseases. So far, most of animal studies on neurodegenerative disorders are based on transgenic mice or rats. Although very useful to unravel cellular mechanisms associated to neuronal cell death during the time course of the disease, no mammalian model recapitulates the required age-dependent neurodegeneration, especially in Parkinson’s disease (PD). Of interest, these models failed to replicate the combination of features characterizing PD: α-synucleinopathy associated with dopaminergic cell loss and both motor and non-motor dysfunctions. The absence of adequate *in vivo* experimental models of Parkinson’s disease has severe repercussions for therapeutic intervention success. Natural or ecological models would be an alternative to model PD. Of

interest, among rodent models, *Octodon degus,* a diurnal and highly social South American rodent, might be a very good candidate to study age-associated PD neurodegeneration. Since age-dependent deposition of Aβ-amyloid has been reported in the *Octodon degus*, we posited that this animal could as well be a model of age-related synucleinopathy, and more broadly proteinopathies. No exploratory studies have been undertaken on PD to date. To study this hypothesis, we used a statistically significant group of animals of one, two, three, five and six years old. We thus explored the age-related neurodegeneration observed in the *O. degus* brain by evaluating the progression of pathological markers known to be affected in PD. Analyses have been carried out using biochemical and histochemical techniques in the whole brain. We characterized the pattern of dopaminergic loss in the striatum and in the substantia nigra, the regional distribution of α-synuclein immunoreactivity in several brain structures (i.e. within hippocampus, striatum, substantia nigra, and cortex), as well as its pathological state (i.e. S129 phosphorylation) and occurrence of intracellular inclusion formation. Overall, we observed age-related differences in several brain areas related to PD pathophysiology.

Disclosures: **S. Dovero:** None. **M. Bourdenx:** None. **E. Bezard:** None. **B. Dehay:** None.

Poster

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Parkinson's Disease Foundation

Title: Alpha-Synuclein splice variant and C-terminal truncation analysis in yeast: Implications for sporadic and familial PD

Authors: *S. BELLO ROJAS[1], K. HAMID[2], N. KUKULKA[2], K. CAMPBELL[2], P. SCHRAG[2], L. GRAHAM[2], M. MUNOZ[2], C. ALVARADO[2], S. CHIREN[2], J. JAMES[2], A. ROMAN[2], S. K. DEBBURMAN[2];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disease caused by the death of midbrain dopaminergic neurons linked to misfolding and aggregation of the protein alpha-synuclein. Familial PD accounts for 10% cases and is linked to at least seven genes. Six different mutations on alpha-synuclein itself cause PD. While the full-length alpha-synuclein (which is 140 amino acids long) is the major misfolded form in all PD forms, smaller versions of alpha-synuclein were recently discovered, including three alternatively spliced variants (syn126, syn112, and syn98) and several carboxyl-terminal truncation variants (syn103, syn110, syn120, and syn123). Truncation variants are better studied, but in the wild-type alpha-synuclein background only, and in cell culture and *in vitro* systems; current splice variants studies are limited to just one report. Evaluation of both variant types needs to be extended to additional model systems. Furthermore, properties of neither type of variants are known for the six familial mutant alpha-synuclein backgrounds. We tested the hypotheses that both types of variants would possess a higher pathogenic potential compared to the full-length form, and that this increased pathogenicity would be further potentiated on the six familial mutant backgrounds. We used our well-developed budding yeast model to test our hypotheses. Here, we firstly describe the creation, subcloning, and yeast transformation of all splice variant forms of alpha-synuclein and familial splice variants needed for this study. Thus far, we have examined both types of variants in the wild-type alpha-synuclein background. With them, we describe evidence that the truncation variants have altered solubility, membrane association and aggregation potential; the degree of change depends on how much of the C-terminus is removed. Finally, we show that splice variants possess reduced membrane association and increased aggregation, depending on the variant. We are currently comparing truncation and splice variant properties in familial mutant backgrounds. Our longer-term expectation is that our completed studies will provide a fuller picture of the contributions of the diversity of naturally occurring alpha-synuclein variants in familial and sporadic PD pathogenesis.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

Title: Effects of α-synuclein on behavioral dysfunction in the Parkinson's disease model *Drosophila* melanogaster

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Abstract: The present study involves using *Drosophila melanogaster* to look at behavioral dysfunction associated with specific genes linked to Parkinson's Disease (PD) known as A30P and A53T genes. The genes promote the formation of protein α-synuclein and the overexpression of this protein promotes the formation of toxic filamentous inclusions. Ultimately, the build up of inclusions leads motor dysfunction due to severe loss of dopaminergic neurons. Research has suggested a deep homology of the arthropod and vertebrate brain structures that have similar functions. Particularly, the arthropod central complex and vertebrate basal ganglia are the homologous structures that mediate the selection and maintenance of behavioral actions. Testing *Drosophila* that overexpress this protein can inform us about how abnormalities in human A30P and A53P genes account for behavioral deficits. Currently, the assay that has been used to investigate motor dysfunction in flies is a simple negative geotaxis climbing assay. However, geotaxis climb may not fully represent the movement dysfunction observed in PD patients. PD patients have difficulty negotiating obstacles due to impairment of voluntary motor control. For this reason we developed a gap negotiation and an obstacle avoidance assay that may be better suited to study PD in the fruit fly model and allow for investigation into more complex motor control. Using GAL4 lines targeting the ellipsoid body or mushroom bodies and UAS lines carrying A30P or A53T, *Drosophila* were genetically altered to express α-synuclein protein in specific brain regions known to be associated with sensory integration and locomotor abilities in insects. In this study, gap-crossing and visually guided obstacle avoidance tests were examined in flies using a high-speed camera and tracking system. We quantified the locomotor abilities of control and mutant flies expressing α-synuclein in terms of the changes in approach speed towards the gap or obstacle, the speed of negotiating the gap or obstacle and the changes in success of negotiating the gap or obstacle on repeated trials. Our results suggest that flies expressing α-synuclein had significantly reduced locomotor ability, resulting in low success rate in gap crossing and impaired obstacle avoidance. Our behavioral assays and results provide new insight into our understanding of behavioral deficits and α-synuclein pathology associated with human neurological diseases, such as PD.

Disclosures: D. Hwang: None.

Poster

132. Parkinson's Models

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Title: Evaluation of alpha-synuclein nitration in yeast models

Authors: *M. N. MARSHALL, C. ALVARADO, K. SOLVANG, Y. ZAYATS, S. K. DEBBURMAN;
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder linked to the death of midbrain dopaminergic neurons and alpha-synuclein misfolding and aggregation. Alpha-synuclein is a highly post-translationally modified protein, including phosphorylation, sumoylation, and nitration. Nitration sites on alpha-synuclein occur on tyrosine residues -39, -125, -133, and -136. To add to the growing literature on alpha-synuclein nitration contributions to PD pathogenesis, we created nitration-mimic (Y39C, Y125C, Y133C, Y136C) and nitration-deficient (Y39F, Y125F, Y133F, Y136F) mutants of alpha-synuclein to test the hypothesis that nitration of alpha-synuclein will increase aggregation and cell toxicity, and decrease membrane binding. We evaluated these eight mutants in budding and fission yeast models. Our results demonstrate that nitration does regulate alpha-synuclein localization, aggregation and toxicity. In budding yeast, nitration at Y133 affected toxicity the most, while nitration at Y39 regulated membrane localization. In fission yeast, alpha-synuclein nitration at Y39 and Y125 most affected its toxicity, while nitration on all four sites altered intracellular localization. We are currently evaluating double/triple/quartet combination mutants in our budding and fission yeast models, and also in yeast genetically altered for nitrative stress. Our assessment of nitration supplements our insight into the effects of alpha-synuclein post-translational modifications as they relate to PD.

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Poster

132. Parkinson's Models

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Support: RFFI #13-04-00327a

RSF #14-15-00942

Title: GDNF gene containing cells and sleep-wake cycle in a mouse model of Parkinson's disease

Authors: *V. M. KOVALZON[1], G. V. PAVLOVA[2], A. V. REVISHCHIN[2], Y. V. UKRAINTSEVA[3], V. B. DOROKHOV[3], L. S. MOISEENKO[4];
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Abstract: We found that transgenic GDNF containing HEK cells implanted into striatum of C57Bl/6 mice can effectively smooth changes in the characteristics of sleep-wake cycle induced by injection of proneurotoxin MPTP. HEK293 cells transfected by plasmid constructions containing an isoform of human GDNF gene without pre- and pro-sites were implanted (under chloral-hydrate anaesthesia) into caudatum/putamen area of C57Bl/6 mice preliminary adapted to 12/12 LD circadian rhythm. Simultaneously, the epidural electrodes for EEG registration were implanted to the same mice. Three days later animals were subjected to subcutaneous injection of 40 mg/kg dopaminergic proneurotoxin MPTP. Just before the injection, 7 and 14 days after it the 24-hr continuous recording of sleep-wake EEG and motor activity have been performed. After the completion of the experiment animals were tested in Rotarod. Then the animals were narcotised, perfused, brains were extracted and fixed. The amount of dopaminergic neurons of substantia nigra/pars compacta and ventral tegmentum area was studied in brain slices. Our previous studies revealed an increase in motor activity and percentage of waking state during the dark period of nychthemeron at the 7th and especially 14th day since MPTP administration as compared to the controls. Consequently, there was a decrease in percentage of NREM and REM sleep at the 12-hr dark period. Now we have found that preliminary implantation of transgene HEK293/mGDNF cells in amount of about 200000 cell bodies in 1 microliter of solution into striatum of mice results in smoothing of above-mentioned behavioral and EEG effects of MPTP. These data were also confirmed by histochemical studies. We can suppose that the transgenic cells developed and used in our experiments might be regarded as neuroprotective ones also in *in vivo* situation. Supported by RFFI #13-04-00327a and RSF #14-15-00942.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

Title: traumatic brain injury leads to development of Parkinson's disease in mice

Authors: ***E. ESPOSITO**, D. IMPELLIZZERI, M. CORDARO, G. BRUSCHETTA, R. CRUPI, S. CUZZOCREA;
Univ. of Messina, Messina, Italy

Abstract: Central nervous insults, such as traumatic brain injury (TBI), are among the leading causes of mortality and morbidity worldwide. TBI is an insult to the brain from the application of external physical force that leads to temporary or permanent structural and functional impairment of the brain. Traumatic brain injuries are reported as risk factors for sporadic neurodegenerative diseases. Parkinson's disease (PD) is a late-onset neurodegenerative disorder caused by degeneration of dopaminergic neurons in the substantia nigra (SNc). In that regard, the aim of this study was to investigate the possible development of PD following experimental model of TBI. Specifically, TBI was induced in mice by controlled cortical impactor. At different time points behavioral tests (Open field, Elevated plus maze tests and Barnes maze) were performed; the animals were sacrificed 30 days after TBI (corresponding to 5-6 years in human) and the brains were collected. Our results showed that following TBI there was decreased expression of tyrosine hydroxylase (TH) and dopamine transporter (DAT) in the SNc, which are specific markers of PD, and significant behavioral alterations. In addition, a strong increase in neuroinflammation evaluated as GFAP, TNF-α, COX-2, iNOS expressions, IκB-α degradation, and NF-κB translocation, was evident. Also, neurotrophic factors such as BDNF, NT3, NGF, GDNF were also decreased at 30 days after TBI. Interestingly, our results showed a significant accumulation of αsynuclein (αsyn) in microglia compared to astrocytes, where we couldn't find any difference; the same situation was found in TH expression. In conclusion, in this study, we suggested that there are currently under-appreciated biological mechanisms linking brain injury and neurodegenerative diseases.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson’s Disease

Title: The antioxidant N-acetyl-L-cysteine exerts strong neuroprotective effects in both *in vitro* and *in vivo* models of Parkinson’s disease

Authors: *E. ANDRIAMBELOSON, C. NEVEU, N. KADOUCI, F. LAUGA, E. POIRAUD, B. HUYARD, S. WAGNER;
NEUROFIT, ILLKIRCH, France

Abstract: Parkinson's disease (PD) is a devastating neurodegenerative disorder for which there is no cure. It is caused by the loss of dopaminergic (DA) neurons in the striatum, producing disabling motor symptoms. In the present study, the neurotoxin 6-hydroxydopamine (6-OHDA) was used for the modeling of Parkinson's disease in both *in vitro* and in-vivo experiments. In primary culture of mesencephalic neurons, 15µM of 6-OHDA induced selective death of the DA neuron population. Unilateral injection of 6-OHDA in the medial forebrain of the rat induced ipsilateral depletion (90% reduction) of nigrostriatal dopamine along with ipsilateral rotation of rat following injection of apomorphine. In addition, 6-OHDA-injected rats showed marked motor deficit in different behavioral tests such as beam walking and forelimb use asymmetry tests. *In vitro*, Baclofen ($GABA_B$ receptor agonist), Z-vad-fmk (caspases inhibitor) and PD150606 (calpain inhibitor) elicited 30, 50 and 55% inhibition of the death of DA neurons, respectively. Complete inhibition of cell death was observed with the antioxidant N-acetyl-L-cysteine (NAC). Furthermore, treatment of 6-OHDA rats with NAC prevented the loss of nigrostriatal dopamine and markedly reduced apomorphine-induced rotation. These rats showed improved performance in beam walking and in the forelimb use asymmetry tests. The above data underline the pivotal role of the oxidative stress pathway in 6-OHDA -mediated death of DA neurons. In addition, they indicate the relevance of an *in vitro* model to predict in-vivo neuroprotection.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

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Research on rare and intractable diseases (039), Japan

Research on rare and intractable diseases (025), AMED, Japan

Title: Knockdown of ESCRT-0 disrupts protein quality control and promotes ER stress-mediated neuronal cell death

Authors: *T. HASEGAWA[1], R. OSHIMA[1], N. SUGENO[1], K. TAMAI[2], S. YOSHIDA[1], J. KOBAYASHI[1], A. KIKUCHI[1], A. TAKEDA[3], N. TANAKA[2], M. AOKI[1];
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Abstract: Objectives: ESCRT (endosomal sorting complex required for transport) plays a key role in the later step of the autophagosome, amphisome, and endosome maturation. The aim of this study is to investigate as to whether the ESCRT dysfunction is associated with the abnormal accumulation of protein aggregates and subsequent neurodegeneration in animal model. Methods: We specifically deleted the ESCRT-0 component, hepatocyte growth factor-regulated tyrosine kinase substrate (hrs), in neurons of the adult forebrain by using conditional knockout mice on calcium/calmodulin-dependent protein kinase II alpha (CaMKII)-Cre-expressing background. The locomotor activity was evaluated by footprint analysis, hind-limb extension and hanging wire tests. The neuronal cell loss was determined by hematoxyline-eosin staining. The intraneuronal accumulation of ubiquitinated proteins, p62, and neurodegenerative disease-related proteins such as α-synuclein, TDP-43, tau, and huntingtin was examined by immunostaining and Western blot analyses. In parallel with *in vivo* studies, RNAi-mediated silencing of Hrs was conducted in HEK293 and PC12 cells to validate the autophagic function, involvement of ER stress and its downstream MAPKs pathway. Results: The locomotor performance in the hrsflox/flox; CaMKII-Cre mice was significantly impaired compared to that in hrs+/+; CaMKII-Cre mice. Histological analysis showed the prominent neuronal loss in CA1/CA3 regions of the hippocampus. Furthermore, we observed a striking accumulation of detergent-insoluble α-synuclein, TDP-43, tau, and huntingtin as well as ubiquitinated proteins and p62 in the brain of hrsflox/flox; CaMKII-Cre mice. Tracking of autophagy in RFP-GFP-LC3B-expressing HEK293 cells revealed the impairment of autophagic flux at later stage. The neuronal cell death induced by Hrs knockdown was accompanied by the striking induction of ER stress markers and the

activation of SAPK/JNK signaling pathway. Conclusions: These findings suggest that the neuron-specific disruption of ESCRT-0 compromises autophagic/lysosomal degradation of aggregate-prone proteins and acquires ER stress-mediated neuronal cell death.

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Poster

132. Parkinson's Models

Location: Hall A

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Topic: C.03. Parkinson's Disease

Support: NIH R01NS082565

Title: Mechanisms of nigral neuron loss in PINK1 knockout rat models of Parkinson's disease

Authors: ***S. K. BARODIA**, J. WATSON, R. B. CREED, V. M. TAPIAS, M. S. GOLDBERG; Dept. of Neurology, Ctr. for Neurodegeneration and Exptl. Therapeut, Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Parkinson's disease (PD) is characterized by motor symptoms caused by selective, progressive and robust loss of dopaminergic neurons in the substantia nigra. Mutations in genes including Parkin, DJ-1, PINK1, LRRK2 and alpha-Synuclein are causally linked to inherited forms of PD. PINK1 is a serine/threonine kinase with a mitochondrial targeting motif at its N-terminus. Recent studies indicate that PINK1 normally functions by recruiting Parkin to selectively target dysfunctional mitochondria for clearance by autophagy. PD-linked mutations in PINK1 are recessively inherited and result in the loss of functional PINK1 protein, consistent with a loss-of-function mechanism. We used PINK1 knockout rats and neurons cultured from wild-type and PINK1 knockout rats to test potential mechanisms by which PINK1 mutations cause early onset PD. We hypothesized that PINK1 deficiency causes an accumulation of dysfunctional mitochondria due to impairment of mitochondrial autophagy (mitophagy) resulting in enhanced oxidative stress. PINK1 knockout rats show age-dependent loss of greater than 50% of nigral dopamine neurons by age 8 months. We observed mitochondrial abnormalities in PINK1 knockout rats, suggesting that this might be the mechanism underlying the loss of nigral dopamine neurons caused by PINK1 mutations. Analysis of susceptibility of wild-type and

PINK1 knockout neurons to PD-relevant stresses may further define the mechanisms of neurodegeneration in familial and sporadic PD.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

Support: Canadian Institutes of Health Research IC100171

Title: Partial neurorestorative action of DHA after a lesion of the mouse dopaminergic system

Authors: *K. COULOMBE[1,2], M. SAINT-PIERRE[1], G. CISBANI[1,3], I. ST-AMOUR[1,3], C. GIBRAT[1,3], A. GIGUÈRE-RANCOURT[1,2], F. CALON[1,2], F. CICCHETTI[1,3];
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Abstract: BACKGROUND: Parkinson disease (PD) is characterized by a massive loss of dopaminergic (DAergic) projections from the substantia nigra pars compacta (SNpc) to the striatum, which is responsible for motors symptoms. The underlying causes of the diseases have not been clearly identified. Although symptomatic treatments exist, curative therapy is yet to be found. Recent data from our team in the MPTP mouse model of PD support the use of omega-3 polyunsaturated fatty acids (n-3 PUFA)-enriched diet as a neuroprotective intervention. However, the usefulness of n-3 PUFA in diagnosed symptomatic patients, after the 70-80% DAergic neuronal death, remains to be evaluated. OBJECTIF: In this study, we investigated the effects of a n-3 PUFA rich diet using a neurorestorative paradigm METHODOLOGY: C57Bl6 mice fed a control diet were submitted to a striatal stereotaxic injection of the neurotoxin 6-hydroxydopamine (6-OHDA) to induce DAergic denervation. Three weeks post-lesion, the mice received either an n-3 PUFA rich or a control diet for a period of 6 weeks. We investigated the effect of dietary intake on the motor phenotype and DAergic nigrostriatal system. RESULTS: Although no improvement in the motor behavior was observed, HPLC analyses revealed a 39% increase of striatal dopamine levels in the n-3 PUFA group compared to ctrl ($P<0.05$). Furthermore, the n-3 PUFA diet led to a 52% rise of tyrosine-hydroxylase (TH) - positive terminals in the striatum ($P<0.05$). Interestingly, despite the fact that n-3 PUFA did not restore

the number of TH-positive neurons in the SNpc, morphological analyses uncovered increased perimeters (+ 21% vs ctrl) and areas (+ 7 % vs ctrl) of DAergic cell bodies. CONCLUSION: Collectively, our results suggest a mild neurorestorative effects or a potentiation of DAergic neurorecovery mechanisms following a n-3 PUFA - enriched diet, and support further studies to investigate the potential of a diet-based intervention in PD.

Disclosures: K. Coulombe: None. **M. Saint-Pierre:** None. **G. Cisbani:** None. **I. St-Amour:** None. **C. Gibrat:** None. **A. Giguère-Rancourt:** None. **F. Calon:** None. **F. Cicchetti:** None.

Poster

132. Parkinson's Models

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Topic: C.03. Parkinson's Disease

Support: National Key Basic Research Program of China (Grant 2012CBA01304)

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Title: Mutant alpha-synuclein causes age-dependent neuropathology in monkey brain

Authors: *W. YANG[1], G. WANG[1,2], C.-E. WANG[2], X. GUO[1], P. YIN[1], J. GAO[1], Z. TU[1], Z. WANG[3], J. WU[3], X. HU[3], S. LI[2], X. LI[1,2];
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Abstract: Parkinson's disease (PD) is an age-dependent neurodegenerative disease that often occurs in those over age 60. Although rodents and small animals have been used widely to model PD and investigate its pathology, their short life span makes it difficult to assess the aging-related pathology that is likely to occur in PD patient brains. Our recent work has established transgenic PD monkey model that expresses mutant alpha-synuclein and shows early non-motor symptoms. Because the neuropathology of PD is age-dependent, we want to investigate how aging influences PD neuropathology. We first examined the expression of Parkin, PINK1, and alpha-synuclein, which are known to cause PD via loss- or gain-of-function mechanisms, in the brain tissues of monkeys at 2-3, 7-8, and >15 years of age. We found that alpha-synuclein is

increased in the older monkey brains, whereas Parkin and PINK1 are decreased or remain unchanged. Stereotaxic injection of lentiviral vectors expressing mutant alpha-synuclein (A53T) into the substantia nigra of monkeys revealed that aging increases the accumulation of A53T in neurites and its associated neuropathology. A53T also causes more extensive reactive astrocytes and axonal degeneration in monkey brain than in mouse brain. We also found that A53T interacts with neurofascin, an adhesion molecule involved in axon subcellular targeting and neurite outgrowth, and that aged monkey brain tissues show an increased interaction of neurofascin with A53T. Overexpression of A53T causes neuritic toxicity in cultured neuronal cells, which can be attenuated by transfected neurofascin. These findings from non-human primate brains reveal age-dependent pathological and molecular changes that could contribute to the age-dependent neuropathology in PD.

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Poster

132. Parkinson's Models

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Topic: C.03. Parkinson’s Disease

Support: PDF-IRG-131

Title: Non invasive imaging of retinal morphology and mitochondrial dysfunction by optical coherence tomography during progression of Parkinsonism in a nonhuman primate model

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Abstract: Optical coherence tomography (OCT), a non-invasive procedure for analysis of retinal morphology, has been effectively used as a measure of disease progression in multiple sclerosis

(MS) and may be a surrogate measure for brain damage. OCT may also be a valuable tool for longitudinally following other neurodegenerative conditions such as Parkinson's disease (PD) and potentially for monitoring disease progression as well as assessing efficacy of neuroprotective treatments. Studies using OCT to assess retinal histology *in vivo* in PD patients have reported significant inverse correlations between foveal or peripapillary retinal nerve fiber layer (RNFL) thickness and Unified Parkinson's Disease Rating Scale scores, and have even suggested the possibility of differentiating between rigid/akinetic and tremor dominant PD patients by differences in retinal morphology between these subgroups. In a recent study using a non-human primate model of Parkinsonism, we identified statistically significant reductions in RNFL thickness (particularly in nasal and inferior RNFL quadrants) in chronically Parkinsonian (MPTP-treated) animals vs. controls as well as significant foveal thinning and decreases in macular volume. Recent developments in OCT have enabled imaging of retinal flavoprotein fluorescence (FPF), a putative marker of oxidative stress and mitochondrial dysfunction in the retina. FPF occurs due to changes in oxidative stress impairing enzymatic complexes of the electron transport chain and reduction in mitochondrial membrane potential, leading to flavoproteins linked to these enzymatic complexes becoming oxidized and, when excited by blue light, emitting green flavoprotein fluorescence. As such FPF imaging, in conjunction with OCT retinal imaging may be used as a non-invasive biomarker for oxidative stress associated with neurodegenerative diseases such as PD. In the present study we have assessed the changes from normal baseline in retinal morphology, together with with FPF imaging, during the development of Parkinsonism in 10 adult male cynomologous MPTP-treated monkeys. Results indicate that there are early changes in both retinal thickness measures and in FPF expression following MPTP exposure and preceding development of overt Parkinsonian symptoms as well as long-lasting increases in FPF signal. These data suggest the potential value of these non-invasive techniques for early detection of a neurodegenerative process, for monitoring disease progression and for evaluating potential neuroprotective therapies in PD.

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Poster

132. Parkinson's Models

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LABEX BRAIN ANR-10-LABX-43

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Fondation pour la Recherche Médicale

Fondation de France

Title: *In utero* delivery of scAAV9 mediates widespread brain transduction in rats and monkeys : towards new models of Parkinson's disease

Authors: *B. DEHAY[1], M. BOURDENX[1], L. CHANSEL-DEBORDEAUX[1], S. DOVERO[1], V. GROUTHIER[1], J. URANGA[1], N. DUTHEIL[1], S. BRUN[1], A. ESPAGNA[2], L. GROC[2], Q. LI[3], C. JIMENEZ[1], E. BEZARD[1];
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Abstract: Transgenic mammals allow to study brain function and diseases. However, technical difficulties associated to classical transgenesis approaches in species such as rats and monkeys have prevented this long awaited technological development for the study of brain function to be further developed in such species. The development of the viral-mediated delivery of genes in the past few years has led to the identification of viruses with unique characteristics. The adeno-associated virus serotype 9 (AAV2/9) crosses the blood brain barrier, is capable of transducing developing cells and neurons after intravenous injection in many species, and mediates a long-term stable transduction. Ability to transduce brain decreases over time, being maximum at P1 and decreasing dramatically by P10 already suggesting a developmental period in which AAV2/9 transduction has maximal efficacy. While P1 is an attractive (and easily accessible) time point, *in utero* gene delivery has clearly demonstrated that a wide transduction of neurons is possible at embryonic stages compatible with the development of the targeted area. To test this hypothesis, we injected intracerebroventricularly (i.c.v.) high-titer bolus of AAV2/9 carrying either mutant human α-synuclein (A53T) or enhanced green fluorescent protein (EGFP) under the synapsin or CMV immediate enhancer/β-actin (CAG) promoter respectively, at embryonic day 16.5 for rat and around 100 days fetal age for monkeys under ultrasound imaging guidance. Animals after birth have then been behaviourally followed-up and were terminated at regular interval to access the brain pathology. We characterized the regional distribution of GFP immunopositivity in brain structures and peripheral organs. We observed transduction of neurons in most regions of the brain – i.e. within hippocampus, thalamus, spinal cord, striatum, globus pallidus, substantia nigra, choroid plexus, cerebellum, and cortex- 25 days after injection in rats. Moreover, the efficacy of transduction seems dependent on the brain structure. Transduction of mutated α-synuclein was then characterized as was defined the specific brain lesions in both rats and monkeys. Overall, these results indicate that an engineered scAAV9 injected *in utero* in rats or rhesus macaques results in efficient, neuronal and widespread transduction of the brain. Altogether, this proof of concept study could facilitate and offer unique opportunities for

modelling brain diseases in rats and rhesus macaques by targeting mutant genes with tissue-specific gene expression, not mentioning future clinical applications for *in vivo* correction of monogenic diseases or abnormalities in humans.

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Poster

132. Parkinson's Models

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Title: Deficits in motivation, increased anxiety, and increased daytime sleepiness in a nonhuman primate MPTP model of Parkinson's disease

Authors: *K. GANAPATHY SUBRAMANIAN[1,2], C. ADAM[3], A. M. DETTMER[4], A. DOYLE[3], T. LIU[3], S. CULVER[3], S. BENJI[3], M. RAGOZA[3,5], C. BRANDON[3], J. MORGAN[3], D. PYRDEK[6], N. ROCKCASTLE[6], N. D. RYAN[6], Z. ZHANG[7], R. PENN[8], M. SAARMA[9], J. L. CAMERON[6];
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Abstract: Movement problems including bradykinesia, tremor and rigidity have been studied in detail using a variety of animal models of Parkinson's disease (PD), including the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated nonhuman primate model. However, non-motor problems including decreases in motivation, increases in anxiety, changes in social behaviors and increases in daytime sleepiness occur frequently in PD and have profound

negative impacts on the quality of life. This study was performed to determine whether these non-motor problems were apparent in the low dose (0.14-0.16 mg/kg, intracarotid injection), unilateral rhesus monkey MPTP model of PD. Thirty adult female rhesus monkeys (Maccaca mulatta), aged 17-20 years (equivalent to 50-80 human years) received a single unilateral MPTP injection. Motivation to work for a reward was significantly decreased post-MPTP (19% decrease as measured by progressive ratio testing, $p=0.005$), motivation to eat as indicated by a 5% decrease in body weight with ad libitum food available was decreased, $p<0.001$, and latency to take a novel food was increased, $p=0.013$, but no decrease in social motivation was noted (proportion of time spent in close proximity to a penmate, $p=0.48$). Low reactivity, or behavioral inhibition, in response to a threatening stimulus is a common indicator of increased anxiety in children and monkeys. MPTP led to a significant decrease in reactivity, $p=0.001$. Daytime sleep duration, measured by actigraphy, increased from 100.77±9.04 min/day to 248.19±20.37 min/day, $p<0.001$, with confirmation of daytime sleep assessed by videography. No changes in nighttime sleep were found. As these monkeys received a unilateral MPTP injection and none of these non-motor measures were dependent on use of the affected hand, we conclude that the low dose, unilateral MPTP monkey model will be useful for further studies to better understand the etiology of these non-motor impairments and for testing effective therapies to improve these non-motor impairments.

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Poster

132. Parkinson's Models

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PAPCA-IZTACALA UNAM-2014-16

PAPCA-IZTACALA UNAM2014-18

Title: Manganese Acetate and Manganese chloride specifically damage SNc dopaminergic neurons

Authors: A. TRUJILLO MARTÍNEZ[1], V. ANAYA-MARTÍNEZ[2], A. GUTIÉRREZ-VALDEZ[2], E. MONTIEL-FLORES[2], L. REYNOSO-ERAZO[2], L. REYNOSO-ERAZO[2], F. HUERTA-OLIVAREZ[2], F. HUERTA-OLIVAREZ[2], M. IBARRA[2], C. LOZANO-VILLALOBOS[2], J. SÁNCHEZ-BETANCOURT[2], P. ALEY-MEDINA[2], J. ESPINOSA-VILLANUEVA[2], *M. AVILA-COSTA[3];
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Abstract: Our group recently reported that mice and rats that were inhalationally exposed to a mixture of manganese chloride (MnCl2) and manganese acetate (MnOAc3) developed movement abnormalities, significant loss of Substantia nigra pars compacta (SNc) dopaminergic neurons, dopamine depletion and improved behavior with L-Dopa treatment as Parkinson disease (PD) patients. However, it is not yet evaluated Mn selectivity for SNc dopaminergic neurons. In the present study we directly exposed mice and rats brain slices to the Mn mixture. The brain slices contained the mesencephalon, striatum, Globus Pallidus, hippocampus and Parietal cortex. The slices were incubated for one hour in 0.02M MnOAc3 and 0.04M $MnCl_2$ and the control tissue were exposed to Krebs solution for one hour. Afterwards, we performed immunohistochemistry for NeuN and Tyrosine hydroxylase (for SNc and ventral tegmental area). We found in both, rats and mice, significant cell death mainly in SNc, in comparison with control tissue and the other brain structures evaluated. Our findings strongly suggest that this Mn mixture specifically damage SNc dopaminergic neurons.

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Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.01/E14

Topic: C.03. Parkinson's Disease

Support: DFG Grant WE5375/1-1

Title: Nigral stimulation desynchronizes cortical theta activity in gait freezers with Parkinson's disease

Authors: *M. A. SCHOLTEN[1,2,3,4], R. B. GOVINDAN[5], C. BRAUN[6], C. PLEWNIA[7], A. GHARABAGHI[3,8], R. KRUEGER[9], D. WEISS[1,2,3];
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Abstract: Introduction Freezing of gait is a debilitating symptom of Parkinson's disease (PD) and constitutes a major unmet therapeutic need. Interleaved deep brain stimulation of the subthalamic nucleus (STN-DBS) and the substantia nigra pars reticulata (SNr) may improve freezing of gait.[1] SNr stimulation alone was described to have a positive effect on axial symptoms.[2] However the mechanism is largely unknown. Here, we characterized cortical activity in PD patients during walking and its modulation with differential neurostimulation therapy. Methods We analyzed eleven PD patients with STN-DBS (7 male, age 62.5 ± 9.7 years). They were instructed to walk at their own comfortable pace fort and back on a straight walkway of nine meters. Patients were measured in three conditions: with stimulation of the STN (STN_On, with stimulation of the SNr (SNr_On) and without stimulation (StimOff). We measured a 48-channel EEG and analyzed cortical activity during straight walking offline. Results Preliminary results show that cortical activity is differently modulated with STN_On and SNr_On. STN_On shows enhanced cortical activity over bilateral supplementary motor area, premotor area and sensorimotor area in the theta frequency band (4-7 Hz) compared to StimOff. Conversely, SNr_On shows attenuated cortical activity over the midline of the frontal, motor and occipital areas in the theta range compared to StimOff. Discussion The effect of SNr stimulation on cortical activity highly differs from STN stimulation showing desynchronization in the theta band during walking over the midline of the frontal, motor and occipital areas. Interestingly, increase of midline cortical activity was identified in transition periods from walking to freezing and during the freezing episode itself.[3] References 1) Weiss D et al., Nigral stimulation for resistant axial motor impairment in Parkinson's disease? A randomized controlled trial. Brain. 2013;136:2098-108. 2) Chastan N et al., Effects of nigral stimulation on locomotion and postural stability in patients with Parkinson's disease. Brain. 2009;132:172-84. 3) Shine JM et al., Abnormal patterns of theta frequency oscillations during the temporal evolution of freezing of gait in Parkinson's disease. Clin Neurophysiol. 2014;125:569-76.

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Poster

133. Gait Disturbances and Freezing

Location: Hall A

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Program#/Poster#: 133.02/E15

Topic: C.03. Parkinson's Disease

Title: Investigation of neuronal circuit responsible for gait

Authors: ***N. FARHANI**[1,2], R. RAJAKUMAR[3], M. S. JOG[4];
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Abstract: Background In advanced stages of Parkinson Disease (PD), gait, balance abnormalities, and impaired control of complex movements emerge. These symptoms are not prominent at early stage of PD despite significant dopaminergic neuronal loss. Gait abnormalities are largely not responsive to levodopa. Therefore, the postural and gait abnormalities are believed to not be a consequence of dopaminergic cell loss. One of the candidates for abnormal gait is the dysfunction in the cholinergic neurons of the Pedunculopontine Nucleus (PPN) located in the midbrain. However, no study has comprehensively investigated the connections between the PPN, other brainstem nuclei and the Central Pattern Generators (CPGs) of the spinal cord. Our aim in this study is to delineate these pathways responsible for gait control. Methods Cholera Toxin β subunit (CTB), was microinjected into the lumbar area of the anterior horn of the spinal cord in rats, neurons in the Gigantocellular Nucleus (GiN) were labeled. Then CTB was injected in the GiN of another group of rats and a group of neurons in the Dorsal Raphe Nucleus (DR) were detected. In the next step, we had three groups of rats: in the first group, cannulas were inserted in PPN bilaterally; in the second and third groups, single cannulas were inserted in DR nucleus and GiN respectively. Lidocaine (0.5µl of 4% solution) was injected through the cannulas in PPN and DR and then rats were placed on a catwalk system and their gait was recorded. In the third group of animals, different receptor agonists and antagonists were injected in GiN and their gait was recorded. Results A.DR neurons send projections down to the GiN and GiN sends projections down to the anterior horn of the lumbar spinal cord where CPGs for gait are located. B.Lidocaine injection in DR, caused a significant increase in body speed variation in rats on the catwalk. C.Lidocaine injection in PPN, did not cause any significant change in different aspects of gait on

catwalk. D. Methiothepin maleate (0.5 µL of 2.5 µM solution), a 5HT1,2, and7 antagonist affects many aspects of gait cycle including speed, body movement variability, stand and swing phases significantly. Conclusion Our results suggest a significant role of serotonergic neurons in DR nucleus and their projections to GiN in gait. This study can be a foundation for future pharmacological studies to identify the role of serotonergic neurons in gait and locomotion.

Disclosures: N. Farhani: None. **R. Rajakumar:** None. **M.S. Jog:** None.

Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.03/E16

Topic: C.03. Parkinson's Disease

Support: NIH Grant AG006457

VA RR&D Merit Review SF 424

KL2 grant KL2TR000152

Title: Freezing of gait in Parkinson's disease: a stopping deficit?

Authors: *K. SMULDERS[1], D. S. PETERSON[1,2], M. M. FLEMING[1], N. PAL[1,2], J. G. NUTT[1], F. B. HORAK[1,2], B. W. FLING[1];
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Abstract: Recent studies suggest that patients with Parkinson's disease (PD) with freezing of gait (FOG) are impaired in response inhibition (e.g. Stroop task, Go-NoGo task). Moreover, neuroimaging studies show that patients with FOG have loss of white matter in nodes that are part of the "stopping" network, comprising the pre-supplementary motor area (preSMA), right inferior frontal gyrus (rIFG) and subthalamic nuclei (STN). This network is particularly important for global stopping of motor actions as assessed in stop signal reaction tasks (SSRT). We evaluated the performance of PD patients with and without FOG on the SSRT and related stopping performance to the structural integrity of the neural stopping network. Methods: 14 patients with FOG (FOG+) and 9 PD patients without (FOG-) completed the SSRT. Probabilistic structural connectivity of the right hemisphere's stopping network was performed to identify quantity and quality of fiber tract connections between 1) preSMA - IFG, 2) preSMA - STN and 3) IFG - STN. Results: There were no significant differences between FOG+ and FOG- patients on the SSRT (p=.467). Also, microstructural integrity of fibers comprising the stopping network

did not differ between FOG+ and FOG- (all p's>.60). Across all participants, we observed a negative association between the SSRT and connectivity quality of the rIFG (r=-.452, p=.030) and preSMA (r=-0.509, p=.013), but not with STN (-.323, p=.133). These significant associations between SSRT and connectivity values were driven by the FOG+ group (r = -0.55, p = 0.04 for both; Fig. 1); there were no significant correlations between SSRT and structural integrity of the stopping network in the FOG- group alone. Conclusion: Our results do not support a global stopping deficit in PD patients with FOG. Similar to previous work in healthy subjects, in patients with FoG+ we report a strong association between inhibitory network structural integrity and SSRT performance. This suggests that integrity of the tracts to/from the rIFG and preSMA is related to the ability to stop a prepared motor action in PD patients with FOG.

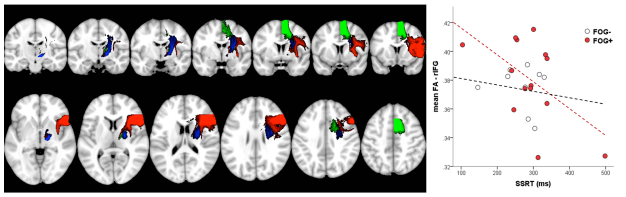

Fig. 1 - Left: Fiber tracts of the stopping network in the right hemisphere. Blue: tracts connecting the STN with IFG and preSMA. Green: tracts connecting the preSMA with STN and IFG. Red: tracts connecting the IFG with STN and preSMA. Right: Correlations between connectivity quality between IFG and performance on SSRT for PD patients with (red filled) and without (white) freezing of gait.

Disclosures: K. Smulders: None. **D.S. Peterson:** None. **M.M. Fleming:** None. **N. Pal:** None. **J.G. Nutt:** None. **F.B. Horak:** None. **B.W. Fling:** None.

Poster

133. Gait Disturbances and Freezing

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Topic: C.03. Parkinson's Disease

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NIA Grant AG08415

Title: Relationship between circadian rhythm disruption and gait initiation impairment in Parkinson's disease

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Abstract: Effective gait initiation is dependent on the appropriate planning and execution of anticipatory postural adjustments (APAs). In patients with Parkinson's disease (PD), APAs may be prolonged, reduced in amplitude, or absent, contributing to impaired gait initiation. In addition to motor symptoms, disturbance of circadian activity rhythm (CAR) is one of the common non-motor symptoms of PD. We investigated whether changes in CAR would influence APAs during gait initiation task. We hypothesized that CAR disruption in PD patients would worsen their ability to appropriately generate APAs during gait initiation in the afternoon compared with the morning. To explore this hypothesis, APAs were compared across two cohorts of subjects (n= 10 for each group): (1) patients with PD, and (2) matched control subjects, at two different time of day (9:00am and 2:30pm). The time between dopaminergic medication intake and gait initiation studies was controlled in PD group. Ground reaction forces (GRFs), center of pressure excursions (CoPs) and tibialis anterior (TA) EMG activity were recorded. Gait initiation tasks included visually cued and self initiated stepping trials. All participants were asked to wear an acti-watch for seven consecutive 24-h periods. Wrist actigraphy was used as a measure of CAR, and two parameters were derived from an extended cosine model: amplitude, which is the distance between the lowest and the highest points of the model, and f-statistic, which is a measure of the goodness-of-fit of the model; higher value of each indicates stronger CARs. Gait initiation studies demonstrated that only in PD group, the amplitude of anterior/posterior and medial/lateral CoP excursions were significantly reduced ($p<0.05$ & $p<0.01$ respectively), the delay in the onset of TA EMG was significantly increased ($p<0.05$), and the duration of the first burst of TA EMG activity was shortened ($p<0.05$), in the afternoon compared with the morning session across all trials. In PD patients, peak loading or unloading GRFs were also reduced markedly in the afternoon but these changes did not reach statistical significance ($p>0.05$). Actigraphy results showed that the CAR f-statistic and amplitude were significantly decreased in the PD group ($p<0.05$), which suggest that the PD group suffered from CAR disruption. Consistent with previous studies, PD subjects demonstrated impaired gait initiation and disrupted CAR. The novel observation of this study was that the PD patients' inability to effectively initiate a gait pattern was significantly worsened in the early afternoon compared with the morning; while the possible effect of medication fluctuation was omitted by study's design.

Disclosures: G. Bachman: None. **J. Stewart:** None. **C. Cooper:** None. **F. Boman:** None. **L. Liu:** None. **S. Ancoli-Israel:** None. **L. Alibiglou:** None.

Poster

133. Gait Disturbances and Freezing

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS035032

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Title: Impaired postural synergies in patients with Parkinson's disease without clinical signs of postural instability

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Abstract: We used the framework of the uncontrolled manifold (UCM) hypothesis to quantify multi-muscle postural synergies in Parkinson's disease (PD) patients without postural instability signs (Hoehn-Yahr, HY stage-II). PD patients and age-matched controls (n = 11 in each group) performed tasks such as quiet standing, cyclic body sway at 0.5 Hz, quick discrete body sway in the anterior-posterior (AP) direction, and releasing a load from extended arms while standing on a force platform. Surface EMG signals were recorded from 13 muscles on the right side of the body. Integrated indices of muscle activation were subjected to principal component analysis with rotation and factor extraction resulting in four eigenvectors in the muscle activation space (four M-modes). Linear regression analysis was used to link changes in M-modes to shifts in the center of pressure in the AP direction (COPAP). Further, a synergy index was computed reflecting the difference between the inter-trial M-mode variance in two spaces, preserving COPAP (UCM) and leading to its changes (ORT). The PD group tested on-drug showed three main differences from the controls: (1) a lower amount of variance in the muscle activation space accounted for by the four M-modes; (2) significantly lower synergy indices; and (3) significantly reduced anticipatory synergy adjustments (ASAs) in preparation to action. In a follow-up study, 10 PD patients (5 in HY stage-II and 5 in HY stage-III) were tested in the morning prior to taking their medication (off-drug) and one hour after medication ingestion (on-drug). Off-drug, the described differences from healthy subjects became significantly larger. In addition, the amount of M-mode variance within the UCM was significantly lower off-drug. This was consistent across the stage-II and stage-III patients. We conclude that analysis of muscle synergies can detect problems in postural control in PD patients before such problems are recognized based on clinical tests. Further, impairments in postural synergies and their feed-forward adjustments (ASAs) are sensitive to dopamine replacement therapy. The reduced ASAs

may be related causally to later appearing signs such as episodes of freezing. Our results emphasize the importance of the basal ganglia in the neural control of stability of movement and posture.

Disclosures: **A. Falaki:** None. **X. Huang:** None. **M.M. Lewis:** None. **M.L. Latash:** None.

Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.06/E19

Topic: C.03. Parkinson's Disease

Title: Bi-hemispheric phase synchronization in subjects with Parkinson's disease during stance, gait and upper limb motor tasks

Authors: ***M. PLOTNIK**[1,4], Y. MIRON[4], S. HASSIN[5,4], O. S. COHEN[5,4], I. BLATT[2], E. CHERNIAK[3], R. INZELBERG[4,3], J. W. KANTELHARDT[6];
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Abstract: Background: Gait and postural disturbances, predominant clinical features of Parkinson's disease (PD), significantly affect patients' quality of life. The neural pathophysiology that underlies walking impairments in PD is not fully understood. The objective of this study was to begin exploring if and how bi-hemispheric cortical phase synchronization (PS), as reflected by electroencephalography (EEG) during free over ground walking, is associated with gait disturbances in PD in comparison to healthy elderly subjects. Methods: We examined 11 patients with idiopathic PD (68 ± 8 y; 3 women) and 7 elderly subjects (64 ± 9 y; 4 women) while performing 1) two min quiet standing; 2) back and forth straight line corridor walking (turns and freezing episodes, if occurred, were omitted from the analysis); 3) alternating and 4) simultaneous hand tapping. EEG signals were recorded with a 32 electrode array (sampling rate 2048 Hz). The Fourier-mode PS method was used to quantify synchronization in periodic cortical activation between the two brain hemispheres (PS ranges from 0 to 1, representing no to maximal synchronization, respectively). The theta (3.9-7.8Hz), alpha (7.8-15.6 Hz) and beta (15.6-31.2 Hz) bands were studied. Non-parametric statistics were used for the analyses. Results: Bi-hemispheric PS was significantly stronger among PD patients versus

control subjects while standing. Mean PS values (± SEM) (grand averaged across the three bands) were 0.55 ± 0.04 and 0.45 ± 0.01, respectively ($p < 0.001$). This difference in bi-hemispheric PS became even larger during walking, mainly due to increased PS values among the PD group, namely 0.61 ± 0.04 for the PD and 0.43 ± 0.01 for the control group, ($p < 0.001$). The mean ratios of the gait PS to the stance PS were 1.13 ± 0.07 and 0.96 ± 0.02 for the PD and control groups, respectively ($p = 0.056$). Alternating and simultaneous hand tapping also led to increased PS, but differed less between the groups ($p \geq 0.098$). In particular, higher PS values were seen in the simultaneous tapping (0.64 ± 0.04) vs. alternating tapping (0.58 ± 0.03) only for the PD group ($p=0.062$). The mean ratios of PS for simultaneous tapping to alternating tapping - PS ratio were 1.10 ± 0.04 for the PD and 1.03 ± 0.03 for the control group ($p=0.35$). Conclusions: Our findings suggest that excessive bi-hemispheric cortical synchronization may contribute, or alternatively be consequential to stance and gait disturbances in PD.

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Poster

133. Gait Disturbances and Freezing

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.07/E20

Topic: C.03. Parkinson's Disease

Support: 7th EU programme V-TIME GA 278169

Title: The effect of motor-cognitive combined rehabilitation on gait performance during dual task in Parkinson's disease

Authors: *E. PELOSIN[1], C. OGLIASTRO[1], G. LAGRAVINESE[1], A. RAVASCHIO[1], A. MIRELMAN[3], J. HAUSDORFF[3], G. ABBRUZZESE[1], L. AVANZINO[2];
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Abstract: Multitasking situations exacerbate gait impairments and increase the risk of falling among people with Parkinson disease (PD). These situations include dual-task paradigm. The dual- task (DT) paradigm highlights the cognitive contribution to gait control. When subjects are asked to walk while performing another task, the change in walking performance indicates the extent of the cognitive demand. The aim of the present study was to assess the effects of a motor-

cognitive combined rehabilitative approach on gait performance during normal walking and dual-task in PD. This study involved 14 PD patients (mean age: 73.3 ±5) with a history of 2 or more falls recruited for the V-TIME study[1]. Motor-cognitive combined rehabilitation consisted of a multi-modal treadmill-training program augmented by virtual reality in order to deal with both the motor and cognitive aspects of fall risk and to promote motor learning[1]. Participants were trained for 12 weeks, 3 times a week. Gait evaluations were performed at baseline (V1), after 6 weeks of treatment (V2), at the end of the 12 weeks training program (V3) and 1 month after the end of the treatment (V4). Gait speed (GS) under single-task and dual-task conditions was measured using an electronic walkway. The dual task required participants to perform a verbal fluency task while walking. Gait data collected at V1, V2, V3 and V4 were entered in a RM ANOVA with VISIT and TASK as main factors. Statistical analysis showed first a significant effect of TASK ($p=0.002$). PD patients exhibited a lower gait speed during dual task with respect to normal gait. However we also found a significant effect of VISIT ($p=0.008$) and post hoc analysis showed that mean gait speed across different conditions reduced already after 6 weeks of treatment ($p=0.045$) and remained lower than baseline at the end of the 12 weeks training program ($p=0.014$) and 1 month after the end of the treatment ($p=0.021$). No difference was found between GS recorded at V2 vs V3 vs V4. We did not found any significant interaction VISIT*TASK. In conclusion, our data showed that a motor-cognitive combined rehabilitation induced an improvement in gait speed in PD patients not only during normal walking, but also when patients are requested to execute a cognitive task during walking (dual task). This latter result may suggest that a multimodal training program is likely to have a beneficial effect on those environmental situations that increase the risk of falling in patients with PD. References Mirelman A et al. V-TIME: a treadmill training program augmented by virtual reality to decrease fall risk in older adults: study design of a randomized controlled trial. BMC Neurol. 2013

Disclosures: **E. Pelosin:** None. **C. Ogliastro:** None. **G. Lagravinese:** None. **A. Ravaschio:** None. **A. Mirelman:** None. **J. Hausdorff:** None. **G. Abbruzzese:** None. **L. Avanzino:** None.

Poster

133. Gait Disturbances and Freezing

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.08/E21

Topic: C.03. Parkinson’s Disease

Support: PSC

FRSQ

Title: Impact of a proprioceptive training program on reaching movement accuracy and postural stability limits in Parkinson's disease

Authors: ***S. BERGERON**[1,3], P. BLANCHET[2,4], D. MONGEON[1], M. BLANCHET[1], M. JEAN-DÉSILETS[1], J. TREMBLAY[1], F. PRINCE[1], J. MESSIER[1,3];
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Abstract: Evidence suggests that the pathophysiology of movement disturbances in PD includes deficits in proprioceptive processing. Several studies indicated that dopaminergic medication either did not normalize or even worsened sensorimotor performance and/or proprioceptive processing in PD. The aim of this study was to examine the impact of a proprioceptive 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 healthy controls (HC) of similar age in a three-dimensional (3D) reaching movements (n=6 PD, n=9 HC) and a postural stability limits tasks (n=6 PD, n=6 HC). In the reaching task, subjects performed 3D reaching movements in two conditions in which the target location and hand positions were 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. Subjects 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 in vision and no vision-proprioceptive conditions. Accuracy 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 reaching errors in the proprioceptive condition ($p<0.05$) as well as significantly smaller limits of stability relative to healthy volunteers in the visual and proprioceptive conditions ($p<0.05$). The proprioceptive training program increased the average level of spatial accuracy of PD patients in both sensory conditions in the reaching task. Furthermore, the exercise program normalized their reaching accuracy in the proprioceptive condition as well as their stability limits in both the visual and proprioceptive conditions ($p>0.05$). Importantly, the size of the postural stability limits of PD patients was significantly greater in the post-test compared to the pre-test ($p<0.05$). These preliminary findings suggest that a proprioception-based training program improves the proprioceptive control of reaching and postural stability limits in PD.

Disclosures: **S. Bergeron:** None. **P. Blanchet:** None. **D. Mongeon:** None. **M. Blanchet:** None. **M. Jean-Désilets:** None. **J. Tremblay:** None. **F. Prince:** None. **J. Messier:** None.

Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.09/E22

Topic: C.03. Parkinson's Disease

Title: Detection of symmetry and asymmetry of gait during split-belt treadmill walking in Parkinson's disease

Authors: *K. SOWALSKY, J. A. ROPER, C. J. HASS;
Univ. of Florida, Gainesville, FL

Abstract: Parkinson's disease (PD) is a neurodegenerative movement disorder with symptoms that present asymmetrically initially and progress bilaterally although one side of the body remains more affected throughout the course of the disease. These asymmetries contribute to increased temporal and spatial gait asymmetry in persons with PD. Motor deficits in PD gait are well known, however the contribution of sensory deficits on gait are unclear. The purpose of this study was to determine the ability to detect symmetry and asymmetry in gait during split-belt treadmill walking in PD and to identify potential differences between the more affected and less affected limb. Five patients with idiopathic PD diagnosed by a fellowship trained movement disorders neurologist were evaluated. Participants were male, 66 ± 4 y.o., and tested in the on-medication state. Self-selected and fast self-selected gait speeds were 0.6 ± 0.1 m/s and 1.0 ± 0.2 m/s respectively. Six conditions began with both belts at either a slow speed of 0.5 m/s, a fast speed of 1.0 m/s, or a split speed with one at 0.5 m/s and the other at 1.0 m/s. Slow and fast speeds were manipulated to increase and decrease respectively by 0.03m/s every 3 strides. The number of speed manipulations ranged from 1 (best score to detect asymmetry) to 17 (best score to detect symmetry). Conditions and results (average number of speed manipulations ± standard deviation) were as follows: 1) increased tied belt speed detection (5 ± 3), 2) decreased tied belt speed detection (1 ± 1), 3) split increase in 1 belt from 0.5 m/s to detection of asymmetry (6 ± 3), 4) split decrease in 1 belt from 1.0 m/s to detection of asymmetry (4 ± 2), 5) split start increase in 1 belt to detection of symmetry (12 ± 3), 6) split start decrease in 1 belt to detection of symmetry (11 ± 3). Paired t-tests revealed more sensitivity to detection of decreased tied belt speed compared with increased tied belt speed (p-value < 0.05). Asymmetric belt speed detection was also more sensitive to decreased speed compared with increased speed (p-value < 0.05). Speed change detection in both increasing and decreasing conditions were more sensitive in tied conditions relative to split conditions. In the split start decrease to detection of symmetry condition, manipulation of the more affected limb revealed a significant difference (p-value <

0.05) relative to the less affected limb manipulation. We conclude that persons with PD are more sensitive to decreases in speed as approaching their self-selected pace than increases in speed as approaching their fast self-selected pace. The sensitivity is greater with manipulation of both limbs versus one. Inter-limb detection differences may also be present.

Disclosures: K. Sowalsky: None. **J.A. Roper:** None. **C.J. Hass:** None.

Poster

133. Gait Disturbances and Freezing

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Topic: C.03. Parkinson's Disease

Support: #CRO112051

NRF-2014R1A2A1A01005128

NRF-2006-2005330

Title: Effects of dual-mode non-invasive brain stimulation on freezing of gait in patients with Parkinson's disease

Authors: *S.-W. KIM, W. CHANG, J.-W. CHO, J.-Y. YOUN, Y. KIM, E. PARK, A. LEE, Y.-H. KIM;
Samgsung Med. Ctr., Seoul, Korea, Republic of

Abstract: Introduction: Freezing of gait (FOG) in Parkinson's disease (PD) would be considered as multiple aspects of motor, cognitive and affective factors. Our previous study demonstrated the favorable effect of repetitive transcranial magnetic stimulation (rTMS) over both the dominant primary motor cortex of lower leg (M1-LL) and the left dorsolateral prefrontal cortex (DLPFC). The aim of this study was to investigate the effects of dual-mode non-invasive brain stimulation (NBS) on FOG using both rTMS and tDCS in patients with PD. Methods: This study was a randomized controlled double-blind study comparing the effect of single vs. dual-mode stimulation. Thirty-two patients (20 males, 12 females) with PD that featured FOG were included and divided into two groups. For the dual-mode stimulation, 10 Hz rTMS over the dominant M1-LL and the anodal tDCS over the left DLPFC were simultaneously applied for 20 mins per session, 5 sessions in a week. For the single stimulation, rTMS were applied at the same manner, however, tDCS were given only during 30 sec. and then fade-off. Behavioral, neurophysiologic and cognitive outcomes were measured before, after, and 1 week after the

intervention by the Timed Up and Go (TUG) test, Standing Start 180° Turn (SS-180° Turn Step/Time) test, Unified Parkinson's Disease Rating Scale (UPDRS) part III, FOG Questionnaire (FOG-Q), motor evoked potential, Korean version of Montreal Cognitive Assessment (K-MOCA), Digit span-Forward/Back and Trail Making-A/B. Results: There were significant improvements in TUG, SS-180° Turn Step, UPDRS part III and FOG-Q in both the dual-mode and the single stimulation group ($p<0.05$). The dual-mode stimulation also resulted in significant improvements in SS-180° Turn Time ($p=0.008$), K-MOCA ($p=0.004$), Digit span-Forward ($p=0.015$) and Trail making-B test ($p=0.010$), whereas single stimulation did not. Furthermore,improvement of Trail making-B test was significantly greater after the dual-mode stimulation than the single stimulation ($p=0.024$). Conclusion: The dual-mode stimulation using the 10 Hz rTMS over the dominant M1-LL and the anodal tDCS over the left DLPFC was more effective than single stimulation for improving FOG in combination with cognitive improvement in patients with PD.

Disclosures: S. Kim: None. **W. Chang:** None. **J. Cho:** None. **J. Youn:** None. **Y. Kim:** None. **E. Park:** None. **A. lee:** None. **Y. Kim:** None.

Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.11/E24

Topic: C.03. Parkinson's Disease

Support: John A. Blume Foundation

Medtronic, Inc. provided the investigational devices only

Title: Effects of low (60 Hz) and high (140 Hz) frequency STN DBS on gait and STN beta band power in Parkinson's disease

Authors: *Z. BLUMENFELD[1], T. E. PRIETO[1], M. H. TRAGER[1], E. J. QUINN[1], A. VELISAR[1], C. H. HALPERN[2], J. M. HENDERSON[2], H. BRONTE-STEWART[1]; [1]Neurol., [2]Neurosurg., Stanford Univ., Stanford, CA

Abstract: Objective: High frequency (HF) subthalamic nucleus (STN) deep brain stimulation (DBS) attenuates resting state STN local field potential (LFP) power in the 13 - 30 Hz (beta) band and improves the cardinal motor signs of Parkinson's disease (PD). There is evidence to suggest that low frequency (LF) STN DBS may improve aspects of gait in certain patients. We

report preliminary data on the effect of LF versus HF DBS on STN beta band power as well as on gait during free ambulatory walking using synchronized neural and kinematic recordings outside of the operating room. Methods: Three PD subjects (six STNs), off-medication at least one month after DBS implantation surgery, performed a free ambulatory walking task without DBS (baseline) and during randomized presentations of bilateral LF (60 Hz) and HF (140 Hz) DBS (2.5 - 3V; PW = 60 μsec). STN LFPs were recorded from electrodes 0 - 2 or 1 - 3 of the DBS lead (model 3389, Medtronic, Inc.) and from the investigational Activa® PC+S system via telemetry (FDA-, IDE-, IRB-, and CA Medicare-approved) while DBS was administered through electrode 1 or 2, respectively. One STN was excluded from LFP power analysis due to band-wide artifact. Lower limb kinematics were recorded using wireless Opal® sensors (APDM, Inc.). Gait cycle time (GCT) and shank angular range (SAR) were calculated in LabVIEW (National Instruments, Inc.) and MATLAB (The MathWorks, Inc.). Results: Both GCT and SAR increased during 60 Hz DBS compared to baseline in all three subjects. Preliminary results from LFP power analysis demonstrate that both 60 Hz and HF DBS attenuated both resting state and movement beta band power in all three subjects. Two subjects demonstrated concomitant beta band power attenuation and improvement in both GCT and SAR regardless of DBS frequency. However, one subject's GCT and SAR were worse during HF DBS than during baseline, whereas the subject's GCT and SAR improved during 60 Hz DBS compared to baseline. Conclusions: Sixty Hz DBS improved these gait measures in all three subjects whereas HF DBS was detrimental to gait in one of the subjects, though both frequencies attenuated STN beta band power. This suggests that attenuation of STN beta band power may not be causal to improving the axial symptoms of Parkinson's disease in certain patients.

Disclosures: Z. Blumenfeld: None. **T.E. Prieto:** None. **M.H. Trager:** None. **E.J. Quinn:** None. **A. Velisar:** None. **C.H. Halpern:** None. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical, Nevro Corp.. F. Consulting Fees (e.g., advisory boards); Intelect Medical, Nevro Corp.. **H. Bronte-Stewart:** None.

Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.12/E25

Topic: C.03. Parkinson's Disease

Support: John A. Blume Foundation

Medtronic, Inc. provided the investigational devices only

Title: Synchronized neural and kinematic recordings during freezing episodes in a stepping in place task in Parkinson's disease

Authors: *M. MILLER KOOP[1], M. H. TRAGER[1], E. J. QUINN[1], A. SRIVATSAN[1], Z. BLUMENFELD[1], A. VELISAR[1], M. MALEKMOHAMMADI[1], C. KILBANE[1], J. M. HENDERSON[2], C. H. HALPERN[2], H. M. BRONTE-STEWART[1];
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Abstract: Objective: The neural correlate of freezing of gait (FOG) is unknown although increased high beta band (21-35 Hz) oscillations in the subthalamic nucleus (STN) have been reported in Parkinson disease (PD) Freezers at rest (Toledo et al., 2013). We analyzed synchronized STN neural and kinematic data during standing and a stepping in place (SIP) task in PD Freezers and non-Freezers. Methods: Five PD subjects (3 Freezers and 2 non-Freezers, none with tremor) off meds and OFF DBS, at least 1 month after bilateral DBS surgery, performed a SIP task on adjacent force plates (Neurocom, Inc.). Synchronized force plate data (normalized to body weight) and STN local field potentials (LFP) (model 3389, Medtronic, Inc.) using the investigational Activa® PC+S system via telemetry (FDA-, IDE-, and IRB-approved) were collected. Freezing episodes (FEs) were detected with a custom algorithm (MATLAB, The MathWorks, Inc.) and were confirmed using synchronized videography. Results: Figure 1 demonstrates synchronized neural and kinematic data during SIP for a Freezer (A) and non-Freezer (B). Power spectral density (PSD) plots comparing the FEs to regular stepping show increased synchrony in both leads in the high beta band during FEs compared to regular stepping (Fig. 1A). Total beta band power was lower during regular stepping compared to standing in both the Freezer and non-Freezer. The PSD during SIP that included FEs showed increased high beta band power compared to that during SIP without FEs. Conclusions: Increased beta band power was evident during standing compared to stepping in place in all patients. There was increased power in the high beta band during SIP that included FEs compared to regular stepping. Future investigation will determine whether any trends observed in these cases can be applied to a larger cohort.

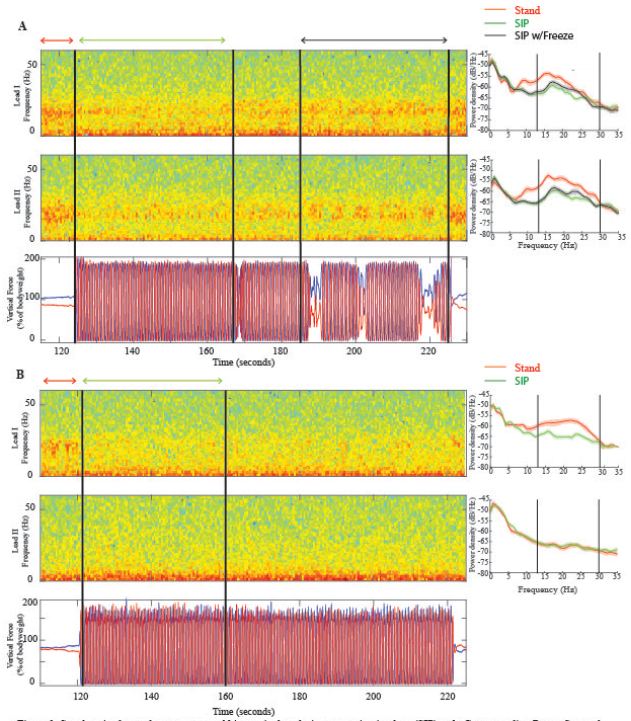

Figure 1. Synchronized neural spectogram and kinematic data during a stepping in place (SIP) task. Corresponding Power Spectral Density (PSD) of the spectograms are shown to the right. (A) Freezer: PSD epochs- stand period is 93-122 s, regular stepping period is 125-166 s and stepping with freezing period is 184-226 s. (B) Non-Freezer: PSD epochs - stand period is 85-117 s, regular stepping period is 120-160 s. Duration of time intervals were selected to maintain consistency in SIP and stand periods between Freezer and nFreezer. Lead II in the nFreezer was not stimulated as the patient does not exhibit PD symptoms on the contralateral body side.

Disclosures: **M. Miller Koop:** None. **M.H. Trager:** None. **E.J. Quinn:** None. **A. Srivatsan:** None. **Z. Blumenfeld:** None. **A. Velisar:** None. **M. Malekmohammadi:** None. **C. Kilbane:** None. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical, Nevro Corp.. F. Consulting Fees (e.g., advisory boards); Intelect Medical, Nevro Corp. **C.H. Halpern:** None. **H.M. Bronte-Stewart:** None.

Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.13/E26

Topic: C.03. Parkinson's Disease

Support: IZKF-255

Title: Subthalamic nucleus activity at gait initiation in Parkinson's disease: report of three cases

Authors: *N. G. POZZI[1], G. ARNULFO[1,3], F. STEIGERWALD[1], V. MALTESE[1], C. PALMISANO[1,4], J. BRUMBERG[2], M. M. REICH[1], E. PAVAN[4], J. VOLKMANN[1], I. U. ISAIAS[1];
[1]Dept. of Neurol., [2]Dept. of Nuclear Med., Uni. Hosp. and Julius-Maximilian-University, Wuerzburg, Germany; [3]Dept. of Informatics, Bioenginering, Robotics and Syst. Engin., Univ. of Genoa, Genoa, Italy; [4]Movement Biomechanics and Motor Control, TBM lab, DEIB-Politecnico di Milano, Milan, Italy

Abstract: Background: Gait initiation is defined as the transient state between standing and walking. It is affected in Parkinson's disease (PD) and its impairment is a hallmark of disease progression. Despite Deep Brain Stimulation of the Subthalamic nucleus (STN DBS) has shown to remarkably improve PD symptoms, its effect on gait and posture is still debated. Actually, the physiological role of STN in gait and posture is largely unknown. For the first time we were able to record STN activity (Local Field Potential, LFPs) during locomotion. **Methods:** LFPs were recorded in three patients by means of the Activa PC+S® (Medtronic® Inc., Minneapolis, USA). We also measured striatal dopaminergic innervation by means of [^{123}I]FP-CIT and SPECT. Gait initiation was defined with motion capture system (SIMI Motion System®, Muenchen, DE) and force plates (Kistler, AG) measurements in meds-OFF and -ON state (L-dopa challenge test). Subjects stood upright and started walking, at a natural (preferred) speed when receiving a visual cue. Four phases were identified: (i) *standing* (ST); (ii) *anticipatory postural adjustment (APA):* form ST to instant of heel-off of the leading foot (HO_{ld}); (iii) *gait initiation (GI):* from HO_{ld} to toe-off trailing foot (TO_{tr}); (iv) *walking*. Beta band was defined (±5Hz to patient frequency peak) and data from each subject were normalized to their *standing* condition. **Results:** The main finding was a beta activity reduction during *GI* compared to *APA* (STN_{right}: -93% *GI* vs. *APA*, -1% *GI* vs. *walking*; STN_{left}: -43% *GI* vs. *APA*, -1% *GI* vs. *walking*). No clear difference was observed with regards of the STN ipsi- or contralateral to the leading foot. Interestingly, the right STN, with greater beta activity reduction, happened to be -in all subjects- ipsilateral to the striatum with less dopaminergic innervation loss. Lastly, we confirmed the effect of L-Dopa in suppressing the beta over-activity in all the gait phases. **Conclusion:** Despite being preliminary and in a limited number of subjects, our data suggest a selective involvement of the STN in different gait phases, corroborating the hypothesis of ß-synchrony reduction in movement initiation. In PD subjects, dopamine depletion may lead to an STN inter-neuronal over-activity, which enforces neuronal synchrony in the ß-frequency range. Accordingly, in our study a greater STN modulation was found ipsilateral to the striatum with greater dopaminergic innervation.

Disclosures: N.G. Pozzi: None. **G. Arnulfo:** None. **F. Steigerwald:** None. **V. Maltese:** None. **C. Palmisano:** None. **J. Brumberg:** None. **M.M. Reich:** None. **E. Pavan:** None. **J. Volkmann:** None. **I.U. Isaias:** None.

Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.14/E27

Topic: C.03. Parkinson's Disease

Support: Canadian Institute of Health Research

Title: Deep brain stimulation: measuring gait parameters objectively using kinematic technologies in Parkinson's disease patients

Authors: *G. GILMORE[1], M. DELROBAEI[2], S. TRAN[2], T. STRATTON[2], M. JOG[3]; [1]Clin. Neurolog. Sci., Western Univ. Hosp., London, ON, Canada; [2]Western Univ., London, ON, Canada; [3]London Hlth. Sci. Ctr., London, ON, Canada

Abstract: Background and Aim: Gait impairments are a hallmark symptom of PD often contributing to patient falls and reducing quality of life. Specifically, it remains unclear whether PD gait improves or worsens with DBS intervention over a long-term. Currently gait changes are monitored using the Unified Parkinsons Disease Rating Scale, which is subjective and qualitative in nature. An objective and quantitative assessment of gait, using kinematic technology, will allow the clinician to more effectively determine whether gait is affected by DBS. Kinematic sensor technologies that are targeted to specifically studying gait are a new avenue being explored to monitor the gait changes with DBS. The goal of the present study is to assess the progression of gait changes, following DBS intervention, over a year long period using kinematically based gait measures. **Methods:** 24 PD participants undergoing bilateral STN-DBS alongside 24 healthy age-matched controls will be used in the study. The participants will be recruited from the Movement Disorder Clinic at the University Hospital in London, Ontario. STN-DBS participants are assessed one week pre-operatively and then up to one year post-operatively. The participants gait is captured using the Zeno walkway and the PKMAS software. The carpet provides spatio-temporal gait parameters, including: stride length, stride width, gait cycle, center of mass/pressure and single/double support time. The study has been approved by the university ethics committee and each participant has signed informed consent. **Results:** 10 of the total patients have completed the 6 month session. Preliminary data has shown an improvement in important areas of gait performance up to 6 months post-operatively. Stride length (Pre-op: *M*=99.87 cm, 6 months: *M*=112.22 cm, z = 2.427, p = .015) and step length (Pre-op: *M*=47.53 cm, 6 months: *M*=59.89 cm, z = 2.501, p = .012.) both increased from pre-operative baseline. Double support time decreased from PD pre-operative baseline (Pre-op: *M*=.30 sec, 6 months: .26 sec, z = -2.259, p = .024). There was no change in stride width (Pre-op: *M*=7.86 cm, 6 months: *M*=8.56 cm, z = .224, p = .823) and cadence (Pre-op: *M*=110.82, 6 months: *M*=112.70,

z = .709, p = .478). **Conclusions:** Preliminary data analysis suggests STN-DBS intervention may improve gait over a 6 month period post-operatively. Further analysis, up to 1 year post-operation, will better elucidate the long-term efficacy of DBS intervention on gait. Our method provides the first objective and quantitative measure of long-term gait variances in PD patients undergoing DBS treatment.

Disclosures: G. Gilmore: None. **M. Delrobaei:** None. **S. Tran:** None. **T. Stratton:** None. **M. Jog:** None.

Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.15/E28

Topic: C.03. Parkinson's Disease

Title: DBS improves gait speed in Parkinson's disease: a meta-analysis

Authors: *J. ROPER[1], J. H. CAURAUGH[1], N. KANG[2], J. BEN[1], M. OKUN[1], C. J. HASS[1]; [1]Univ. of Florida, Gainesville, FL; [2]Univ. of Florida, University of Florida, FL

Abstract: Gait speed is often considered a clinical vital sign. In Parkinson's disease (PD), slow gait speed is significantly related to clinical ratings of disease severity, impaired performance of daily activities, as well as increased overall disability. We conducted a systematic review and meta-analysis that analyzed the effects of deep brain stimulation (DBS) surgery on gait speed in patients with PD to gain fundamental insight into the nature of therapeutic effectiveness. A random effects model meta-analysis on 28 studies revealed a significant overall standardized mean difference medium effect size equal to 0.61 (SE = 0.06; p < 0.0001; Z = 10.80; I2 = 0.00%). Analysis of the 12 studies that tested the effects of DBS on gait speed in participants on medication showed an overall effect of 0.56 (SE = 0.09; p < 0.0001; Z = 6.32; I2 = 0.00%). Analysis of the 16 off medication studies revealed a significant medium effect size (ES = 0.64; SE = 0.09; p < 0.0001; Z = 8.77; I2 = 0.00%). Seven studies reported the effects of unilateral stimulation, and the analysis indicated a significant ES = 0.57 (SE = 0.09; p < 0.0001; Z = 6.14; I2 = 0.00%). Further, analysis of the 21 bilateral DBS studies revealed a significant standardized effect = 0.63 (SE = 0.07; p < 0.0001; Z = 8.89; I2 = 0.00%). Thus, DBS provided simultaneously to both hemispheres shows a slightly higher ES. The effects of DBS on gait speed were similar in the data collection sessions after surgery (n=22) (DBS on vs. off) than data collection before surgery (n =6). Both subgroup analyses indicated significant standardized mean effects: (a) pre-surgery: ES = 0.62; SE = 0.19; p = 0.001; Z = 3.33; I2 = 43.51% and (b) post-surgery data

collection only: ES = 0.62; SE = 0.06; p < 0.0001; Z = 9.95; I2 = 0.00%). Based on our synthesis of the 28 studies, we determined the following: 1) DBS improves gait speed in persons with PD; 2) DBS improved gait speed regardless of whether the patients were tested off medication or on medication; 3) Bilateral DBS led to a slightly higher improvement in gait speed compared to unilateral DBS; and 4) The effects of DBS on gait speed were greater in the data collection sessions after surgery (DBS on vs. off) than comparisons made to gait speed before surgery (before surgery vs. DBS after surgery). In sum, the current analysis provides robust evidence that DBS provides a therapeutic benefit to gait speed in persons with PD.

Disclosures: J. Roper: None. **J.H. Cauraugh:** None. **N. Kang:** None. **J. Ben:** None. **M. Okun:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Royalties for book publications. **C.J. Hass:** None.

Poster

133. Gait Disturbances and Freezing

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 133.16/E29

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Temporal, but not spatial, gait variability is affected in persons with Essential Tremor

Authors: *S. AMANO[1], R. ROEMMICH[3], J. W. SKINNER[4], S. HONG[2], C. J. HASS[4]; [2]Biomed. Sci., [1]Ohio Univ., Athens, OH; [3]Kennedy Krieger Inst., Baltimore, MD; [4]Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL

Abstract: This experiment compared gait patterns of individuals with Essential Tremor (ET, n = 11; 66.0±7.7 years, 175.4±13.1cm, 92.2±23.6kg) and healthy older adults (HOA, n = 12; 63.6±7.8 years, 170.9±7.2cm, 75.7±13.3kg). Kinematic data were collected at 120 Hz using a 10-camera motion capture system while participants walked for 5 minutes on a split-belt treadmill at their preferred speed. Step length was calculated as the distance between ankle markers along the anteroposterior axis at heel-strike, and step time was calculated as the time between contralateral heel-strikes. Entropy of step length and step time were computed on the dominant (D) and non-dominant side (ND) individually to measure the unpredictability of step patterns of each side. Joint Entropy of step length and step time was then computed to measure the combined uncertainty across both limbs, allowing us to compute mutual information. Mutual information was normalized by joint entropy. Independent samples t-tests were performed on the dependent variables with significance set at p=0.05. Persons with ET exhibited significantly

higher step time entropy (D: 3.47±.59, ND:3.53±.58) than HOA (D: 2.64±.27, ND:2.56±.34; both p's<.01) on both limbs. However, step length entropy in the ET group (D: 4.25±.0.25, ND: 4.20±.27) did not differ from the HOA group (D: 4.16±.30, ND: 4.30±.17; both p's>.05). Similarly, persons with ET exhibited significantly higher joint entropy (6.20±.80) and mutual information (0.13±.08) associated with step time than the HOA group (4.86±.45; p<.01, and 0.07±.04; p=.05, respectively), while joint entropy (7.05±.40) and mutual information (0.20±.09) in step length for the ET group did not differ from those for the HOA group (7.21±.25, and 0.17±.03 respectively; both p's>.05). An important finding is that ET seems to affect temporal (step time), but not spatial (step length) variability in gait patterns. These results indicate that stepping patterns are more uncertain in persons with ET if examined at the level of the individual limb. However, higher mutual information values indicate that the entropy in gait patterns is actually shared across both limbs. Thus, while each limb behaves more unpredictably, their collective uncertainty is more restricted than in HOA, perhaps reflecting dedifferentiation of temporal motor patterns. These results are consistent cerebellar deficits (impaired temporal control of movement) in gait in ET.

Disclosures: S. Amano: None. **R. Roemmich:** None. **J.W. Skinner:** None. **S. Hong:** None. **C.J. Hass:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 134.01/E30

Topic: C.03. Parkinson's Disease

Support: NCTR/FDA E7512

Title: Effects of potential therapeutics on the behavior of mice treated with chronic MPTP

Authors: *S. A. FERGUSON, D. LAW, S. SARKAR;
Natl. Ctr. Toxicological Res., Jefferson, AR

Abstract: The chronic MPTP+probenecid (MPTPp) treatment paradigm has been used to successfully model the neurochemical, neuropathological, and behavioral effects associated with Parkinson's Disease. Here, the effects of 6 different potential therapeutic compounds to attenuate MPTPp-related behavioral alterations were assessed. Adult male C57Bl/6 mice (n=6/group) were injected ip with 25 mg/kg MPTP and 250 mg/kg probenecid (MPTPp) or probenecid only twice weekly for a total of 10 injections. Prior to the MPTPp treatment, mice were exposed to one of 6

potential therapeutic agents which continued until the last MPTPp injection: morin (10 mg/kg/day, oral), tetramethylpyrazine (TMP) (20 mg/kg/day, ip), tauroursodeoxycholic acid (TUDCA) (500 mg/kg/day, ip), pomalidomide (15 mg/kg/day, ip), dimethylaminoethylamino-17-demethoxygeldanamycin (DMAG) (2 mg/kg, 3x/week, ip), trehalose (2% in water bottle daily). Thus, there were 8 treatment groups: 1) MPTPp, 2) probenecid only, 3) morin+MPTPp, 4) TMP+MPTPp, 5) TUDCA+MPTPp, 6) pomalidomide+MPTPp, 7) DMAG+MPTPp, and 8) trehalose+MPTPp. Motor coordination and locomotor activity were assessed before (i.e., baseline) and after therapeutic and MPTPp treatment. Body weight was unaffected by any treatment. Motor coordination performance of the DMAG+MPTPp group was superior to the MPTPp group. Locomotor activity was significantly increased in the second post-treatment session in the TUDCA+MPTPp and trehalose+MPTPp groups relative to the MPTPp group. No other potential therapeutic compound produced significant effects. Effects of DMAG on motor coordination are difficult to interpret as baseline performance was also increased. Increased locomotor activity during the second post-treatment session by the TUDCA+MPTPp and trehalose+MPTPp groups might indicate poor habituation. These results suggest that at these doses, there are few effects of these 6 agents on the behavior of MPTPp-treated male mice. (Supported by NCTR/FDA Protocol E7512)

Disclosures: **S.A. Ferguson:** None. **D. Law:** None. **S. Sarkar:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 134.02/E31

Topic: C.03. Parkinson’s Disease

Title: Squamosamide derivative FLZ attenuates α-synuclein-induced neurotoxicity by activating heat shock protein 70 function in transgenic mouse model of Parkinson's disease

Authors: *D. ZHANG, X. BAO;
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Abstract: Parkinson’s disease (PD) is the second most prevalent neurodegenerative disease. The pathologies of PD are progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta and the presence of distinct intracellular inclusion with the major component of α-synuclein, known as Lewy bodies. Subtantial evidences linked aggregated α-synuclein to familial and sporadic forms of PD. Heat shock proteins (HSPs) are highly conserved molecular chaperones, which are crucial to control protein aggregation in neurodegenerative diseases. In

particular, the 70-kDa heat shock protein (HSP70) was shown to interact with α-synuclein, prevent α- synuclein aggregation and protect neurons against toxicity induced by α- synuclein, thus shows neuroprotective effects in PD models. FLZ (formulated as: N-(2-(4-hydroxy-phenyl)-ethyl)-2-(2, 5-dimethoxy-phenyl)-3-(3-methoxy-4-hydroxy-phenyl)-acrylamide, the code name: FLZ), a natural squamosamide derivative from a Chinese herb, has been shown to protect several *in vivo* and *in vitro* PD models in previous studies, and FLZ was reported as HSPs inducer to protect against MPP^+-induced neurotoxicity, but the mechanism remains unclear. In this study, we investigated the possible beneficial effects of HSP70 induced by FLZ treatment in α-synuclein (A53T) transgenic mice and cells. The results showed that FLZ treatment alleviated motor dysfunction, improved dopaminergic neuronal function of α-synuclein transgenic mice. Treatment of FLZ elicited a pronounced reduction of α-synuclein aggregation and toxicity both *in vivo* and *in vitro*. Moreover, FLZ treatment produced a remarkable production of HSP70 protein expression, together with a significant increase in HSP70 transcriptional activity. Notably, inhibition of HSP70 expression by quercetin or HSP70 siRNA markedly attenuated the neuroprotective effects of FLZ. *In vitro* mechanistic study revealed that the inducible effect of FLZ on HSP70 was mediated by its interaction with the co-chaperone of HSP70. FLZ treatment increased the expression of Hip, one co-chaperone of HSP70, and directly bound to Hip, thus up-regulated HSP70 system function. In conclusion, the present study supported that FLZ exerts neuroprotection against α-synuclein -induced neurotoxicity, which is mediated by binding to Hip and up-regulating HSP70 function. Furthermore, this study defined a critical role of HSP70 and its co-chaperones in neurodegeneration, and suggested that particular approach targeting HSP70 may be a potential treatment for α-synuclein related diseases such as PD.

Disclosures: D. Zhang: None. **X. Bao:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 134.03/E32

Topic: C.03. Parkinson’s Disease

Support: Spanish Ministerios de Economía y Competitividad (SAF2013-48532-R)

PNSD #2012/071

ISCIII, CIBERNED (CB06/05/0055)

Comunidad de Madrid ref. S2011/BMD-2336

Title: Pharmacological blockade of dopamine D3 receptor attenuates l-dopa-induced dyskinesia by targeting d1 receptor-mediated striatal signaling

Authors: *O. SOLÍS CASTREJÓN[1,2], J. R. GARCIA-MONTES[1,2], M. XU[3], R. MORATALLA[1,2];
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Abstract: The dopamine D3 receptor (D3R) belongs to the dopamine D2-like receptor family and is principally located in the ventral part of the striatum. However, previous studies have reported an overexpression of D3R in the dorsal striatum following L-DOPA treatment in dopamine-denervated striatum. This has drawn attention to the importance of D3R in L-DOPA-induced dyskinesia (LID). To study the role of D3R in LID, we assessed the effects of PG01037 (D3R-preferring antagonist) on LID in the 6-OHDA mouse model of Parkinson's disease (PD). Mice were treated with L-DOPA (10 mg/kg) followed by PG01037 (10 mg/kg) after 15 min (to evaluate the effect in the expression and development of LID). Axial, limb and orolingual dyskinetic symptoms were evaluated as abnormal involuntary movements and the rotarod test used as a measure of the antiparkinsonian effect of L-DOPA. Alterations in FosB and histone3 (pAcH3) activation were examined by immunohistochemistry 1 h after the last L-DOPA injection. PG01037 treatment to hemiparkinsonian mice decreased dyskinesia development upon chronic exposure to L-DOPA, attenuating dyskinesia once established, without affecting the antiparkinsonian effect of L-DOPA. The expression of FosB and pAcH3 was associated with the development and expression of dyskinesia. Moreover, PG01037 significantly reduced FosB and pAcH3 when co-administered with L-DOPA. We demonstrate that targeting D3R can modify both the behavioral and molecular consequences of L-DOPA treatment. Together, our results demonstrate that D3R modulates the development of dyskinesia by targeting D1R-mediated intracellular signaling and suggest that decreasing D3R activity may help to ameliorate LID.

Disclosures: O. Solís Castrejón: None. **J.R. Garcia-Montes:** None. **M. Xu:** None. **R. Moratalla:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 134.04/E33

Topic: C.03. Parkinson's Disease

Title: Orally-active highly effective fast-onset long-acting dopamine D3/D2 agonists in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated macaque model of Parkinson's disease

Authors: *G. PORRAS[1], H. STARK[2], T. KOTTKE[2], E. Y. PIOLI[3], A. CROSSMAN[3], E. BEZARD[3];
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Abstract: Introduction. Dopamine replacement therapy with L-DOPA or D2/D3 dopamine receptor agonists remains the gold standard treatment for Parkinson's disease. However, both strategies take several minutes to act and are therefore inefficient to alleviate sudden OFF events in fluctuating patients. While the D2/D3 agonists are less dyskinesiogenic than L-DOPA, they also convey lower antiparkinsonian activity. The ideal profile of a dopamine receptor agonist should thus feature (i) an oral administration to ease administration, (ii) a long-acting profile to reduce daily intake, (iii) a fast onset of action to potentially improve OFF events and (iv) an antiparkinsonian activity comparable if not better than L-DOPA. We here present a new dopamine D2/D3 agonist, ST-836, which fulfils these criteria and shows a promising toxicology profile. Methods. Parkinsonian symptoms were produced in 6 fascicularis macaques by daily administration of MPTP (0.2 mg/kg, i.v.). Regular treatment with L-DOPA resulted in the development of dyskinesia. PD motor disability, dyskinesia and on-time were assessed following oral administration of ST-836 (0.1, 1 and 10 mg/kg) in comparison to L-DOPA, ropinirole and pramipexole at therapeutically relevant doses. Results. ST-836 dose-dependently rapidly improved parkinsonian symptoms despite oral administration, decreased disability score comparably to L-DOPA over time, dramatically increased on-time while also increasing the good on time (i.e. with less dyskinesia) compared to L-DOPA but also compared to ropinirole and pramipexole. Conclusions. ST-836, therefore, bears the potential for becoming the first dopamine receptor agonists therapeutically superior to both L-DOPA and existing agonists, requiring in addition a single daily administration.

Disclosures: **G. Porras:** A. Employment/Salary (full or part-time):; Motac. **H. Stark:** None. **T. Kottke:** None. **E.Y. Pioli:** A. Employment/Salary (full or part-time):; Motac. **A. Crossman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac. **E. Bezard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 134.05/E34

Topic: C.03. Parkinson's Disease

Support: Academy of Finland grant #12677612

Title: Nicotine reduces levodopa-induced dyskinesia but not striatal dopamine release in the intrastriatal 6-hydroxydopamine mouse model of Parkinson's disease

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Abstract: Parkinson's disease is a neurodegenerative motor disorder, where the death of dopaminergic neurons of the nigrostriatal pathway results in loss of dopamine in the dorsal striatum. Treatment with levodopa is effective but often complicated by levodopa-induced dyskinesias (LIDs). The mechanisms of LIDs remain unclear and treatment options are sparse. Among promising drug targets for treating LIDs are nicotinic acetylcholine receptors (nAChRs). Preclinical studies have shown that both nicotinic agonists and antagonists can reduce LIDs, and nAChR subtypes expressed on striatal dopaminergic terminals are implicated in these effects (Quik et al. 2013, Biochem Pharmacol 86). In rats the antidyskinetic effect of nicotine is associated with a decrease in nAChR-mediated release of [3H]dopamine (DA) from striatal synaptosomes (Bordia et al. 2013, J Neurochem 125). Antidyskinetic effects of nicotine have therefore been suggested to result from desensitization of striatal nAChRs. We studied the effects of chronic nicotine treatment on LIDs and striatal nAChR-mediated dopamine release in mice with unilateral dopaminergic denervation. Mice were lesioned with intrastriatal injections of 6-hydroxydopamine (6-OHDA), as the antidyskinetic effect of nicotine after intrastriatal lesioning has not been studied previously. After recovery, mice were given daily injections of levodopa (30 mg/kg) and benserazide (12 mg/kg). Nicotine treatment in drinking water (up to 300 μg/ml) was started simultaneously with the levodopa treatment. LIDs were rated weekly using a validated scale for mice (Cenci & Lundblad 2007, Curr Protoc Neurosci 41). nAChR-mediated dopamine release was investigated by measuring nicotine-induced [3H]DA release from synaptosomes prepared separately from dorsal and ventral striata. Chronic nicotine treatment resulted in lower LID severity in the lesioned mice. 5 weeks after lesioning [3H]DA release from the lesioned hemisphere was markedly reduced. After 7-8 weeks of levodopa and nicotine treatment there were no differences in [3H]DA release compared to mice treated with levodopa only. Interestingly, after the chronic drug treatments the release of [3H]DA from the lesioned hemisphere had partially recovered, with the recovery being less prominent in mice treated with both levodopa and nicotine. In conclusion, while chronic nicotine treatment reduced LIDs in mice lesioned with intrastriatal 6-OHDA injections, striatal dopamine release assays showed no decline in nAChR function. These results suggest that the antidyskinetic effect of nicotine in mice may be mediated at least in part through other mechanisms than nAChR desensitization.

Disclosures: **S. Leino:** None. **L. Aaltonen:** None. **S. Peltola:** None. **O. Salminen:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Retos-Colaboración Subprogram RTC-2014-2812-1

Instituto de Salud Carlos III PI13/01390 co-financed by the European Regional Development Fund "A way to build Europe

Title: Early functional changes induced by overexpression of α-synuclein in mouse dopamine and serotonin neurons: new therapeutic opportunities for antisense oligonucleotide treatment of Parkinson's disease

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Abstract: Pathological changes in end-state of Parkinson's disease (PD) are well characterized from postmortem studies. However, there is an urgent need to identify early functional changes to develop new therapeutic strategies stopping the development of the illness. α-Synuclein is a protein that accumulates in the brain of patients with sporadic PD. Likewise, whole locus multiplications and point mutations in the α-synuclein gene cause a familial form of PD. To better understand the sequence of events occurring in PD, we generated a mouse model overexpressing the human wild-type α-synuclein in dopamine (DA) neurons from the substantia nigra (SN) or serotonin (5-HT) neurons from the raphe nuclei (RN). We used an adeno-associated virus type-5 (AAV5)-α-synuclein vector (kindly donated by MJF foundation), unilaterally injected in SN or RN, respectively. AAV5-α-synuclein mice showed increased human α-synuclein mRNA levels in the ipsilateral SN and RN over time which reached to a

maximal of 278 and 290% of sham mice (8 weeks post-infection), whereas the expression of endogenous α-synuclein remained unaltered. Immunohistochemistry analysis confirmed these results showing the presence of increased human α-synuclein and phospho-α-synuclein levels; but, the mice did not display any loss of tyrosine hydroxylase-(TH)-positive DA neurons or tryptophan hydroxylase-(TPH2)-positive 5-HT neurons 16 weeks post-injection. However, impairments in DA or 5-HT release paralleled development of α-synuclein-positive axonal swelling in the striatum, hippocampus and cerebral cortex at 4 weeks post-injection. Hence, local perfusion by reverse dialysis of the depolarizing agent veratridine (50μM) decreased DA and 5-HT release in the striatum of AAV5-α-synuclein mice injected in SN or RN. In addition, the effects of DA and 5-HT reuptake inhibitors nomifensine (1-10-50μM) and citalopram (1-10-50μM), respectively, on striatal DA or 5-HT levels were profoundly reduced in AAV5-α-synuclein versus sham mice. Moreover, these mice showed motor impairments at 8 weeks post-infection into SN and depressive-like behaviors (tail suspension and forced swim tests) at 4 weeks post-infection into RN. Recent data showed that intranasal treatment (30 days) with a conjugated antisense oligonucleotide targeting α-synuclein in DA and 5-HT neurons (ASO1233) reduced α-synuclein expression in these midbrain nuclei. Synaptic DA and 5-HT dysfunctions and axonopathy would thus be the hallmark of early-stage of PD and suggest that ASO targeted α-synuclein in monoamine neurons may lead to new therapies for PD.

Disclosures: D. Alarcón-Arís: None. **M. Galofré:** None. **E. Ruiz-Bronchal:** None. **A. Ferrés-Coy:** None. **A. Montefeltro:** A. Employment/Salary (full or part-time):; nLife Therapeutics, S.L.. **F. Artigas:** None. **I. Fariñas:** None. **M. Vila:** None. **A. Bortolozzi:** A. Employment/Salary (full or part-time):; Andres Montefeltro - Nlife Therapeutics S.L..

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

Location: Hall A

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Program#/Poster#: 134.07/E36

Topic: C.03. Parkinson’s Disease

Support: Nutricia Research

Title: Dietary interventions with strong preventive and therapeutic effects on both motor and non-motor symptoms in a mouse model of Parkinson’s disease

Authors: *L. M. BROERSEN[1,2], P. PEREZ PARDO[2], H. DOUNA[2], E. M. DE JONG[2], N. VAN WIJK[1], A. ATTALI[1], J. GARSSEN[1,2], B. OLIVIER[2], A. D. KRANEVELD[2];

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Abstract: Introduction. Administration of dietary precursors and cofactors for membrane synthesis may enhance synapse formation relevant for neurodegenerative disorders. This approach may be relevant for PD, as preventive treatment with the precursors UMP and DHA reduced rotational behavior in unilateral 6-OHDA model (Cansev2008). The aim of the present study was to test the efficacy of dietary interventions in the rotenone model of PD, inducing both motor and non-motor symptoms, including gastrointestinal dysfunction. In addition, we tested the effects of an extended nutritional formula based on the same precursors. Method. Rotenone exposure in rodents is a frequently used model of PD since it reproduces brain and gut-related pathology and induces both motor and non-motor symptoms. Male C57Bl/6J mice received rotenone (5.4 ug) unilaterally in the striatum. Dietary interventions started either 1 week before (preventive) or 3 weeks after surgery (therapeutic). Readout parameters included behavioral tasks (rotarod, novel object discrimination, grip strength, and inverted screen test) and histological examination of brain and gut. Results. Rotenone induced clear deficits in all parameters, whereas dietary interventions clearly alleviated symptoms. Preventive treatment with precursors improved rotarod performance and intestinal transit time. Therapeutic treatment additionally alleviated rotenone-induced deficits in novel object discrimination, grip strength, and inverted screen test. Furthermore, our extended nutritional intervention was more effective than the diet providing membrane precursors only. Conclusion. This is the first study demonstrating a therapeutic effect of specific dietary interventions in a mouse model of PD. The improved efficacy of the extended nutritional intervention suggests synergies within our specific multi-nutrient approach. Brain and gut histology are currently being investigated.

Disclosures: **L.M. Broersen:** A. Employment/Salary (full or part-time):; Nutricia Research BV. **P. Perez Pardo:** None. **H. Douna:** None. **E.M. de Jong:** None. **N. van Wijk:** A. Employment/Salary (full or part-time):; Nutricia Research BV. **A. Attali:** A. Employment/Salary (full or part-time):; Nutricia Research BV. **J. Garssen:** A. Employment/Salary (full or part-time):; Nutricia Research BV. **B. Olivier:** None. **A.D. Kraneveld:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Program#/Poster#: 134.08/E37

Topic: C.03. Parkinson’s Disease

Title: The therapeutic effects of environmental and physical enrichment on motor behavior in a 6-ohda rat model of Parkinson's disease

Authors: *R. A. WORTMAN[1], A. STAVNEZER[2];
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Abstract: Previous studies have indicated that an enriched environment and exercise are neuroprotective and promote the survival of dopaminergic neurons in the 6-OHDA rat model of Parkinson's Disease (PD). In this study, we demonstrate that exposure to a combination of environmental and physical enrichment improves performance on several behavioral measures. Environmental enrichment was characterized by group housing, in a multi-tiered environment with a variety of toys. Physical enrichment was characterized by forced treadmill exercise at 11 m/min for 20 minutes each day. For the Prevention+Treatment condition, the intervention of environmental and physical enrichment was started 21 days before a right hemisphere substantia nigra 6-OHDA lesion, and was then continued for 21 more days following a one-week recovery period. For the Treatment only condition, the intervention of environmental and physical enrichment was 7 days after a right hemisphere substantia nigra 6-OHDA lesion and continued for 21 days. Both groups demonstrated some improvement on balance beam, rotarod and cylinder task, but the Prevention+Treatment group performed better and was near the No lesion control performance on several tasks. This suggests that the Prevention + Treatment protocol strategy has a greater impact on building cognitive brain reserve, fostering cellular plasticity, and improving motor behavior in the 6-OHDA rat model of PD.

Disclosures: R.A. Wortman: None. **A. Stavnezer:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Topic: C.03. Parkinson's Disease

Support: NIH-NINDS grant R03-NS088502

Rosalind Franklin University

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Parkinson's Disease Foundation

Title: Repeated pharmacological inhibition of the sGC-cGMP signaling pathway is equally effective as L-dopa in ameliorating motor deficits in experimental Parkinsonism

Authors: *V. R. JAYASINGHE[1], B. PHAM[2], V. VERMA[3], R. YOGENDRAN[3], A. R. WEST[3], K. Y. TSENG[2];
[2]Cell. and Mol. Pharmacol., [3]Neurosci., [1]Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: L-DOPA is by far the most effective pharmacological option currently available for treating motor symptoms in Parkinson's disease (PD). However, chronic treatment with L-DOPA often results in the development of side effects termed L-DOPA-induced dyskinesias. Here we compared the therapeutic action of L-DOPA to the potential utility of a non-dopaminergic target in ameliorating motor deficits using the well-known 6-OHDA rat model of parkinsonism and stepping behavior. One such target is the striatal sGC-cGMP signaling pathway, which becomes abnormally upregulated following chronic dopamine depletion. We found that systemic administration of the sGC-cGMP inhibitor ODQ improved stepping performance in 6-OHDA-treated rats over the course of the 7-day treatment period in a dose-dependent manner (5-20 mg/kg/day, i.p.). Notably, the stepping improvement was sustained for up to 72 h following the last treatment, particularly in the 20 mg/kg dose group. Systemic administration of L-DOPA also resulted in comparable dose-dependent improvements in stepping performance over the course of 7 days (2.5 and 5 mg/kg/day, i.p.) to those observed with ODQ. However, approximately 50% of rats that received the 5 mg/kg dose of L-DOPA developed dyskinesias by the end of the 7-day treatment period. Collectively, these results demonstrate that repeated inhibition of the sGC-cGMP signaling is as effective as L-DOPA in improving motor behavior in dopamine-depleted rats with the advantage that it does not induce dyskinesias. Thus, pharmacological downregulation of striatal sGC-cGMP-PKG signaling may be a superior non-dopaminergic therapy for treating motor dysfunction observed in patients with PD.

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Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

Location: Hall A

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Program#/Poster#: 134.10/E39

Topic: C.03. Parkinson's Disease

Title: Evaluation of the effects an implant TiO2DA inserted in the caudate in rat model hemiParkinsonism on motor behavior and its relationship to the levels of catecholamines

Authors: ***A. S. ROJAS**[1], G. VALVERDE AGUILAR[3], M. PALOMERO RIVAS[4], M. VELAZQUEZ PANIAGUA[2], I. SANCHEZ CERVANTES[2], I. LOPEZ MARTINEZ[2], R. MAYEN DIAZ[2], D. VAZQUEZ MATIAS[2], R. GONZALEZ TREJO[2], K. PINEDA ROMERO[2], P. VERGARA ARAGON[2];
[2]Physiol., [1]Fac. of Medicine, Natl. Autonomous Universit, Mexico City, Mexico; [3]CICATA Legaria, IPN., Mexico City, Mexico; [4]Cell. Physiol. Institute, UNAM., Mexico City, Mexico

Abstract: Introduction. Parkinson's disease(PD) is characterized by motor symptoms (rigidity, tremor, postural instability and bradykinesia) and non-motor symptoms: anxiety, depression, chronic fatigue and sleep disorders. Anxiety and depression affect 40% of PD patients mainly associated with three neurotransmitters; dopamine, norepinephrine and serotonin. It has been established that the decrease in dopamine transporters in the mesolimbic pathway structures contribute to anxiety disorder. Currently they are looking for alternative treatments to counteract the symptoms in PD. Objective. Determine the effects that an implant TiO2DA inserted in the caudate in a rat model hemiparkinsonism on motor behavior and its correlation with the levels of dopamine, adrenaline and serotonin. Material and/or methods. Male wistar rats(250-300gr) were used, which were randomly divided into 4 groups: a)control b)injury (Lx); c)Lx+implant; d)implant. Post-injury for 21 days anxiety behavior and locomotor activity of the rats of each group through the open field test was evaluated. The test was conducted in an acrylic box (with transparent walls and floor), whose floor is divided with painted black lines formings squares and iluminated with floodlights. The test was recorded for five minutes, the following measurement parameters were assessed: total distance traveled and the number of crossed lines marked on the floor. The tests were recorded. In each group, determinations adrenaline levels, 5HT and striatal dopamine were performed, by HPLC. Results. In the open field test it was found that the group of rats Lx group had lower locomotor activity relative to the control group rats. The Lx+implant group showed significant changes in locomotor activity compared to the group with Lx group being similar in the control group and the implant group showed an increase in locomotor activity producing a state of hyperactivity. HPLC Lx group showned a decreased DA, A and 5-HT in striatum. Conclusions. Implant placement (TiO2DA) in rats hemiparkinsonian improved locomotor activity due to increased levels of DA in striatum and increased in the concentrations of 5HT in striatum, that correlating with an improvement in the condition of adaptability, characterized by decreased anxiety state.

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Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Topic: C.03. Parkinson's Disease

Support: Agence Nationale de la Recherche ANR-12-BSV4-0001-01

Agence Nationale de la Recherche LABEX BRAIN ANR-10-LABX-43

European Union 7th Generation Framework grant NeuWalk (CP-IP 258654)

Title: The effects of clinically used anti-Parkinsonian drugs on whole-body kinematics in the MPTP-treated macaque model of Parkinson's disease: therapeutic validation of a translational platform for movement disorder research

Authors: *D. W. KO[1], I. VOLLENWEIDER[2], L. BAUD[2], Q. LI[1], G. COURTINE[2], E. BEZARD[3];
[1]Motac Neurosci. Ltd, Greater Manchester, United Kingdom; [2]Ctr. for Neuroprosthetics and Brain Mind Inst., Swiss Federal Inst. of Technol., Lausanne, Switzerland; [3]Inst. of Neurodegenerative Dis., Univ. of Bordeaux, Bordeaux, France

Abstract: Anti-parkinsonian treatment therapies are often developed in preclinical studies that utilise animal models of Parkinson's disease but translation into clinical benefit remains uncommon. A major contributing factor to this predicament is an inconsistency in methodological approaches between preclinical and clinical research. There is a lack of consensus on the most appropriate measures for rating motor performance and defining useful experimental endpoints. In this study, we employ the translational experimental platform of whole-body kinematic analyses in MPTP-treated macaques, the gold-standard model for parkinsonian motor symptoms, for evaluating clinically proven anti-parkinsonian agents. Animals were first trained to perform unconstrained locomotor tasks, following which the effects of pharmacological treatments were tested in the same conditions. The functional impact of drug treatment on gait, posture and limb dysfunction were determined using quantitative biomechanical analyses and objective statistical procedures. These unbiased quantitative analyses of motor function during unrestricted movement execution allowed mechanistic identification of clinically effective treatments against parkinsonian motor disabilities (i.e. L-DOPA, pramipexole, ropinirole, amantadine and istradefylline). The high resolution of these analyses dissociates specific clusters of motor control parameters that are improved from those that remain affected. For example, L-DOPA enhanced movement velocity and stride length

during walking, but had no effect on stance duration or foot elevation. These recording methodologies and analytical tools for assessment of motor control capacities are readily transferable to a clinical setting, offering an efficient and reliable avenue for predicting and evaluating therapeutic benefit of novel treatment strategies. This experimental platform and methodological approach, which has been validated in human patients, bridges the gap between preclinical and clinical research. Together, these tools support (i) greater translational efficacy of novel therapeutic interventions and (ii) objective fine-tuning of existing drug treatments used in patients with parkinsonian or other neuro-motor disorders.

Disclosures: **D.W. Ko:** A. Employment/Salary (full or part-time):; Motac Neuroscience. **I. Vollenweider:** None. **L. Baud:** None. **Q. li:** A. Employment/Salary (full or part-time):; Motac Neuroscience. **G. Courtine:** None. **E. Bezard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac neuroscience.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Topic: C.03. Parkinson’s Disease

Support: NRC funding

Title: Cannabis resin extract in Parkinson’s disease: behavioral, neurochemical, and histological evaluation

Authors: *S. A. EL SHEBINEY;
Narcotics, Natl. Res. Ctr., Cairo, Egypt

Abstract: Levodopa is the first therapeutic strategy for the treatment of Parkinson’s disease (PD) but motor drawbacks associated with long-term use limit its usefulness, emphasizing the search for other agents. The current study aimed at evaluating the modulatory role of cannabis resin extract in a rotenone model of PD based on a clinical report providing evidence for the amelioration of motor deficits in some PD patients by cannabis. Male Swiss mice received rotenone (1.5 mg/kg/48h, s.c., 6 injections). Cannabis resin extract (5, 10, and 20 mg/kg expressed as Δ9-tetrahydrocannabinol, i.p.) or levodopa (25 mg/kg, p.o.) was given daily alone or concomitantly with rotenone for 15 days. Rotenone induced marked PD features, characterized by depressed motor behavior, assessed by wire hanging test. This was

accompanied by decrease in striatal dopamine, norepinephrine, glutathione, adenosine triphosphate (ATP), γ-aminobutyric acid (GABA), paraoxonase 1 (PON1) activity, and tyrosine-hydroxylase immunoreactivity (TH-ir). On the other hand, elevation in glutamate, serotonin, lipid peroxides, nitric oxide, and lactate dehydrogenase (LDH) contents were noticed. Rotenone evoked a memory deficit as recognized by water maze test. Though cannabis resin extract did not affect memory impairment nor histopathological changes induced by rotenone, it improved the motor deficit induced by rotenone exposure at the highest dose level. These effects were associated by restored striatal catecholamines content, as well as decreased oxidative and nitroactive stress markers that were reflected by restored brain LDH, ATP, and PON1 levels. However, cannabis resin extract neither affected TH-ir nor the concentrations of glutamate, serotonin, and GABA. The present work displays that cannabis resin extract ameliorates motor deficits elicited by rotenone, via enhancement of dopamine in the striatum, its antioxidant and antinitrosative properties but without improvement in memory or degenerative changes.

Disclosures: S.A. El Shebiney: None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Support: Michael J Fox Foundation for Parkinson's Research, Rapid Response Innovation Award

Binghamton University, Center for Development and Behavioral Neuroscience

Title: D-512, a novel dopamine D2 / D3 receptor agonist, demonstrates superior anti-Parkinsonian efficacy over ropinirole in Parkinsonian rats

Authors: *D. LINDENBACH[1], S. MEADOWS[1], Y. AVNOR[1], E. NUSS[1], M. MELKHOV-SOSIN[1], L. GROSS[1], N. VILCEUS[1], N. SCHUMAN[1], B. DAS[2], A. K. DUTTA[2], C. BISHOP[1]; [1]Psychology, Binghamton Univ., Binghamton, NY; [2]Pharmaceut. Sci., Wayne State Univ., Detroit, MI

Abstract: Parkinson’s disease (PD) is a movement disorder commonly treated with dopamine receptor agonists, such as ropinirole, which target D2 and D3 receptors. Although symptomatically effective, D2/D3 agonists typically have moderate half-lives and provide little

to no disease-modifying effects. Recently, a novel D2/D3 agonist, D-512, was shown to provide protracted motor activation and neuroprotection in rodent models of PD (Santra et al., 2013, Shah et. al., 2014). Extending upon this work, in the present experiment, D-512 and the clinically-employed agonist ropinirole were directly compared for: degree and length of anti-Parkinsonian effects, propensity to evoke dyskinesia (a drug side effect), and ability to restore dopamine cell viability. To examine these effects, we used the 6-hydroxydopamine lesion rat model of PD. Three weeks after lesion, rats were given daily injections of ropinirole (0.7 - 5.1 μmol/kg), D-512 (1.0 - 8.6 μmol/kg) or vehicle for 22 d and monitored for PD status, spontaneous movement and dyskinetic behavior. The next day, animals were transcardially perfused with formaldehyde for examination of the number of remaining DA cells in the substantia nigra pars compacta. Results show that, compared to ropinirole, D-512 provided a longer duration of motor activation and anti-Parkinsonian benefit. Both drugs elicited similarly mild levels of dyskinesia. Investigations into potential neurorestorative effects of D-512 are ongoing. Our data provide pre-clinical evidence that D-512 is superior to an available dopamine receptor agonist, suggesting further investigations are warranted.

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Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Topic: C.03. Parkinson’s Disease

Support: Faculty of Medicine

Title: Effects of a titanium dioxide dopamine loaded microimplant on Susbtantia Nigra neuron density and rotational behavior in a hemiParkinsonian rat model

Authors: *R. MAYEN DÍAZ[1,2], I. LOPEZ MARTINEZ[3], I. SÁNCHEZ CERVANTES[2], D. VÁZQUEZ MATÍAS[2], E. FRAGOSO ALCALÁ[3], R. GONZALEZ TREJO[2], K. PINEDA ROMERO[2], A. SOLANA ROJAS[2], M. VELAZQUEZ PANIAGUA[2], M. PALOMERO RIVAS[4], M. VALVERDE AGUILAR[5], P. VERGARA ARAGÓN[2];
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Abstract: The purpose for this study was to evaluate the effects produced by microimplant TiO2DA, placed in striatum on a model of hemiparkinsonian rats, over the motor alterations, neuronal density in Substantia Nigra and determine whether exists an association between neuron density and induced spin behavior . Materials and Methods: For this study were used 40 male Wistar rats divided in 4 groups: A) 10 control rats: (Control), B) 10 injured rats by administration of the dopaminergic specific neurotoxin (6-OHDA 8µg/4µl) by stereoscopic surgery in left or right median forebrain bundle: (Lx), C) 10 rats with the same lesion and the colocation of an titanium dioxide implant: (Lx+TiO2DA) , and D) 10 rats only with the colocation of implant: (TiO2DA). 21 days after the injury all the groups underwent to apomorphine induced rotation test (0.05 mg/kg, sc) during 50 minutes to determine damage in dopaminergic system, this test was performed again 360 days after the colocation of the implant. Once finalized the test, the animals were slaughtered to obtain the brains and the neuron density was analyzed by optical microscopy. p values <0.0001 were considered significant. Results: In induced rotation test there were 5 significant results, Lx group showed more spins realized, than implanted and control groups: control vs Lx (4.4±2.6 vs 301.3±19.0), control vs Lx+TiO2DA (4.4±2.6 vs 31.8±14.9), Lx vs Lx+TiO2DA (301.3±19.0 vs 31.8±14.9), Lx vs TiO2DA (301.3±19.0 vs 9.5±7.1), and Lx+TiO2DA vs TiO2DA (31.8±14.9 vs 9.5±7.1). In the assessment of neuron density on the left side there were 5 significant results where the implanted and control groups, which displayed higher density than injured groups: Control vs Lx (8.3±1.5 vs 0.5±0.5), control vs Lx+TiO2DA (8.3±1.5 vs 5.7±1.0), Lx vs Lx+TiO2DA (0.5±0.5 vs 5.7±1.0), Lx vs TiO2DA (0.5±0.5 vs 9.5±1.5), Lx+TiO2DA vs TiO2DA (5.7±1.0 vs 9.5±1.5). On the right side there were 3 significant results where the implanted and control groups had higher neuron density than injured groups: control vs Lx (8.8±1.2 vs 4.8±0.8), Lx vs Lx+TiO2DA (4.8±0.8 vs 7.8±1.5), and Lx vs TiO2DA (4.8±0.8 vs 9.6±3.0). The Pearson correlation between neurons on the left and right side and spins on the induced rotation test showed a negative association: r= -0.9458; r= -0.9641. Conclusion: The microimplant achieves to revese the hemipakinsonims, as observed in our Lx+TiO2DA rats who diminish their number of spins. Likewise, Lx+TiO2DA rats displayed a higher neuron density than injured rats. The correlation analysis reported an inverse relation between neuron density and the number of spins.

Disclosures: **R. Mayen Díaz:** None. **I. Lopez Martinez:** None. **I. Sánchez Cervantes:** None. **D. Vázquez Matías:** None. **E. Fragoso Alcalá:** None. **R. Gonzalez Trejo:** None. **K. Pineda Romero:** None. **A. Solana Rojas:** None. **M. Velazquez Paniagua:** None. **M. Palomero Rivas:** None. **M. Valverde Aguilar:** None. **P. Vergara Aragón:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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LABEX BRAIN ANR-10-LABX-4

Title: Drp1 inhibition amelioratesα-synuclein-mediated neurodegeneration in rats

Authors: *S. BIDO[1], L. ARCURI[1], M. HELLEY[2], R. FAN[2], K. TIEU[2], E. BEZARD[1]; [1]CNRS UMR 5293, Inst. des Maladies Neurodégénératives, Bordeaux, France; [2]Plymouth Univ., Plymouth, United Kingdom

Abstract: In Parkinson's disease the α-synuclein (α-syn) accumulation in the dopaminergic neurons can overburden the clearance mechanisms, increase production of reactive oxygen species, and induce imbalanced mitochondrial dynamics, thus eliciting the necrosis/apoptosis pathways that finally culminate in cellular death. Fusion and fission play an important role in the regulation of mitochondria dynamics. The disruption of this equilibrium can lead to the induction of apoptosis/necrosis pathway. It has been reported that inhibition of the mitochondrial fission dynamin-related protein 1 (Drp1) has protective effects in different *in vitro* and in-vivo models with mitochondrial dysfunction. In order to investigate whether inhibition of Drp1 could be effective in reducing the neurotoxicity caused by the overexpression of α-syn, we used rats overexpressing the mutated form of α-syn (A53T) specifically in the substantia nigra pars compacta (SNc) and treated them chronically with the Drp1 inhibitor mdivi-1. After eight weeks of daily injection of mdivi-1 (twice a day) or vehicle, we sacrificed the animals and processed the brain for the immunohistochemistry assays. Rats that received mdivi-1 displayed a significant reduction of the phenotype and neurotoxicity induced by A53T compared with the vehicle treated animals. The mechanisms of this neuroprotection are being investigated. Using ecdyson-inducible dopaminergic neuronal cells, mdivi-1 improved mitochondrial function 48h after α-syn expression. Furthermore, because mdivi-1 blocks Drp1, which induces apoptosis, we try to understand which necrotic/apoptotic pathway is mostly regulated by the mitochondrial fission/fusion process. With this study we provide new insights on the relationships occurring between mitochondria physiology and α-syn accumulation, thereby providing an already-exploitable pharmacological tool to counteract the neurodegenerative process.

Disclosures: S. Bido: None. **L. Arcuri:** None. **M. Helley:** None. **R. Fan:** None. **K. Tieu:** None. **E. Bezard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac neuroscience.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Title: Therapeutic potential and antidyskinetic activity of the combined antagonism of adenosine A2A receptors and stimulation of 5HT1A/1B receptors in models of Parkinson's disease : acute and chronic studies

Authors: *A. PINNA[1,2], G. COSTA[2], N. SIMOLA[2], E. TRONCI[2], M. CARTA[2], M. MORELLI[2];
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Abstract: Recent report demonstrated that mixed serotonin 5-HT1A/1B receptor agonist, eltoprazine, produces a near to full suppression of dyskinetic-like behaviors in animal models of Parkinson’s disease (PD). However, eltoprazine resulted in a partial reduction of motility induced by L-dopa, both in rodents and in non-human primates. Moreover, in a recent clinical trial, the partial 5-HT1A agonist sarizotan has been found to be only partially effective. Preclinical and clinical studies showed that adenosine A2A receptor antagonists as preladenat, significantly increase L-dopa efficacy in PD, without exacerbating dyskinetic-like behaviors. On this basis, we hypothesize that combination of eltoprazine with preladenant may produce prevention or suppression of L-dopa-induced dyskinesia, without impairing the efficacy of L-dopa in relieving motor symptoms. Unilateral 6-hydroxydopamine-lesioned rats, L-dopa-naïve or rendered dyskinetic by repeated-L-dopa-treatment, were administered with eltoprazine (0.3 or 0.6 mg/kg) and preladenant (0.3 or 1 mg/kg), alone or in combination with L-dopa (4 or 6 mg/kg), and rotational behavior, as index of motility, and abnormal involuntary movements (AIMs) as index of dyskinesia, were evaluated. Results show that combined administration of L-dopa (4 mg/kg) plus eltoprazine (0.6 mg/kg) plus preladenant (0.3 mg/kg) significantly prevented or reduced dyskinetic-like behaviors, as revealed by AIMs test without impairing the motor activity, as revealed by similar number of contralateral and ipsilateral rotations. Moreover, acutely, the combined treatment appears to prevent worsening of the motor performance induced in L-dopa-naïve animals by eltoprazine plus L-dopa in the adjusting step test and the initiation time of stepping, two tests with high predictive validity of PD associated motor disability. Analogous results were obtained with the vibrissae-evoked forelimb placing test, which is used to specifically evaluate sensory-motor impairment. Overall these data suggest that combination

of L-dopa (4mg/kg) with eltoprazine (0.6mg/kg) and preladenant (0.3mg/kg) could be a new therapeutic strategy for treating motor symptoms and dyskinesia in PD.

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Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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PAPIIT Grant IN204715

Title: Extremely low frecuency magnetic fields exposition decreases L-DOPA induced-dyskinesias in a rat model of Parkinson's disease

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Abstract: Parkinson's disease (PD) is a frequent and incapacitating neurological disorder. Dopamine (DA) precursor molecule, 3,4-dihydroxyphenyl-L-alanine (L-DOPA) is currently the most effective pharmacotherapy for PD. However, the prolonged use of L-DOPA along with the progressive degeneration of nigrostriatal pathway, induces the development of incapacitating movement complications known as L-DOPA-induced dyskinesia (LID). Mechanisms underlying LIDs in PD are not fully understood and its medical treatment is generally unsatisfactory. Recently, extremely low frequency magnetic fields (ELF-MF) exposure has been proposed as a non-invasive therapy for PD due to the effects it has on motor system. Several studies suggest that motor effects of ELF-MF exposure might be due to a dopamine system modulation. To the best of our knowledge, this technique has not been explored as a possible therapy for LID. Thus, the aim of the present study was to investigate whether ELF-MF exposure decreases LIDs in a

Parkinson's rat model. In addition, we studied the ELF-MF effects on the expression of FosB in the dorsal striatum as a histological marker for LIDs. Our results showed that global LIDs score significantly decreased after ELF-MF exposure compare to sham-exposure in 6-OHDA lesionaded rats. In order to evaluate changes before ELF-MF, exposure was put off one week. When ELF-MF exposure ceased for a week, LIDs manifestation increased again to a similar value of control group. Afterwards, rats were treated with ELF-MF for a second time, consequently LIDs score significantly decreased compare to sham-exposure. In contrast, no modification of LIDs scores with sham-exposure after the first evaluation was display. LIDs manifestation's decrease was accompanied by a low expression of FosB in the dorsal striatum. Therefore, exposure to these magnetic fields may be an alternative as a non-invasive therapy for LIDs. Nevertheless, further research is needed to elucidate its mechanisms of action.

Disclosures: **M. Ramirez-Lopez:** None. **D. Millán-Aldaco:** None. **M. Palomero-Rivero:** None. **M. Guerra-Crespo:** None. **R. Drucker-Colín:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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PAPIIT Grant IN204612

Title: Comparison between chromosphere and chromaffin grafts in a 6-OHDA rat model of Parkinson's disease

Authors: ***A. BORONAT-GARCÍA**, M. PALOMERO-RIVERO, D. MILLÁN-ALDACO, M. GUERRA-CRESPO, M. N. ZARIÑANA-CAMACHO, R. DRUCKER-COLÍN;
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Abstract: Chromaffin cells, the catecholamine-releasing neuroendocrine cells from the adrenal medulla, and sympathetic neurons, derive from a common fate-restricted sympathoadrenal progenitor. Due to their neuron-like properties and their capacity to synthetize and release

dopamine, chromaffin cells have been employed in Parkinson´s disease cell replacement therapy studies, in which their grafting produced a gradual improvement of functional motor deficits in parkinsonian animal models. However, these cells have not yielded the expected motor results as a consequence of poor graft survival. Recently, Enhart-Bornstein and coworkers developed an isolation and cell-culture protocol, which permits the expansion of chromaffin progenitor-like cells from adult bovine and human adrenal medulla. Under low-attachment conditions, these cells aggregate and grow as spheres, named chromospheres. Chromosphere-cells have the ability of self-renewal and express several sympathoadrenal and neuronal progenitor cell markers. We found that in chromosphere cell cultures there is an increase in cells with dopaminergic phenotype, together with an increase in dopamine release relative to chromaffin cells. Here, we explored the possibility of chromospheres being a better cell source for decreasing motor alterations (evaluated with amphetamine-induced turning behavior), and also having a better survival than chromaffin cells in a 6-OHDA rat model of Parkinson´s disease. Animals with chromaffin grafts showed a 38% decrement in turn-number during the first evaluation, similar to chromosphere-grafted animals. However, the decrement in turn number over the rest of the analyzed time points did not show a significant reduction compared with lesioned animals without graft, whereas chromosphere grafted animals showed a significant reduction of 52% over the 3 months post-grafting that were evaluated. In addition, chromosphere grafts showed a significant 7-fold higher survival than chromaffin cell grafts, with an average number of TH+ cells of 6,441 in chromosphere grafts and 868 in chromaffin grafts. These data suggest that when compared with chromaffin cells, chromosphere grafts have a higher survival and give rise to a better motor improvement.

Disclosures: **A. Boronat-García:** None. **M. Palomero-Rivero:** None. **D. Millán-Aldaco:** None. **M. Guerra-Crespo:** None. **M.N. Zariñana-Camacho:** None. **R. Drucker-Colín:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Topic: C.03. Parkinson’s Disease

Title: Investigation of the therapeutic effects of J-147 in two animal models of Parkinson's disease

Authors: *M. KOPYNETS[1,2];
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Abstract: Human idiopathic Parkinson's disease (PD) is a progressive motor system disorder caused by the extensive depletion of dopaminergic neurons in the nigrostriatal pathway. PD affects approximately one million people in the United States. Here, we tested the therapeutic potential of the neuroprotective compound J147 in two well-established rodent models of PD. J147 was developed on the basis of preventing nerve cell death in a wide range of brain toxicities associated with old age, and it is about to start clinical trials for the treatment of Alzheimer's disease. The hypothesis is that J147 should have therapeutic efficacy in other disorders where nerve cells die. One Parkinson's model was generated by the unilateral injection of 6-hydroxidopamine (6-OHDA) into the striatal pathway, and the other by the intraperitoneal injection of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP). Levels of the neurotransmitter dopamine were assayed by HPLC. Tyrosine hydroxalase (TH) and inflammation markers were determined by Western blotting. Followed by the studies of behavioral changes between the J147-treated and drug-untreated groups. The obtained data showed that J147 treatment reduces the loss of TH-positive neurons, decreases inflammation, and ameliorates the dopamine depletion following the 6-OHDA and the MPTP treatments. In addition, J147 improved some of the behavioral motor deficits assessed by the rotometer and tremor tests. These results plus the outstanding pharmacological properties of J147 suggest that J147 has the potential for the clinical treatment of Parkinson's disease.

Disclosures: M. Kopynets: None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS045926

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Title: Remote control of transplanted human dopamine neurons in Parkinson's disease mice

Authors: *Y. CHEN, M. XIONG, Y. DONG, S.-C. ZHANG;
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Abstract: Cell therapy using human stem cell-derived neurons represents one of the most promising options for treating neurological diseases. Yet, regulation of grafted human cell function in a neural circuitry and hence therapeutic outcomes by non-invasive means has not been achieved. By engineering human pluripotent stem cells to express active or inhibitory form of GPCRs, we found that the function of differentiated midbrain dopamine (DA) neurons was precisely regulated *in vitro* by the pharmacologically inert compound clozapine-N-oxide (CNO). By transplanting the DA neurons into the striatum of Parkinson's disease mice, we found that the mouse behavioral recovery was reversed in animals that received the inhibitory form of GPCR-expressing DA neurons or enhanced in those that received active form of GPCR-expressing DA neurons, upon CNO treatment. By brain slice recording, we found that activation of the grafted DA neurons increased sEPSCs in host striatal neurons, which was abolished by a D1 receptor antagonist, suggesting modulation of glutamatergic synaptic transmission onto striatal GABA neurons in the host striatum. Our findings raise the prospect of human stem cell therapy for neurological diseases by rebuilding and/or modulating neural circuitry.

Disclosures: Y. Chen: None. **M. Xiong:** None. **Y. Dong:** None. **S. Zhang:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Topic: C.03. Parkinson's Disease

Support: Michael J. Fox foundation

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Dept. of Veterans Affairs

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Title: Thiol-repletion therapy for Parkinson's disease guided by *ex vivo* assessment of neuronal anti-oxidant capacity and human CSF analyses

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Abstract: Parkinson's disease is a common neurodegenerative disorder with both genetic and environmental causes. The disorder most prominently affects dopaminergic neurons involved in motor control. Both human and animal studies indicate that this dopaminergic neuronal population is particularly vulnerable to oxidative stress. Glutathione is the most abundant thiol antioxidant in neurons, and in Parkinson's disease glutathione depletion precedes death of these neurons. Pharmacological repletion of glutathione is, therefore, a potential approach for slowing disease progression. We developed a method for evaluating approaches for restoring glutathione content in neurons. The method involves use of the EAAC1$^{-/-}$ transgenic mouse, which has reduced glutathione content selectively in neurons due to a defect in neuronal cysteine transport and a resultant age-dependent loss of dopaminergic neurons. Neuronal glutathione content was evaluated by immunohistochemical quantification of neuronal GSH in brain sections. In parallel, the capacity of the neurons to scavenge reactive oxygen species (ROS) was assessed in ex-vivo brain sections by monitoring the formation of nitrotyrosine during incubation with SIN-1. Neurons in the EAAC1$^{-/-}$ mice showed substantially reduced glutathione content and reduced ROS scavenging capacity relative to wild-type mice. EAAC1$^{-/-}$ mice treated orally with a membrane-permeable analogue of cysteine, N-acetyl cysteine (NAC), showed near-normalization of both neuronal glutathione content and reactive oxygen species scavenging capacity, and a much-attenuated loss of dopaminergic neurons. The cerebrospinal fluid NAC concentration associated with this biological effect was less than 150 nM, thus establishing a dosing target for use in humans. In a parallel study, human subjects with Parkinson's disease were given NAC at doses ranging from 7- 70 mg/kg twice daily. Doses above 35 mg/kg achieved CSF concentrations well above 150 nM. A clinical trial of NAC in Parkinson's disease has been funded on the basis of this pre-clinical work.

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Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Support: Northeastern University Tier 1 Interdisciplinary Grant

Michael J. Fox Foundation for Parkinson's Research

Title: Intranasal hGDNF DNA nanoparticles induce transfection and protein expression by perivascular cells throughout rat brain

Authors: *A. E.-E. ALY[1], B. T. HARMON[1], L. PADEGIMAS[2], O. SESENOGLU-LAIRD[2], M. J. COOPER[2], B. L. WASZCZAK[1];
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Abstract: The therapeutic potential of glial cell line-derived neurotrophic factor (GDNF) has been limited thus far by its inability to cross the blood-brain barrier. We are investigating the intranasal route of administration of PEGylated lysine 30-mer (PEG-CK30) DNA nanoparticles (NPs) encoding GDNF. These NPs, developed by Copernicus Therapeutics, Inc., compact a single molecule of expression plasmid, have a minimum diameter of 10 nm, and provide a non-immunogenic, non-viral vector for CNS gene therapy. We have previously demonstrated that DNA NPs expressing either enhanced green fluorescent protein (eGFP) alone, or eGFP linked with hGDNF (pUGG), transfect cells *in vitro* and brain cells *in vivo.* We further showed that intranasal administration of Copernicus' pGDNF NPs provides neuroprotection of substantia nigra (SN) dopamine neurons in the rat 6-hydroxydopamine model of Parkinson's disease (PD). The goals of the current study were to determine the regional transfection pattern and assess which cell type(s) were transfected 7 days after intranasal administration of these hGDNF DNA NPs. eGFP immunohistochemistry (IHC) was performed on brain sections from rats that received intranasal pUGG NPs, naked pUGG, or saline. The number of fluorescent cells was significantly higher across brain regions in rats given intranasal pUGG NPs compared to saline controls, with the highest number of eGFP+ cells in the midbrain. Double-label IHC was also carried out for eGFP and a cell specific marker, i.e. either rat endothelial cell antigen (RECA-1), glial fibrillary acidic protein (GFAP), the neuronal marker NeuN, or the dopamine neuronal marker tyrosine hydroxylase (TH). Most of the eGFP+ cells found in brain 7 days after intranasal delivery of pUGG NPs were abluminal and immediately adjacent to capillary endothelial cells staining for RECA-1. eGFP+ cells were often found contiguous to GFAP+ astrocytic endfeet enwrapping capillaries, recapitulating their perivascular localization. When eGFP+ cells were observed adjacent to TH+ neurons in the SN, or to NeuN+ cells in any brain region, these neurons were also located within 15 μm of a capillary. Collectively, these results indicate that transgene expression occurred primarily in cells lining the vasculature, most likely pericytes. This is consistent with distribution of intranasally administered agents by perivascular transport. Ongoing studies will examine the dose-response relationship and the time-course of protein expression after intranasal delivery of Copernicus' hGDNF DNA NPs. These studies will determine an optimal dosing regimen for future development of this intranasal pGDNF gene therapy for PD.

Disclosures: A.E. Aly: None. **B.T. Harmon:** None. **L. Padegimas:** A. Employment/Salary (full or part-time):; Copernicus Therapeutics Inc. **O. Sesenoglu-Laird:** A. Employment/Salary (full or part-time):; Copernicus Therapeutics Inc. **M.J. Cooper:** A. Employment/Salary (full or part-time):; Copernicus Therapeutics Inc.. **B.L. Waszczak:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Topic: C.03. Parkinson's Disease

Support: DFG GSC-4

Title: Role of Growth Differentiation Factor-15 in the 6-hydroxydopamine mouse model of Parkinson's disease

Authors: *K. UNSICKER[1,3,4], V. MACHADO[2], B. SPITTAU[2], K. KRIEGLSTEIN[2], S. J. P. HAAS[3], A. WREE[3];
[2]Dept. of Anat. and Cell Biol., [1]Univ. of Freiburg, Freiburg, Germany; [3]Dept. of Anat., Univ. of Rostock, Rostock, Germany; [4]Spemann Grad. Sch. of Biol. and Medicine, Univ. of Freiburg, Freiburg, Germany

Abstract: Growth Differentiation Factor-15 (GDF-15) is a novel member of the transforming growth factor-beta cytokine superfamily. It is one of the most divergent members of this family and is widely expressed in liver, lungs, heart, kidney and exocrine glands. At lower levels, GDF-15 mRNA and protein are ubiquitously found in the CNS, its site of highest expression being the choroid plexus, which secretes the protein into the cerebrospinal fluid. GDF-15 has been shown to be significantly upregulated in lesioned neurons and in activated microglia, suggesting that it may have roles in the lesioned CNS. These results indicate that the molecule may either function in the execution of survival or cell death promoting mechanisms or might be involved in the regulation of microglia activation. Microglia, the resident immune cells of the brain, are known to infiltrate sites of neuronal damage and inflammation. It has been observed that microglia also produce GDF-15, and hence, it is crucial to study the relation and interactions of this molecule. In our study, we used the unilateral 6-hydroxydopamine (6-OHDA) Parkinson mouse model and compared Gdf-15 +/+ and Gdf-15 -/- mice. Using quantitative real-time PCR, we revealed that GDF-15 mRNA levels increase at 2d post-lesion. mRNA levels of inflammatory markers such as TNF-a, iNOS and IL-6 were significantly higher upregulated in Gdf-15 -/- mice. Finally, Gdf-15 -/- mice exhibited decreased midbrain dopaminergic neuron survival and lesser microglia numbers post-lesion. In addition to the *in vivo* studies, we addressed the molecular cues underlying neuron death and the involvement of microglia in the 6-OHDA model by using Gdf-15 +/+ and Gdf-15 -/- mixed neuron-glia and neuron cultures from the ventral midbrain, as well as primary microglia cultures. Exogenous GDF-15 application enhanced survival of midbrain dopaminergic neurons in neuron cultures, but not in mixed neuron-glia cultures, indicating a

direct neurotrophic supportive role for GDF-15 in a 6-OHDA lesion paradigm. Dopaminergic neurons in the mixed neuron-glia cultures had better survival rates as compared to the neuron cultures, as well as elevated IL-6 levels, pointing to a glia-mediated protective effect in this culture model. Taken together, our results indicate that GDF-15 is essential for the survival of midbrain dopaminergic neurons and that its loss impairs the inflammatory response of Gdf-15 -/- mice, following 6-OHDA administration. This study was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) as well as the Excellence Initiative of the DFG (GSC-4, Spemann Graduate School)

Disclosures: **K. Unsicker:** A. Employment/Salary (full or part-time):; Institutes of Anatomy and Cell Biology, Universities of Freiburg and Rostock, D-79104, Freiburg, D-18055 Rostock, Germany. 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.; Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, D-79104, Freiburg, Germany. **V. Machado:** None. **B. Spittau:** None. **K. Krieglstein:** None. **S.J.P. Haas:** None. **A. Wree:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

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Topic: C.03. Parkinson’s Disease

Support: CONACYT 154131

PAPIIT: IN202814

PAPIIT: IN202914

IMPULSA03

Title: AntiParkinsonian drug actions in the striatal microcircuit

Authors: *E. LARA-GONZALEZ, S. CRUZ, M. DUHNE, J. BARGAS;
Univ. Nacional Autónoma de México, México, D.F, Mexico

Abstract: There have been many biochemical, physiological and behavioral studies on pathophysio-logical models of Parkinson's disease. However, L-DOPA is still the drug of choice to treat the disease and the development of drugs such as “dopamine agonists” have not been

tested and compared with L-DOPA in preparations that preserve the workings of the striatal circuit. We used dynamic calcium imaging techniques to record the activity of dozens of neurons simultaneously with single cell resolution. As previously described, the striatal circuitry exhibits time windows with spontaneous co-activation of neurons (network states) that show recurrence, alternation among states and reverberant behavior (Carrillo-Reid et al. 2008). This circuit behavior changed in the Parkinsonian circuit-depleted of dopamine (Jaidar et al. 2010; Plata et al. 2013) revealing an obstruction of reverberant behavior, hy-peractivity, and excessive recurrence of the same state which becomes dominant. The application of L-DOPA eliminated hyperactivity returning the circuit to normal activity. Apormorphine returned the circuit to normal reverberant activity but does not completely eliminate hyperactivity. Pramipexole and ropinirole which mainly act on D2-class receptors had similar actions as L-DOPA. These results show that calcium imaging on striatal slices work as a valid bioassay to test the action of antiparkinsonian drugs.

Disclosures: **E. Lara-Gonzalez:** None. **S. Cruz:** None. **M. Duhne:** None. **J. Bargas:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 134.25/F6

Topic: C.03. Parkinson’s Disease

Title: Boosting of tyrosine hydroxylase activity as a treatment for Parkinson’s disease

Authors: L. P. VAN DER HEIDE, *J. A. VAN HOOFT, M. P. SMIDT;
Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Parkinson’s disease is the second most common neurodegenerative disorder after Alzheimer’s disease. The main features of Parkinson’s disease (PD) are bradykinesia, progressive rigidity, tremor, and loss of postural stability. The clinical manifestations are mainly caused by a loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the midbrain, resulting in a loss of input to the striatum. It is estimated that at the onset of clinical symptoms more than half of the dopaminergic neurons in the SNpc has been lost. Currently, there is no cure for PD. Most therapeutic approaches aim to replenish dopamine levels by supplementation or attenuating dopamine turnover. The dopamine precursor L-Dopa is most frequently used to relief PD motor symptoms. However, L-Dopa treatment is far from ideal as it creates non-physiological situations both within and outside of the dopaminergic circuitry. In the present study, a symptomatic treatment paradigm for PD is proposed that opposes the striatal

dopamine depletion in a more physiological and specific way, namely by acting on tyrosine hydroxylase (TH), the rate-limiting step in dopamine biosynthesis. The activity of the enzyme TH is strictly controlled to maintain dopamine levels, with the post-translational phosphorylation of TH being the principal short-term regulatory mechanism. TH phosphorylation on Ser40 increases its catalytic activity and thereby the rate of dopamine synthesis, whereas Ser31 is suggested to be required for TH stability and subsequent Ser40 phosphorylation. TH Ser40 phosphorylation levels are low under nonstimulated conditions, whereas they are high for Ser31 as detected in MN9D dopaminergic cells. We find that stimulation of MN9D cells with forskolin (an activator of PKA) leads to an increase in Ser40 phopshorylation, but surprisingly leads to a reduction in Ser31 phosphorylation. Inhibition of ERK1/2 signaling under non-stimulated conditions also reduced phopshorylation of Ser31, but not Ser40. Further investigation into the crosstalk between the Ser40 kinase and Ser31 kinase suggest that forskolin-activated PKA leads to an inhibition of ERK1/2 signaling via phosphorylation of Raf on Ser259, leading to Raf inhibition and failure to acivate ERK1/2. Crosstalk between PKA and ERK1/2 pathways is sugested to tightly control the extent of TH phosphorylation and activity and be a manipulatable target to boost TH activity.

Disclosures: **L.P. van der Heide:** None. **J.A. Van Hooft:** None. **M.P. Smidt:** None.

Poster

134. Therapeutics of Parkinson's Disease: Preclinical Studies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 134.26/F7

Topic: D.17. Voluntary Movements

Title: Anodal transcranial direct current stimulation enhances survival and integration of dopaminergic cell transplants in a rat Parkinson’s disease model

Authors: *B. FRITSCH, J. REIS, N. HOFFMANN, C. MÜNKEL, A.-K. GELLNER, H. HULSHOF, M. DÖBRÖSSY, L. FURLANETTI, J. GARCIA, C. WINKLER;
Univ. of Freiburg/ Neurocenter, Freiburg, Germany

Abstract: Background: Restorative therapy concepts such as fetal ventral mesencephalic cell transplantation aim to restitute impaired neurotransmission in neurodegenerative diseases. However, new strategies to enhance grafted cell survival and accelerate graft-derived reinnervation are still needed to enable fast and complete functional recovery. Anodal direct current stimulation promotes BDNF-dependent synaptic plasticity *in vitro*. Here, we assessed whether transcranial direct current stimulation (tDCS) can provide trophic and/or electrotactic

support to grafted stem cells in an *in vivo* rat Parkinson disease model. Methods: Transcranial direct current stimulation (as anodal, cathodal or sham tDCS) was applied daily for 14 days following striatal fetal ventral mesencephalic cells transplantation. Results: Anodal tDCS significantly enhanced graft survival and dopaminergic reinnervation of the surrounding striatal tissue relative to sham treatment control rats. Cathodal stimulation only minimally improved graft survival and had no effect on reinnervation. Both types of stimulation accelerated early motor recovery measured two weeks after transplantation, and behavioral effects correlated with the degree of striatal reinnervation. Discussion: Our results suggest anodal tDCS could be effective to advance restorative therapy in Parkinson disease patients.

Disclosures: **B. Fritsch:** None. **J. Reis:** None. **N. Hoffmann:** None. **C. Münkel:** None. **A. Gellner:** None. **H. Hulshof:** None. **M. Döbrössy:** None. **L. Furlanetti:** None. **J. Garcia:** None. **C. Winkler:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.01/F8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: KAKENHI 26293207

Title: The enhancement of protein degradation systems via autophagy in cellular models of neurodegenerative diseases

Authors: *H. ADACHI[1], Z. HUANG[1], K. OKADA[1], K. OHNARI[1], T. HASHIMOTO[1], T. TOYOTA[1], Y. IWANAKA[1], G. SOBUE[2];
[1]Univ. of Occup. and Envrn. Hlth., Kitakyushu, Japan; [2]Neurol., Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan

Abstract: A common characteristic of neurodegenerative diseases is the accumulation of abnormal proteins. In chronic neurodegenerative disorders such as polyglutamine (polyQ), commonly observed phenotypes include the abnormal accumulation of disease-causing proteins and the formation of nuclear and cytoplasmic inclusions. Under pathologic conditions, the accumulated level of such misfolded and toxic proteins may exceed the protective ability of the proteolytic machinery; the inability to either maintain misfolded proteins in a soluble form or degrade them results in their accumulation and the formation of inclusions. Macroautophagy is a set of bulk degradation processes in which cells form double-membrane vesicles, called

autophagosomes, around a portion of the cytoplasm. These autophagosomes ultimately fuse with lysosomes, resulting in the degradation of their substrates. The transcription factor EB (TFEB) has been reported to regulate autophagy by upregulating genes that belong to the coordinated lysosomal expression and regulation (CLEAR) network, thereby controlling lysosomal biogenesis. We examined the effects of the overexpression of TFEB in cultured cell models of neurodegenerative diseases. NSC34 cells were transfected using Lipofectamine 2000 with plasmids encoding mutant androgen receptor, huntingtin, ataxin-1, ataxin-3 and TFEB. The overexpression of TFEB decreased the expression of each causative protein in the neuronal cell models. The expression of the autophagic marker LC-3 II was significantly elevated in the cells expressing TFEB. These findings demonstrated that the high expression of TFEB induced autophagosome formation and enhanced the degradation of the disease-causative proteins.

Disclosures: **H. Adachi:** None. **Z. Huang:** None. **K. Okada:** None. **K. Ohnari:** None. **T. Hashimoto:** None. **T. Toyota:** None. **Y. Iwanaka:** None. **G. Sobue:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.02/F9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NS04519

Title: Loss of adult myosin heavy chain isoforms in spinal and bulbar muscular atrophy muscle

Authors: ***K. HALIEVSKI**[1], Y. XU[1], M. KATSUNO[2], H. ADACHI[3], G. SOBUE[2], S. M. BREEDLOVE[1], C. L. JORDAN[1];
[1]Neurosci., Michigan State Univ., East Lansing, MI; [2]Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan; [3]Univ. of Occup. and Envrn. Hlth., Kitakyushu, Japan

Abstract: Muscle weakness is a core symptom of spinal and bulbar muscular atrophy (SBMA), an androgen-dependent neuromuscular disease linked to a CAG expansion mutation in the androgen receptor (AR) gene. Recent studies of muscle function have revealed a loss of intrinsic muscle strength that is independent of muscle mass in SBMA mice (Oki et al., Muscle Nerve, (2013)47:823; Oki et al., J Appl Physiol, (2015)118:941). Searching for candidate mechanisms to explain this loss, we now report that diseased muscle is relatively insensitive to the pharmacological inhibitor of skeletal muscle myosin II, N-benzyl-p-toluene sulphonamide (BTS), suggesting defects in myosin heavy chain (MHC), an important mediator of muscle

strength. We used qPCR to investigate the expression of MHC mRNA in muscle of diseased males of two transgenic (tg) mouse models that recapitulate SBMA in a male-biased and androgen-dependent manner, the “myogenic” and “97Q” models. We examined four MHC genes in the prototypical fast (extensor digitorum longus) and slow (soleus) muscles: Myh4 (fast, IIb), Myh7 (slow, β), Myh3 (embryonic), and Myh8 (perinatal). In both SBMA models, we find a dramatic loss of mRNA expression of the adult MHC isoforms in both fast and slow twitch muscles (>45 fold down-regulated) while mRNA encoding the embryonic and perinatal isoforms are up-regulated in both muscle types (2.1-394 fold). We also examined MHC expression in muscle of acutely diseased tg females and find comparable changes in the expression of the adult MHC isoform: mRNA expression of the adult slow isoform (Myh7) is robustly down-regulated (24 fold) in the soleus muscle of acutely diseased myogenic females after only five days of androgen exposure. However, levels of the early forms of MHC (i.e., Myh3 and Myh8) were decreased (1.5-4.4 fold) rather than increased as found in males. These results suggest that expression of adult isoforms of myosin are robustly down-regulated in both fast and slow muscle in the face of disease, potentially contributing to the loss of muscle strength. Up-regulation of early MHC forms in muscles of chronically diseased males, but not in muscles of acutely diseased females, may reflect some compensatory process, which is still not enough to rescue force production, probably due to weak ATPase activity of these early isoforms. That disease impairs the expression of critical contractile proteins in muscle suggests that such proteins may be good therapeutic targets for rescuing muscle function in SBMA patients.

Disclosures: **K. Halievski:** None. **Y. Xu:** None. **M. Katsuno:** None. **H. Adachi:** None. **G. Sobue:** None. **S.M. Breedlove:** None. **C.L. Jordan:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.03/F10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NSC-100-2321-B-001-033

IBMS

Title: MBNL1 deubiquitination results in nuclear translocation and impairs neurite outgrowth in myotonic dystrophy

Authors: *G.-S. WANG, T.-Y. KUO, P.-Y. WANG, Y.-M. LIN, M.-H. TAO;
Academia Sinica, Taipei, Taiwan

Abstract: Myotonic dystrophy (DM) is the most common cause of adult onset muscular dystrophy. Cognitive deficits are found in a high percentage of individuals with DM, type 1 (DM1). The cognitive and behavioral abnormalities include mental retardation, attention deficit and hyperactivity disorder, excessive daytime sleepiness and psychiatric disorders. The genetic basis of DM1 is caused by an expansion of CTG repeats in the 3' untranslated region (UTR) of the Dystrophia Myotonica Protein Kinase (DMPK) gene. DMPK mRNA containing expanded CUG repeats accumulates in nuclear foci and affect nuclear and cytoplasmic functions of RNA binding proteins such as muscleblind like 1 (MBNL1) and CELF1 (CUGBP and ETR3 Like Factor). Structural change in the brain is a DM1 feature; however, the causal mechanism remains unknown. Here we find that dysfunction of cytoplasmic MBNL1 due to deubiquitination may contribute to DM1 histopathological abnormality. Cytoplasmic MBNL1 was functioning in promotion of neurite outgrowth and its cytoplasmic localization was regulated by K63-linked ubiquitination in a brain-specific manner. In DM1 mouse and human brains, the extent of K63 ubiquitination was reduced and reduction of cytoplasmic MBNL1 was accompanied with dendrite fragmentation. Importantly, expanded CUG RNA induced MBNL1 deubiquitintation resulting in nuclear translocation and morphological defects that can be rescued by preventing degradation of K63-linked polyubiquitin chains. In summary, we show that deubiquitination of cytoplasmic MBNL1 has a critical role in DM1 neural pathogenesis through affecting the subcellular localization of MBNL1 and the integrity of neuronal morphology.

Disclosures: G. Wang: None. **T. Kuo:** None. **P. Wang:** None. **Y. Lin:** None. **M. Tao:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.04/F11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R01 NS076919

Title: Analysis of nuclear export of polyglutamine-expanded androgen receptor in a cell model of spinal and bulbar muscular atrophy

Authors: *F. ARNOLD[1], D. MERRY[2];
[2]Biochem., [1]Thomas Jefferson Univ., Philadelphia, PA

Abstract: Spinal and bulbar muscular atrophy (SBMA) is an X-linked neurodegenerative disease caused by a polyglutamine (polyQ) expansion in the androgen receptor (AR). The AR is a steroid hormone receptor that, upon ligand binding of testosterone or dihydrotestosterone (DHT), undergoes a conformational change that induces nuclear localization and subsequently, the transcriptional regulation of AR-target genes. In SBMA, both the presence of hormone and the nuclear localization of the AR are necessary for toxicity, with the formation of intranuclear inclusions of aggregated AR a hallmark of the disease state. Given the importance of nuclear localization in disease-mediated toxicity, we sought to examine whether there are alterations in the nuclear export of polyQ-expanded AR, compared with wild-type AR and, if so, whether enhancing the nuclear export of polyQ-expanded AR is protective in cell models of SBMA. A heterokaryon analysis of PC12 cells expressing human AR under the control of a tetracycline-inducible promoter revealed that polyQ-expanded AR is, in fact, deficient in nuclear export relative to wildtype AR. This difference will be further evaluated using cells expressing human AR fused to photoswitchable DENDRA2 tags, which will allow us to quantify the rate of nuclear export of polyQ-expanded and wildtype AR *in vitro*. Concurrently, we have created nuclear export sequence (NES)-tagged AR constructs to evaluate the effect of enhanced nuclear export on numerous aspects of disease pathogenesis, including: toxicity, intranuclear inclusion formation, and protein degradation. All together, these experiments will provide us with a new understanding of the role of nuclear export in SBMA pathogenesis and new insights into the effect of the polyQ-expansion on nuclear export.

Disclosures: **F. Arnold:** None. **D. Merry:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.05/F12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Grant from Kennedy's Disease Association

NIH grant R01 NS076919

Title: Protein interaction networks in the pathogenicity of Spinal and Bulbar Muscular Atrophy

Authors: ***A. PLUCIENNIK**[1], T. BERGER[1], S. FINKBEINER[2], D. MERRY[1];
[1]Thomas Jefferson Univ., Philadelphia, PA; [2]Gladstone Inst. of Neurolog. Dis., San Francisco, CA

Abstract: Spinal and bulbar muscular atrophy is caused by a loss of brainstem and spinal cord motor neurons (and of the associated innervated muscles). Genetic basis of this disease is an expansion of a polyglutamine-encoding CAG repeat within androgen receptor (AR) gene. Several hypotheses have been put forward to explain the cytotoxic consequences of polyQ expansion at the molecular level, including aberrant protein-protein interactions, altered post-translational modifications, and perturbations to global protein folding homeostasis. Here, we have employed a quantitative proteomics approach involving stable isotope labeling of amino acids in cell culture (SILAC) to identify alterations in the AR interactome due to polyQ expansion. Rat neuronal PC12 cells expressing polyQ expanded AR were labeled with heavy isotopic (13C and 15N) forms of the essential amino acids lysine and arginine, whereas corresponding PC12 cells expressing wild type AR were not labeled. Using the expanded polyQ- and conformation specific antibody 3B5H10, polyQ expanded AR and its protein interaction partners were immunoprecipitated. Our approach has identified several promising protein candidates whose interactions with AR are altered by the polyQ expansion. The potential roles of these AR interacting proteins in pathogenesis are currently being evaluated.

Disclosures: **A. Pluciennik:** None. **T. Berger:** None. **S. Finkbeiner:** None. **D. Merry:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.06/F13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Innovation Fund Denmark

Title: Mass spectrometry analysis of normal and polyglutamine expanded ataxin-3 interaction partners

Authors: *L. V. KRISTENSEN[1,2], R. HARTMANN-PETERSEN[2], K. THIRSTRUP[1];
[1]H. Lundbeck A/S, Valby, Denmark; [2]Dept. of Biol., Univ. of Copenhagen, Copenhagen, Denmark

Abstract: The group of polyglutamine (polyQ) diseases comprises nine different neurodegenerative disorders, characterized by an expanded polyglutamine tract within the respective protein. The disorders include Huntingtons disease (HD) and different spinocerebellar ataxias (SCA-1-3, SCA-6-7, and SCA-17). SCA3 also known as Machado-Joseph disease is the most common dominantly inherited ataxia worldwide and is caused by a polyQ expansion within

the ataxin-3 gene. The polyQ expansion confers toxicity to the protein ataxin-3, leading to neuronal dysfunction and loss in various brain areas. How the polyQ expansion confers toxicity to ataxin-3 is currently not very well understood and existing treatment strategies of SCA3 patients are insufficient. Therefore investigations of how the polyQ expansion alters functional properties of ataxin-3 are essential to further increase the understanding of the SCA3 disease pathology. It is conceivable that the polyQ expansion of ataxin-3 alters the repertoire of interacting proteins, which may represent a part of the explanation for its key role in SCA3 pathophysiology. Hence we sought to investigate differences in interaction partners of normal (14Q) and polyQ expanded ataxin-3 (82Q) by mass spectrometry. Flag-ataxin-3 variants were expressed in HEK293 cells and purified by Flag-immunoprecipitation. Samples were then analyzed by mass spectrometry to assess differences between normal and polyQ expanded ataxin-3. In the present study we report the data obtained from this mass spectrometry analysis and discuss how these results can further increase our understanding of the pathological role of polyQ expanded ataxin-3.

Disclosures: **L.V. Kristensen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **R. Hartmann-Petersen:** None. **K. Thirstrup:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.07/F14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R21NS084392-01A1

Title: Impaired neuronal activity in hippocampal and neocortical slices from a mouse model of Juvenile Batten disease

Authors: N. KARPUK[1], M. BURKOVETSKAYA[1], *T. KIELIAN[2];
[2]Dept. of Pathology and Microbiology, [1]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Juvenile Neuronal Ceroid Lipofuscinosis (JNCL or Juvenile Batten Disease) is a debilitating lysosomal storage disorder caused by an autosomal recessive mutation in CLN3. JNCL symptoms typically present between 5-10 years of age, beginning with blindness and progressing to seizures, motor incoordination, cognitive deficits, and early death (late teen-early 20s). JNCL is typified by progressive neurodegeneration in thalamocortical structures, which has

been suggested to occur from excessive excitatory and impaired inhibitory synaptic function. However, these conclusions were made by *ex vivo* analysis of neurotransmitter levels and treatment of JNCL mouse models with glutamate receptor antagonists and direct analysis of synaptic communication using electrophysiology methods has not yet been performed. Here we evaluated evoked field potentials (FP) in acute hippocampal and neocortical slices from a mouse model of JNCL (i.e. CLN3Δex7/8) and wild type (WT) animals. FP measurements were compared between CLN3Δex7/8 and WT slices at 3, 7, and 12 months of age to identify the onset of synaptic defects and alterations with advancing disease. Our findings demonstrated that FP paired-pulse facilitation (PPF) was significantly attenuated in CLN3Δex7/8 hippocampus (CA1 to CA3) that was already evident at 3 months of age. This finding is intriguing since neuronal loss is not evident in CLN3Δex7/8 mice until 12 months and suggests that synaptic changes are an early disease manifestation. Changes in FP were more complex in the neocortex, namely PPF and FP amplitude were significantly increased in the upper layers of the visual neocortex (i.e. layers I-III) of CLN3Δex7/8 mice, whereas these values were slightly reduced in deeper neocortical layers (i.e. layers IV-V). These FP changes in the CLN3Δex7/8 brain may be one mechanism to explain impaired hippocampal memory and neuronal afferent/sensory signaling in the neocortex that are hallmarks of JNCL in affected children.

Disclosures: **N. Karpuk:** None. **M. Burkovetskaya:** None. **T. Kielian:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

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Program#/Poster#: 135.08/F15

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Darwin Trust of Edinburgh

BBSRC

EBRC

Title: Identifying potential peripherally accessible biomarkers in batten disease

Authors: *M. LLAVERO HURTADO[1], T. W. MARCHANT[2], S. L. EATON[2], H. F. FULLER[3,4], A. TAVENDALE[5], D. J. LAMONT[5], T. H. GILLINGWATER[6,7], J. D. COOPER[8], T. M. WISHART[2,7];
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Edinburgh, Edinburgh, United Kingdom; [3]Wolfson Ctr. for Inherited Neuromuscular Dis., Oswestry, United Kingdom; [4]Keele University, Inst. for Sci. and Technol. in Med., Keele, United Kingdom; [5]FingerPrints Proteomics Facility, Col. of Life Sciences, Univ. of Dundee, Dundee, United Kingdom; [6]Ctr. for Integrative Physiology, Univ. of Edinburgh, Edinburgh, United Kingdom; [7]Euan MacDonald Ctr. for Motor Neuron Dis. Research, Univ. of Edinburgh, Edinburgh, United Kingdom; [8]Inst. of Psychiatry, King's Col. London, London, United Kingdom

Abstract: Batten disease or Neuronal ceroid lipofuscinosis (NCL) is the most frequent autosomal-recessive neurodegenerative disease of childhood [1]. There are 14 forms of NCL caused by mutations in different genes affecting lysosomal function. All of them lead to the same features of clinical progression including, the accumulation of autofluorescent storage material in the lysosome (the main hallmark of these conditions), early synaptic loss and premature death [2, 3]. The most prevalent forms of this devastating condition are the CLN1 disease variant or infantile NCL (INCL) and the slightly later onset CLN3 disease or Juvenile NCL (JNCL) variant [4, 5]. Here we have used a proteomic based approach in murine models of NCL disease to demonstrate that although the gross appearance of CLN3 KO muscle fibres remains unchanged at early disease stages, there are significant molecular perturbations detectable at early time points. These include but are not limited to pathways involved in small molecule biochemistry and mitochondrial dysfunction. Thalamic synapses are an early pathological target in NCL [6, 7, 8]. We hypothesise that a tractable biomarker will be something which is constitutively expressed, but which changes in response to a mutation of interest in a tissue specific manner. We therefore compared the changes observed in muscle and thalamic synaptic proteomes. Constitutively expressed proteins were examined for higher order functional clustering and candidates were temporally tracked using label free proteomic profiling of CLN1 KO muscle throughout the time course of CLN1 disease progression. Here we identify multiple potential peripherally accessible biomarkers of disease progression which are mitochondrial in origin and whose expression is conserved in both CLN1 and CLN3 forms of NCL disease. References: 1.PMID: 8576551. 2. PMID: 12644737. 3. PMID: 15965709. 4. PMID: 7553855. 5. PMID: 12125808. 6. PMID: 16242638. 7. PMID: 18091563

Disclosures: **M. Llavero Hurtado:** None. **T.W. Marchant:** None. **S.L. Eaton:** None. **H.F. Fuller:** None. **A. Tavendale:** None. **D.J. Lamont:** None. **T.H. Gillingwater:** None. **J.D. Cooper:** None. **T.M. Wishart:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.09/F16

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: An animal disease model for episodic ataxia 6

Authors: *P. KOVERMANN, V. UNTIET, C. FAHLKE;
Inst. for complex systems 4, Forschungszentrum Jülich Gmbh, Juelich, Germany

Abstract: Mutations in the SLC1A3 encoding the glial excitatory amino acid transporters 1 (EAAT1) are associated involved in several neurological diseases such as Tourette syndrome (Adamczyck et al. 2011, Psychiatr. Genet. 21, 90-97.1) or episodic ataxia type 6 (Jen et al. 2007, Brain 130, 2484-2493). Recently, a point mutation in SLC1A3 was found in a patient with a severe form of episodic ataxia type 6, who suffered from long lasting attacks of ataxia and seizures (Jen et al. Neurology 65, 529-534). This mutation predicts the exchange of a highly conserved proline to arginine at position 290 (P290R) in EAAT1. Functional analysis of mutant transporters in heterologous expression systems revealed gain-of-function of the EAAT1-associated ion channel (Winter et al. 2012, Brain, 135, 3416-3425) as well as reduced glutamate transport via dramatic deceleration of a conformational change associated with sodium binding to the glutamate-free mutant transporters (Hotzy et al. 2013, J. Biol. Chem. 288, 36492-36501) in P290R EAAT1. However, the cellular mechanisms by which changes in glutamate transport or EAAT- associated anion channel activity might result in ataxia and seizures are still insufficiently understood. We here study the consequences of this mutation on cerebellar morphology and motor function in a knock-in animal disease model carrying the P290R mutation. P290R knock-in mice exhibit impaired balance and prominent ataxia as well as spontaneous generalized seizures and premature death. The changes in motor coordination are accompanied by dramatic changes in the morphology of the cerebellar cortex. Confocal microscopy of slices from cerebellar hemispheres of P290R knock-in mice show a reduced number of Bergmann glia cells in the Purkinje cell layer and a substantial number of Purkinje neurons with ectopic localization. Moreover, we observed highly increased density of neurons in the molecular layer. We conclude that changes in EAAT1/GLAST function impair the developmental migration of cerebellar granule cells and enhance the incidence of other types of neuronal cells in the molecular layer.

Disclosures: **P. Kovermann:** None. **V. Untiet:** None. **C. Fahlke:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.10/F17

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R21NS075645

Title: Regulation of neuronal hemoglobin expression and potential roles in mitochondrial respiration

Authors: S. LI, N. SINGHAL, R. GEGORY, J. MCDONOUGH, *E. J. FREEMAN;
Kent State Univ., Kent, OH

Abstract: It has been demonstrated in multiple sclerosis (MS) cortex that mitochondrial function is compromised and is associated with increased markers of oxidative damage. A previous analysis of the mitochondrial proteome in diseased and control postmortem cortex revealed an increase in the presence of the β subunit of hemoglobin (Hb) in MS cortex. In addition, in the CNS, Hb mRNA and protein have been localized to neurons and a potential role for Hb in mitochondrial respiration has been proposed. Indeed, Hb may function to ensure adequate delivery of oxygen, but may also serve to protect against oxidative damage, since Hb has been shown to express high affinity binding for other gas molecules, including nitric oxide (NO), as well as, act as a scavenger of peroxynitrite. Therefore, we examined the extent and distribution of Hb expression in brain and its regulation in response to various challenges, as well as, investigated its role in mitochondrial respiration. Using immunofluorescent staining, western blotting and flow cytometry we identified the presence of Hb throughout many brain regions in rat and confirmed its expression in primary neuronal cultures. Expression of Hb was regulated by oxygen concentration, inhibition of electron transport, and oxidative stress. Neurons cultured under hypoxic conditions (2-10% O_2) demonstrated increased Hb expression (80-100%) compared to those cultured under atmospheric conditions. In addition, treatment of neurons with low levels of rotenone or peroxide resulted in modest increases in Hb expression (25-30%), while increased concentrations reduced expression by 70-80% of controls. In respirometry experiments, we noted that rotenone, peroxide, as well as exposure to the NO-donor sodium nitroprusside (SNP) significantly attenuated mitochondrial respiration. Conversely, overexpression of Hb induced by erythropoietin (EPO) or transfection increased neuronal respiration through interactions with mitochondria and up-regulation of mitochondrial gene expression. We observed that NO-induced reductions in mitochondrial respiration could be reversed in transfected cells or those pretreated with EPO. Further, in cells transfected with Hb we identified increases in histone H3K4 trimethylation that was associated with increased expression of complex V of the electron transport chain. MS cortical tissue has been linked with mitochondrial dysfunction and oxidative stress, thus based on our observations, we suggest that the increase in Hb expression identified in MS cortex may occur in response to these influences to promote increased mitochondrial function and protect from oxidative damage.

Disclosures: **S. Li:** None. **N. Singhal:** None. **R. Gegory:** None. **J. McDonough:** None. **E.J. Freeman:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.11/F18

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: KAKENHI (25340104)

MEXT-Supported Program for the Strategic Research Foundation at Private Universities,2013-2017

Kansai University Research Grants: Grant-in-Aid for Encouragement of Scientists, 2014.

Title: Involvement of specific nur77 family genes during neurite outgrowth induced by forskolin and a histone deacetylase inhibitor in PC12 cells

Authors: *K. SHIMOKE[1], R. YAMAZOE[2], T. TOMIOKA[2], K. TSUMURA[2], Y. NISHIHATA[2], H. MARUOKA[3];
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Abstract: Neurite outgrowth is involved in neuronal differentiation. In this process, specific genes are expressed during neurite formation. Identification and functional analyses of these genes are important in developing a new strategy for regenerative therapy. We have demonstrated that forskolin (FSK), an intracellular cAMP producer, or valproic acid (VAL), a histone deacetylase inhibitor, both induce the nur77 gene, which belongs to the Nur nuclear receptor family, along with two other genes, within 4 hours in PC12 cells. We have previously revealed that nur77 protein induces neurites in PC12 cells. In the present study, we investigated which genes belonging to the Nur family are important in elongation of the neurites in the presence of FSK or VAL. Knock-down experiments showed that siRNA against nur77 or nurr1 mRNA suppressed neurite elongation in response to treatment with both FSK and VAL, while siRNA only suppressed the elongation induced by the treatment with FSK, suggesting that nur77 and nurr1 are essential for neurite outgrowth in the presence of FSK or VAL. We also found that epigenetic regulation via histone H3 modification (i.e., acetylation of lysine) was important for the FSK- or VAL-induced neurite outgrowth. These results show that up-regulation of nur77 and

nurr1 genes are involved in neurite outgrowth induced by FSA or VAL through acetylation of histone H3 at the lysine residue, and suggest that different mechanisms are also involved among the nur77 family genes in inducing neurite outgrowth.

Disclosures: K. Shimoke: None. **R. Yamazoe:** None. **T. Tomioka:** None. **K. Tsumura:** None. **Y. Nishihata:** None. **H. Maruoka:** None.

Poster

135. Non-Huntington's Disease Ataxias and Other Repeat Diseases

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 135.12/F19

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MEXT KAKENHI Grant Number 23390291

Title: Chorein /HDAC6 interaction confers resistance to nutrient deprivation in chorein-overexpressing HEK293 cells

Authors: *M. NAKAMURA, N. SASAKI, A. KODAMA, N. SHIOKAWA, A. SANO; Kagoshima Univ, Dept of Psychiatry, Kagoshima, Japan

Abstract: Loss of chorein function causes an autosomal recessive neurodegenerative disorder chorea-acanthocytosis, which is characterized by striatal neurodegeneration and erythrocyte acanthocytosis. Detailed physiological role of chorein at the molecular level remains unclear. Recently autophagy pathway had been implicated in several neurodegenerative diseases. In the present study, chorein-overexpressing human embryonic kidney (HEK) 293 cells demonstrated increased cell viability following nutrient starvation. Co-immunoprecipitation assays using chorein stably overexpressing HEK 293 cells revealed that chorein interacts with HDAC6, which is known as a central component of basal autophagy. Selective HDAC6 inhibitor tubacin decreased cell viability of chorein stably overexpressing HEK293 cells under nutrient starvation condition. These results suggest that chorein/HDAC6 interaction may have an important role in autophagic pathway and the collapse of this mechanism may be one of the molecular pathogenesis of chorea-acanthocytosis.

Disclosures: M. Nakamura: None. **N. Sasaki:** None. **A. Kodama:** None. **N. Shiokawa:** None. **A. Sano:** None.

Poster

136. Ischemia: Perinatal

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 136.01/F20

Topic: C.08. Ischemia

Support: Uludag University Scientific Research Projects Commission (Grant number: KUAP(T)-2013/76)

Title: Uridine protects against hypoxic-ischemic brain injury by reducing histone deacetylase activity in neonatal rats

Authors: *T. ALKAN[1], T. KOYUNCUOGLU[1], M. TURKYILMAZ[2], B. GOREN[1], M. CANSEV[2];
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Abstract: A significant cause of neurological disability in newborns is hypoxic-ischemic encephalopathy (HIE), a disorder which involves an enhancement in histone deacetylase (HDAC) activity among underlying pathological mechanisms. We showed recently that exogenous administration of uridine to newborn rats with HIE reduced brain injury in a dose-dependent manner. The present study was performed to investigate whether uridine modulates histone acetylation/deacetylation balance in a neonatal rat model of HIE. Newborn rats that were subjected to hypoxic-ischemic (HI) insult on postnatal day 7 (P7) were injected intraperitoneally with either saline or uridine (500 mg/kg) for three consecutive days. One day after completion of treatment, brains of pups were collected for evaluation of brain infarct volume, apoptosis, HDAC activity and acetylated-Histone H3 (Ac-H3) and H4 (Ac-H4) protein levels. Results revealed that uridine administration reduced infarct volume, active Caspase-3 levels and HDAC activity while increasing the expressions of Ac-H3 and Ac-H4 proteins. We conclude that one mechanism by which uridine provides neuroprotection in neonatal rat HIE model involves reduction in HDAC activity. Experimental protocol was approved by the Ethical Committee on Experimental Animals in Uludag University, Bursa, Turkey (2013-14/03), and the experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 and EC Directive 86/609/EEC.

Disclosures: **T. Alkan:** None. **T. Koyuncuoglu:** None. **M. Turkyilmaz:** None. **B. Goren:** None. **M. Cansev:** None.

Poster

136. Ischemia: Perinatal

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Program#/Poster#: 136.02/F21

Topic: C.08. Ischemia

Support: Shriners Hospitals for Children 85500-PHI-14

Shriners Hospitals for Children 84298-PHI

Title: Long-term white matter instabilities after neonatal hypoxic-ischemic brain injury

Authors: *H. JEONG[1], R. KHAWAJA[1], J. D. ROTHSTEIN[2], S. H. KANG[1,3];
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Abstract: Periventricular leukomalacia (PVL) is the predominant form of white matter brain injury associated with premature birth, and such injury often leads to long-term neurological disabilities, such as cerebral palsy. Oligodendrocytes (OLs), the CNS myelinating glia, are known to be particularly vulnerable to various injury-causing insults in the developing brain. Accordingly, this form of brain injury has been attributed to early death of OLs and/or maturation failure of developing OLs. However, given the abundance of highly regenerative OL progenitors (OLPs) in the developing brain, the mechanisms underlying post-injury, long-term white matter deficits remain elusive. In the present study, we followed the fate of NG2-expressing OLPs, and examined the growth of the OL population until adulthood in a mouse model of perinatal hypoxic-ischemic (HI) injury, utilizing various OL lineage-specific transgenic mice. Following a surgical procedure that induced unilateral HI into the brain at P7, the injured hemisphere exhibited marked gliosis and ventricle enlargement, which persisted to adult ages. Despite an instant loss of subsets of OLs, there followed a robust proliferation of OLPs, with enhanced differentiation into OLs in the injured areas. Unexpectedly, the number of mature OLs in the injured side was even larger than in the control side at P15, and it remained elevated for at least one month after HI, indicating an active OL development, as well as an intact regenerative response of OLPs. However, the activated OLP responses gradually subsided to a normal level afterwards, and the number of OLs was significantly decreased in the injured white matter by two months after HI. In parallel, the degree of myelination in the injured areas also followed this multi-phasic OL growth pattern. Furthermore, many surviving OLs in the injured white matter failed to express monocarboxylate transporter-1 (MCT1), a lactate transporter that may mediate metabolic support for axons, as assessed by a reporter expression in MCT1-tdTomato mice. Maturation of nodes of Ranvier was also impaired in the adult brain. Taken together, these results suggest that the long-lasting white matter deficits observed after perinatal HI, are not

primarily caused by early OL loss and/or developmental maturation arrest, but rather are attributable to shorter survival of mature OLs, and structural and functional impairments of OLs in the adult brain. Insufficiency of OL-mediated metabolic support for axons, as well as delayed demyelination, may play a role in the long-lasting neuronal dysfunctions associated with perinatal white matter injury.

Disclosures: H. Jeong: None. **R. Khawaja:** None. **J.D. Rothstein:** None. **S.H. Kang:** None.

Poster

136. Ischemia: Perinatal

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: C.08. Ischemia

Support: NIH-NINDS 1K08NS083736

Title: Nicotinamide Mononucleotide Adenylyltransferase 3 protects the mouse neonatal hippocampus from the effects of neonatal hypoxia-ischemia

Authors: *R. GALINDO[1], M. B. GREENBERG[2], T. ARAKI[3], D. M. HOLTZMAN[2]; [1]Neurol., Washington Univ. In St. Louis, Saint Louis, MO; [2]Neurol., Washington Univ., St. Louis, MO; [3]Dept. of PNS Res., Natl. Inst. of Neurocience, Kodaira Tokyo, Japan

Abstract: Neonatal cerebral hypoxia-ischemia (H-I) is a major cause of death and disability in the pediatric population. There are only limited treatments available for this condition and the mechanisms that lead to acute and chronic cerebral dysfunction are poorly understood. The family of NAD-synthetizing enzymes, nicotinamide mononucleotide adenylyltransferases (NMNATs), consist of three isoforms and they all have been implicated in the protection of axons and neurons after different forms of neuronal injury. However, it is not known whether NMNAT3 protects immature neurons or axons following neonatal cerebral injury. In this study, we find that endogenous NMNAT3, although present at low levels in immature healthy neurons, is strongly induced following neonatal cerebral hypoxia and ischemia. Utilizing NMNAT3 overexpressing transgenic mice, we find that NMNAT3 overexpression decreases overall neonatal mortality following H-I and also decreases cerebral tissue injury in the neonatal hippocampus and cortex. We then sought to determine the mechanism via which NMNAT3 was exerting its neuroprotective effects. Utilizing biochemical assays, we show that NMNAT3 overexpression decreases the activation of caspase-3. NMNAT3 overexpression was also associated with decreases in calpain- and caspase-mediated cleavage of brain alpha-spectrin

following cerebral ischemia. We then investigated whether the above effect was downstream of the activation of key signaling pathways involved in the regulation of cell death of injured neurons. The calpain-inhibitor protease, calpastatin, has been recently implicated in the neuroprotective mechanism of NMNAT1 after peripheral axonal injury. Furthermore, calpastatin has also been implicated in the modulation of caspase activation in ischemic neurons. Therefore, we asked whether NMNAT3 overexpression results in decreases in injury-dependent proteolytic degradation of calpastatin. NMNAT3 overexpression prevented the degradation of calpastatin after neonatal H-I. Taken together, the present data indicates that NMNAT3 protects ischemic immature neurons by inhibiting apoptotic and necrotic associated cellular damage possibly via a calpastatin-mediated mechanism. Work funded by NIH NINDS 1K08NS083736-02

Disclosures: R. Galindo: None. **M.B. Greenberg:** None. **T. Araki:** None. **D.M. Holtzman:** None.

Poster

136. Ischemia: Perinatal

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Topic: C.08. Ischemia

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FONDECYT 1120079

FONDECYT 1110263

FONDECYT 1150744

ER-M was Conicyt PhD Fellow 21090557

Title: Systemic administration of YC-1 prevents morphological changes and functional impairments induced by perinatal asphyxia in hippocampus of rats

Authors: *E. ROJAS-MANCILLA[1,5,2], T. NEIRA-PEÑA[1,2], G. LORCA[1], B. MORALES[6], P. ROJAS[6], P. GEBICKE-HAERTER[1,7], D. BUSTAMANTE[1], J. L. VALDÉS[2,3], P. MORALES[1,2], L. LEYTON[4,2], M. HERRERA-MARSCHITZ[1,2];
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Univ. de Santiago de Chile, Santiago, Chile; [7]Deptartment of Psychopharmacology, Central Inst. of Mental Hlth. J5, Mannheim, Germany

Abstract: Perinatal asphyxia is a main cause of long-term neurological damage, requesting novel therapeutic ideas to prevent its deleterious consequences. HIF-1α is a key molecule in the signalling elicited by hypoxia, triggering neuroprotective or deleterious cascades depending upon the intensity of the insult. Here, we show that severe PA increases HIF-1α translocation, leading to astrocyte reactivity and neuronal impairments in rat hippocampus, prevented by systemic treatment with the HIF-1α inhibitor, YC-1. Asphyxia was induced by immersing G22 foetuses into a water bath at 37°C for 21 min. Surviving and control pups were treated with YC-1 (2 mg/kg, i.p.) or saline (1 mL/kg, i.p.), 10 min after delivery. For preparing primary cultures of neurons or astrocytes, hippocampi from control and asphyxia-exposed rats were dissected 6 h or 1 day after delivery. Sibling animals were given to surrogate dams pending further analysis. It was found that perinatal asphyxia: (i) increased HIF-1α protein levels, translocated to the nuclei of neurons and astrocytes immediately after delivery; (ii) increased astrocyte reactivity, assessed by Bystin, Aldh1L1 and GFAP markers, 1, 8 and 24 h after-asphyxia; (iii) reduced the number of neurons, neurite length, and pre- and post-synaptic dots assessed by MAP-2, Synaptophysin and PSD95, respectively, in cultures at DIV 7-18; (iv) impaired synaptic structure, reducing the number of synapses in CA3 at P 7-22; (v) decreased long term potentiation at P24, and (vi) impaired learning and non-spatial memory at P90. YC-1 treatment precluded several of the morphological and functional impairments elicited by perinatal asphyxia. The present results provide evidence that HIF-1α is a pivotal molecule responding to asphyxia, leading to astrocyte reactivity, and long-term synaptic, neuronal and functional deficits affecting the plasticity of hippocampus, prevented by systemic YC-1 treatment. Thus, HIF-1α is a prominent target for therapeutic strategies aiming the deleterious effects of perinatal asphyxia.

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Poster

136. Ischemia: Perinatal

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 136.05/F24

Topic: C.08. Ischemia

Title: Effects of normobaric oxygen treatment on brain injury after hypoxia- ischemia in newborn mice

Authors: *T. KELESTEMUR[1], A. B. CAGLAYAN[2], M. C. BEKER[2], U. KILIC[3], S. ALTUNAY[2], B. CAGLAYAN[2], E. YALCIN[2], R. Z. GUNDUGDU[2], E. KILIC[2];
[2]Physiology, Regenerative and Restorative Med. Res. Ctr., [3]Med. Biol., [1]Istanbul Medipol Univ., Istanbul, Turkey

Abstract: Hypoxic-ischemia (HI) is a widely used animal model to mimic the preterm or perinatal sublethal hypoxia. It causes diffuse neurodegeneration in the brain and results in mental retardation, hyperactivity, cerebral palsy and epilepsy. Normobaric oxygen (NBO) enhances post-ischemic tissue re-oxygenation and promotes neuronal survival. However, its therapeutic effect is compromised by reactive oxygen species which are formed in response to oxygen. In this contex, we investigated effects of NBO combined with free radical scavenger melatonin after newborn hypoxic-ischemia. For this aim we anesthesized 7 days old mice with 1% isoflurane (30% O_2; reminder N_2O) and exposed to 8% Oxygen for 1 hour after right carotid artery ligation which controlled by laser speckle imaging and evaluated effects of normobaric oxygen (70% or 100% over 120 min), administered either alone or in combination with melatonin (4 mg/kg, i.p.), on apoptotic cell death, neuronal survival, infarct volume, brain swelling and cell signalling. Combination of oxygen and melatonin treatment decreased infarct volume, neuronal injury, brain swelling more strongly than oxygen or melatonin alone. As compared with Oxygen and melatonin treatment, phosphorylation of SAPK/JNK-1/2 was reduced and ERK-1/2, CREB phosphorylation were increased in NBO/melatonin combined animals. Here, we provided evidence that NBO treatment is beneficial after newborn HI, which was associate with improved neuronal survival and ERK-1/2 and CREB activities. This data encourages proof-of- concept studies in human hypoxic-ischemia treatment.

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Poster

136. Ischemia: Perinatal

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 136.06/F25

Topic: C.08. Ischemia

Support: CHRCDA K12 HD068322

Vikings Children's Fund

Title: Intrauterine growth restriction results in lower cortical glutamine concentrations in the adult rat brain

Authors: *M. ALEXANDER[1], I. TKAC[2], G. OZ[2], R. RAO[1], A. MALISZEWSKI-HALL[1]; [1]Pediatrics, Univ. Minnesota, Minneapolis, MN; [2]Ctr. for Magnetic Resoance Spectroscopy, Univ. of Minnesota, Minneapolis, MN

Abstract: Background: Children who are born with intrauterine growth restriction (IUGR) are at increased risk for long-term neurodevelopmental deficits including motor, cognitive and attention impairments. The nature of these deficits suggests that the cerebral cortex and hippocampus are particularly vulnerable to injury, however the mechanisms of injury are unknown. Using *in vivo*, high-field (9.4T) 1H MRS, we recently showed lower concentrations of several metabolites responsible for energy metabolism and glutamatergic neurotransmission in the cerebral cortex of postnatal (P) day 7 IUGR rats versus normally grown (NG) controls. Whether these early metabolite changes persist long-term is unknown. Objective: To evaluate the neurochemical profile of the cortex and hippocampus in adult IUGR and NG rats using *in vivo* 1H MRS at 9.4T. Methods: IUGR was induced using bilateral uterine artery ligation at gestational day 19 (term=22.5d) in pregnant Sprague Dawley dams. MR spectra were obtained from the cortex and hippocampus at P60 (adulthood) in IUGR (N=8) and NG (N=7) pups. All spectra were acquired as previously described (Maliszewski-Hall et al., 2015). Differences in neurochemical concentrations in each region were compared between IUGR and NG groups using a two-way ANOVA and follow-up students T-test. Results: In the P60 cortex, IUGR resulted in lower concentrations of Gln compared to NG. There was no effect of IUGR on neurometabolite concentrations in the hippocampus. Conclusion: IUGR differentially effects the neurochemical profile in a regional dependent manner in the adult rat brain. Specifically Gln concentrations were lower in the IUGR cortex but unchanged in the hippocampus compared to NG. Using1H MRS Gln concentrations are considered a good measure of the turnover of glutamate involved in neurotransmission. Lower Gln concentrations in the IUGR cortex suggest disruptions in glutamate-glutamine neurotransmission. We speculate a lower concentration of Gln may be one underlying mechanism leading to cortex-based long-term cognitive impairments in human IUGR infants.

Disclosures: M. Alexander: None. **I. Tkac:** None. **G. Oz:** None. **R. Rao:** None. **A. Maliszewski-Hall:** None.

Poster

136. Ischemia: Perinatal

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 136.07/F26

Topic: C.08. Ischemia

Support: Canadian Partnership for Stroke Recovery Catalyst Grant

Title: Effects of metformin and enriched rehabilitation on recovery following neonatal hypoxia-ischemia

Authors: *S. ANTONESCU[1,2], M. JEFFERS[2], J. LIVINGSTON-THOMAS[2], C. MORSHEAD[3], D. CORBETT[2,4];
[1]Neurosci., [2]Univ. of Ottawa, Ottawa, ON, Canada; [3]Univ. of Toronto, Toronto, ON, Canada; [4]Canadian Partnership for Stroke Recovery, Ottawa, ON, Canada

Abstract: Neonatal hypoxia-ischemia (HI) is one of the most common causes of mortality and morbidity in children, often leaving survivors with profound physical and cognitive disabilities. Effective treatments capable of supporting long-term recovery and reducing the severity of disabilities are needed. Previous research using adult animal models of stroke has shown that metformin, an antidiabetic drug, promotes neurogenesis, oligogenesis and angiogenesis to enhance motor and cognitive function following injury. This study aims to determine whether metformin, enriched rehabilitation (ER) or a combination of the two could provide a clinically relevant therapeutic option for enhancing motor function following neonatal HI. At post-natal day (PND) 7, Sprague-Dawley rats were assigned to two groups: sham or hypoxia-ischemia. The Rice-Vannucci model was used to induce unilateral injury, in which HI animals had their left carotid artery permanently ligated prior to being placed in a hypoxia chamber (8% O2) for 90 minutes. At weaning (PND 21), animals assigned to ER were housed in an enriched environment and received reach training for 4 weeks. All other animals were standard housed. Once weaned, pups received subcutaneous metformin (200mg/kg/day) or saline injections for 4 weeks. Motor function was assessed pre- and post-combined therapy using the following tests: adhesive-strip removal, ladder-walking and Montoya staircase. Following four weeks of treatment, rats receiving ER made fewer errors with their impaired forelimbs and hindlimbs on the ladder-walking test and displayed a decreased latency to contact the adhesive strip on their impaired forelimb. In addition, animals receiving either metformin or enriched rehabilitation showed an improved learning curve on the Montoya staircase. In conclusion, enriched rehabilitation promoted motor recovery following HI, while the effects of metformin on recovery are further being investigated. Future experiments will be examining the effects of metformin and enriched rehabilitation on cognitive function following HI.

Disclosures: **S. Antonescu:** None. **M. Jeffers:** None. **J. Livingston-Thomas:** None. **C. Morshead:** None. **D. Corbett:** None.

Poster

136. Ischemia: Perinatal

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 136.08/F27

Topic: C.08. Ischemia

Support: Health Labour Sciences Research Grant

Title: Magnetic resonance imaging and O-15 gas positron emission tomography in immature rats with hypoxic-ischemic brain injury

Authors: *M. TSUJI[1,2], J. ENMI[3], T. MORIGUCHI[3], K. KOSHINO[3], M. OHSHIMA[2], H. IIDA[3];
[2]Dept Regen Med. Tissue Eng, [3]Dept Invest Radiol, [1]Natl. Cerebral and Cardiovasc. Ctr., Suita, Osaka, Japan

Abstract: Background & Objectives: Hypoxic-ischemic (HI) brain injury results from birth asphyxia in perinatal period and from cardiopulmonary arrest with heart diseases or near-drowning in childhood. Magnetic resonance imaging (MRI) and positron emission tomography (PET) are used to evaluate the brain damage and to predict the outcome in those neonates and children. However, precise temporal changes in MR/PET imaging are not known, and their predictive values are not high. Recently we have developed a non-invasive MR/O-15 gas PET imaging system for small animals that does not require tracheal intubation/tracheostomy and arterial blood sampling. Objectives of this study are; 1) to clarify temporal profiles of brain injury and hemodynamics in immature rats with HIE by using the MR/PET imaging; 2) to analyze correlations between early parameters in the imaging and the later morphological damage to see if there is a highly predictive parameter. Methods: Postnatal day 21 rats were subjected to unilateral common carotid artery ligation followed by 80 min hypoxic insult. Each of nine rats was sequentially evaluated with 7-tesla MR/O-15 gas PET imaging system at 2-3h, 24h, 48h, 7, and 41 days after the HI insult. We measured three parameters in the MRI, i.e. T2-weighted signal (T2WS), apparent diffusion coefficient (ADC), and fractional anisotropy (FA), and three parameters in the PET, i.e. cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO2), and cerebral blood volume (CBV). We set regions of interest in the cortices, and calculated the ipsilateral/contralateral ratios of each parameter. The brains were removed at 42 days after the HI and evaluated morphological damage by calculating the ipsilateral/contralateral ratios of hemispheric volume. Results: At 2-3h after the HI, the mean ratio of CBF was the only parameter distinctively altered, i.e. decreased. At 24h after the HI, the mean ratio of T2WS increased, and that of ADC decreased, while the ratio of CBF returned to

normal. At 48h after the HI, the ratios of FA and CMRO2 decreased. T2WS at 24h and ADC at 24h and 48h after the HI significantly correlated with the later brain damage. CMRO2 at 24h and 48h after the HI exhibited trend in correlation with the later brain damage. Other than those parameters at the specific time points, no parameters correlated with the later brain damage. Conclusions: Our MR/PET imaging revealed temporal changes in brain injury and hemodynamics in immature rats with HI. T2WS, ADC, and CMRO2 at subacute phase were predictive of the later brain damage. This study showed that predictive value of the MR/PET imaging is parameter- and timing-dependent.

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Poster

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Topic: C.08. Ischemia

Support: Waisman P30 HD 03352

NCATS KL2 TR000428

NCATS UL1 TR0000427

Title: Crosstalk of estrogen receptor alpha with TrkB receptors in sexed hippocampal neurons after *in vitro* ischemia

Authors: V. CHANANA[1], W. SUN[1], D. KINTNER[1], E. UDHO[1], P. FERRAZZANO[1], R. A. SHAPIRO[2], J. E. LEVINE[2], *P. CENGIZ[3];
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Abstract: Objective: Hypoxia-ischemia (HI) related brain injury after perinatal asphyxia is a major cause of death and life-long disability. We recently showed in-vivo that the hippocampal ERα expression increases significantly in the female hippocampi compared to males post-HI. In addition, the sexually differentiated TrkB phosphorylation gets ablated in ERα knockout (KO) mice, indicating a role of ERα in conferring responsiveness to the HI and the potent/selective TrkB agonist [7,8 dihydroxyflavone (7,8-DHF)]. Cytoplasmic Src Family kinase, src, got

phosphorylated significantly in the female hippocampus but not in males post-HI and after 7,8-DHF therapy, a response which also got ablated in ERα KO mice in-vivo. We hypothesize that ERα is required for src phosphorylation which in turn results in TrkB phosphorylation in primary sexed hippocampal neurons after *in vitro* ischemia. We intend to define the pathway through which this mechanism works in sexed primary hippocampal neuronal cultures *in vitro* using ER WT and ERα KO mice. Methods: Sexed hippocampal primary neuronal cultures were prepared from 1-day old C57BL/6J ERα WT and ERα KO mouse pups as described previously. Neurons were grown on coverslips after being treated with Ara-C to kill astrocytes and then exposed to either normoxia or OGD (1% O2, 5% CO2, balance N2, 37ºC) for four hours at DIV 7. After 24 hours of REOX, cells were stained by either Hoechst, calcein and propidium iodide (PI) for cell survival studies or with ERα, p-src or p-TrkB. Fluorescent signals from culture dish were obtained by confocal microscope and by imaging 6-9 random fields (20x). The results from each coverslip were averaged. For multiple comparisons ANOVA was used. Results: There were increased ERα and p-TrkB expressions in female primary hippocampal neurons compared to males after OGD-REOX. In addition, TrkB agonist therapy (7,8-DHF) increased the p-TrkB expression further and decreased the cell death only in female hippocampal neurons following OGD-REOX. The p-TrkB expression was ablated in both male and female hippocampal neurons obtained from ERαKO mice. Conclusion: There is sexually differential response of the hippocampal neurons to TrkB signaling mediated by the ERα, favoring females after *in vitro* ischemia. We will be testing the role of the src in mediating the cross-talk between the ERα and TrkB by using an SFK inhibitor and genetically knocking down the src. Identifying the role of ERα-SFK-TrkB interaction in neuronal survival will provide a new target for preventive and therapeutic interventions post-HI.

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Poster

136. Ischemia: Perinatal

Location: Hall A

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Topic: C.08. Ischemia

Support: Basque Government IT 773/13.

BFI-2011-129

Title: Combination of resveratrol and docosahexanoic acid shows a synergic effect raising their neuroprotective effects in perinatal hypoxia-ischemia in rats

Authors: *A. ALVAREZ[1], O. ARTEAGA[2], E. HILARIO[2], L. URIGÜEN[1], M. REVUELTA[2]; [2]Cell Biol., [1]Univ. of Basque Country, Leioa, Spain

Abstract: Neonatal hypoxia-ischemia (HI) brain injury remains one of the major causes of life-long neurological morbidity. Docosahexanoic acid (DHA) and resveratrol (RVT) are antioxidants whose interest has grown due to their neuroprotective possibilities. DHA is a long-chain omega-3 fatty acid, commonly found in fish such as salmon and tuna, while RVT is a natural polyphenol found in different plant species, including grapevines, peanuts and pomegranates. The aim of the present work was to evaluated the protective effects of the combination of resveratrol and docosahexanoic acid when administered before HI brain injury in neonatal rats using the Rice-Vannucci model. Seven-day-old (P7) rats were randomly assigned to: one Control group (n=8), hypoxia-ischemia (HI) group (n=8) and a group of HI animals that received a single dose of a combination of DHA (1 mg/kg) and RVT (20 mg/kg) 10 minutes before hypoxia (RVT+ DHA, n=8). Injury was induced by permanent ligature of the left common carotid artery and then by asphyxia for 2 hours and 15 minutes with 8% O2. On P14 brains were evaluated neuronal and oligondendroglial injury and on P90 we evaluated the long-lasting memory impairments by T-maze and novel object recognition tests. One-way analysis of variance followed by Bonferroni-Dunn correction was performed and $P<0.0001$ was considered to indicate a statistically significant difference. Microscopic photographs and a semi-quantitative neuropathological scoring system demonstrated a damage located at the level of hippocampus and parietal cortex of ipsilateral hemisphere in HI group, but this damage was reduced by RVT+DHA. HI animals also showed a significant decrease in Myelin Basic Protein-immunostaining pattern in comparison with the Control group that was restored with the pretreatment. On PN90, animals pretreated with RVT+DHA performed better at the T-maze and novel object recognition tests than HI animals, reaching similar values to those of control rats. Our results suggest that a pretreatment with a combination of resveratrol and docosahexanoic acid led to a neuroprotective effect by reducing the neuronal damage and preserving myelin in short-term and improving cognitive impairments in long-term.

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Poster

137. Sensory Disorders: Auditory

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Topic: C.13. Sensory Disorders

Support: ONR grant N000141210731

Title: Tinnitus profoundly modifies the dynamics of neuronal rhythms of the thalamo-cortical circuitry of a rodent experimental model of this brain disorder

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Abstract: Tinnitus is a brain disorder characterized by the perception of a phantom ringing sensation. Approximately 1% of adults seek medical treatment for severe debilitating tinnitus. However, there are no FDA approved drugs to treat tinnitus and the lack of safe and effective treatment is due to our poor understanding of the cellular mechanisms underlying tinnitus. The "thalamocortical dysrhythmia model" to explain tinnitus proposes that tinnitus arises from a persistent miscommunication between the thalamus and cortex. However, there is little or no data in animals attempting to explore whether tinnitus changes the thalamo-cortical rhythmic patterns. Here, we performed 32 multi-site simultaneous acute electrophysiological recordings from the Medial Geniculate Body (MGB) and the Primary Auditory Cortex (A1) of anesthetized rodents (n=11) injected with 250 mg/kg of sodium salicylate (SS), a drug that reliably induces tinnitus in rodents and humans. SS induced a threshold shift in the MGB and A1 as well as a neuronal enhancement of suprathreshold response in both structures. Importantly, SS profoundly changed the rhythmic dynamics in the MGB and A1. Spectrogram and power spectrum density analysis in MGB show that SS induced a reduction of oscillatory activity in the theta (4-8 Hz), alpha (8-12 Hz) and beta (15-25 Hz) frequency bands whereas it substantially increased the power of gamma frequency bands (30-80 Hz). Although SS increased the oscillatory activity of theta and alpha as well as a relative increase in theta as compared to alpha frequency bands in some recording sites of the cortex, the most robust finding is that SS decreased the oscillatory activity of theta and alpha frequencies whereas it increased gamma frequency bands. Moreover, spectral analysis of the LFPs in MGB and A1 also revealed a decrease in coherence of low frequency bands (theta, alpha and beta) with a simultaneously increase in coherence at gamma frequency bands in these structures. In conclusion, our results indicate that tinnitus profoundly changes the rhythmic dynamics of large neuronal ensembles in the thalamus and cortex and suggest that high frequency oscillations might be involved in the perception of tinnitus.

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Poster

137. Sensory Disorders: Auditory

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Support: Francqui Foundation fellowship, Belgian American Education Foundation (BAEF)

Tinnitus Research Consortium (TRC)

Title: Effect of modulation of the frontostriatal gating system using transcranial direct current stimulation on tinnitus perception

Authors: *A. J. MAUDOUX[1], P. E. TURKELTAUB[2,3], J. P. RUSCHECKER[1];
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Abstract: Background: Tinnitus is defined as a perception of sound in the absence of any external auditory stimuli. Despite the prevalence of this disorder, tinnitus pathophysiology remains poorly understood. We have proposed previously [1] that tinnitus perception arises only if two conditions are met: (1) a tinnitus signal is being generated within the auditory system, and (2) this uninformative signal fails to be suppressed by a cortico-striatal limbic network encompassing ventromedial prefrontal cortex (vmPFC) and nucleus accumbens (NAc) ("gating theory"). Methods: In our current study, we have chosen to test this model using a combination of transcranial direct current stimulation (tDCS) and functional magnetic resonance imaging (fMRI). In a randomized, doubleblind, shamcontrolled cross-over design, chronic tinnitus subjects are submitted to 15 minutes of 2-mA tDCS anodal stimulation of the vmPFC and cathodal stimulation of the right dorsolateral prefrontal cortex (dlPFC). Participants are scanned before and after each of the tDCS sessions. The locations of anode and cathode are based on a recent study showing that this noninvasive direct stimulation of prefrontal cortex can induce neural activity in the distally connected midbrain, with a direct behavioral effect on an attractiveness rating task [2]. Using this stimulation protocol we aim to target major regions of the gating system, such as the prefrontal region and the NAc. Our primary outcome measure is a change in tinnitus intensity and/or distress assessed with a Visual Analog Scale. We are also looking at how tDCS stimulation modifies the pleasantness rating of a series of affective auditory stimuli [3]. During the fMRI session, subjects are listening to tinnitus-like and non-tinnitus sounds and are making pleasantness ratings of these sounds. Results: We will present our

preliminary results regarding the behavioral effects of the tDCS stimulation on the perception of tinnitus and on the pleasantness rating of affective auditory stimuli. The imaging analysis will examine tDCS-induced changes in neural function associated with the behavioral changes. 1.Rauschecker, J.P., A.M. Leaver, and M. Mühlau, Tuning out the noise: limbic-auditory interactions in tinnitus. Neuron, 2010. 66(6): p. 819-26. 2.Chib, V.S., et al., Noninvasive remote activation of the ventral midbrain by transcranial direct current stimulation of prefrontal cortex. Transl Psychiatry, 2013. 3: p. e268. 3.Stevenson, R.A. and T.W. James, Affective auditory stimuli: characterization of the International Affective Digitized Sounds (IADS) by discrete emotional categories. Behav Res Methods, 2008. 40(1): p. 315-21.

Disclosures: **A.J. Maudoux:** None. **P.E. Turkeltaub:** None. **J.P. Ruschecker:** None.

Poster

137. Sensory Disorders: Auditory

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Topic: C.13. Sensory Disorders

Title: Physiological characteristics of multiple auditory fields in the tinnitus animal model

Authors: *E. BUELL[1], M. S. BORLAND[2], M. P. KILGARD[2];
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Abstract: Specific Aim: To test the hypothesis that hyperactivity in cortical regions outside of A1 is associated with the rat model of tinnitus. Tinnitus is associated with abnormal activity throughout the central auditory system and in many non-auditory structures. The proposed experiments will test the hypothesis that tinnitus is an emergent property of pathological neural activity in multiple brain regions. Recordings were made in inferior colliculus and two non-primary auditory fields. Materials and Methods *Subjects* Fifteen female adult Sprague-Dawley rats were used in this study. Seven were noise-exposed as part of the experimental group and eight remained naïve for the control group. *Auditory Brainstem Recordings* Auditory brainstem recordings (ABRs) were recorded using custom headcap electrodes, BRAINWARE v8.12, and a speaker. Recording and reference screws were placed over cerebellum and cerebral cortex. *Tinnitus Testing* The behavioral correlate of tinnitus was determined using the Turner Gap Detection Method. A 50 ms silent gap inserted into the narrowband noise 100 ms before a 100 dB noise burst. Animals pass the task when they are able to detect the silent gap. Animals are placed in “Tinnitus” group when they fail the task for 2+ consecutive weeks. *Anesthetized Recordings* Four parylene coated tungsten microelectrodes record neuron responses from layer

4/5 of the right primary auditory cortex in barbiturate anesthetized rats. The stimuli used to generate neural responses consist of 1,296 tones at 81 frequencies (1 to 32 kHz) and 16 intensities (0 to 75 dB) Current Results We obtained 839 sites in experimental group and 939 sites in naïve group. Our current data suggests that there are significant increases in spontaneous activity for tinnitus in all fields except the anterior field in tinnitus animal models. Excitability (driven spikes) does not currently show significance as in previous experiments, which indicates more data is needed before forming conclusions. Both the posterior field and inferior colliculus show significant shifts in tonotopicity. Conclusions Results from A1 are consistent with research citing cortical changes in response to noise-exposure and tinnitus. Auditory Brainstem Reponses indicate profound losses at high frequencies (32 kHz), moderate losses at mid-frequencies (10-16 kHz), and no loss at low frequencies (4kHz). Hearing loss at tinnitus frequency is consistent with what is often seen clinically. The anterior shows significant changes in latency and bandwidth only. The posterior field shows changes in tonotopicity, which is consistent with previous work demonstrating this field's susceptibility to shifts.

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Poster

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Topic: C.13. Sensory Disorders

Support: NIDCD grant R03DC012871

Title: Tinnitus detection in three mouse models with salicylate injections, noise exposure or optogenetic stimulation as tinnitus inducers

Authors: *K. T. NAKAMOTO, H. ZUO, D. LEI, J. BAO;
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Abstract: Subjective tinnitus, the perception of a phantom sound, is a common phenomenon in humans. Both operant and reflex based tests have been developed to detect tinnitus-like behavior in mice mainly because of its wide ranges of genetically modified strains for mechanistic studies. Recently, the most widely used behavioral paradigm has been the gap prepulse inhibition of the acoustic startle reflex (GPIAS). In GPIAS the startle reflex is elicited by an abrupt onset of a noise burst. This noise burst is presented on a background of continuous noise and a brief silent interval (gap) is inserted into the background noise. Perception of the gap reduces the startle

response in normal animals, but the reduction is thought to be less in animals with tinnitus. The underlying assumption is that the tinnitus will partially fill in the silent gap, reducing the gap inhibition of the start reflex. However, this assumption has recently come into question (Campolo et al., 2013; Eggermont, 2013; Fournier and Hebert, 2013; Radziwon et al., 2015). This has highlighted the need to validate GPIAS with well-established behavioral methods. In the present study, we assessed the validity of GPIAS to detect tinnitus-like behavior in mice, by comparing it to a well-established operant conditioning (OC) method. We used three known manipulations that can create a "phantom" sound (tinnitus): salicylate injections, noise exposure, or optogenetic stimulation of auditory cortex. By comparing the same animal in both OC and GPIAS methods after salicylate injections we demonstrated that OC method was consistent across salicylate injections, while GPIAS was highly variable. More importantly, we did not find any agreement between OC and GPIAS indication of tinnitus. We then used OC and GPIAS over a four month period to measure behavioral indications of tinnitus after unilateral noise exposure. OC indications of tinnitus were consistent across time, however GPIAS indications of tinnitus were not. Again, we did not find agreement between the OC indication of tinnitus and the GPIAS indication of tinnitus. Finally we optogenetically stimulated the auditory cortex of the mice, inducing a "phantom" sound. Again, we did not find agreement between OC and GPIAS. We believe that this evidence demonstrates that the GPIAS is not a reliable indicator of tinnitus in mice, though it may be still useful to monitor functional changes in the auditory system during tinnitus pathogenesis. In short, this study clearly demonstrated the limitation of GPIAS in future tinnitus studies.

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Poster

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Topic: C.13. Sensory Disorders

Support: Action on Hearing Loss International Project Grant G62

Title: Manipulating endocannabinoid signalling in a guinea pig model of tinnitus

Authors: *B. COOMBER[1], J. I. BERGER[1], S. P. H. ALEXANDER[2], A. R. PALMER[1], M. N. WALLACE[1];
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Abstract: Animal models of tinnitus have revealed long-term hyperexcitability and altered neural synchrony, thought to arise from a pathological imbalance between excitatory and inhibitory neurotransmitter systems. The release of neurotransmitters is regulated by neuromodulators, such as endogenous cannabinoids (endocannabinoids). Endocannabinoids are produced on-demand in response to depolarisation and act to regulate presynaptic neurotransmitter release. Cannabinoid drugs are potent anti-nociceptive agents in models of chronic neuropathic pain, a condition that has substantial parallels with tinnitus, i.e. a phantom sensory percept in the absence of sensory input, initiated peripherally through deafferentation and subsequently involving central mechanisms. In the present study, guinea pigs were subjected to either (1) unilateral acoustic over-exposure (AOE), to induce chronic tinnitus, or (2) sodium salicylate administration, to induce acute tinnitus. In animals subjected to AOE, tinnitus was objectively identified with the gap prepulse inhibition of acoustic startle (GPIAS) behavioural test, eight weeks after AOE. Hearing status was assessed using auditory brainstem responses. Animals were then retested on five occasions after being administered either the cannabinoid CB1 receptor agonist arachidonyl-2'-chloroethylamide (ACEA; 1 mg kg-1, i.p.) or drug vehicle. Treatment with ACEA produced variable effects in animals with AOE-induced tinnitus, but did not produce either a consistent augmentation or an attenuation in tinnitus behaviour across all animals. In the second group of animals, GPs were first implanted with electrocorticography (ECoG) multi-electrode arrays, before being administered with sodium salicylate (350 mg kg-1; i.p.) to induce tinnitus, and either ACEA (1 mg kg-1, i.p.) or drug vehicle. Resting-state and auditory-evoked neural activity recorded in awake, freely-moving animals was compared between groups. In salicylate-treated animals resting-state brain activity was altered and auditory-evoked neural responses were enhanced. Preliminary data collected from animals co-administered ACEA and salicylate indicated a reduction in auditory hypersensitivity (induced by salicylate). These data indicate that manipulating endocannabinoid signalling can affect tinnitus-related neural activity, but these changes may be too subtle to detect using the GPIAS behavioural approach.

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Poster

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Topic: C.13. Sensory Disorders

Title: Salicylate-induced changes in brain activity in awake guinea pigs

Authors: *J. BERGER, B. COOMBER, M. N. WALLACE, A. R. PALMER;
MRC Inst. of Hearing Res., Nottingham, United Kingdom

Abstract: Tinnitus - the perception of phantom sounds - chronically affects an estimated 8-15% of people. Tinnitus is difficult to characterise and frequently intractable, and presents a significant burden to healthcare resources. Studies conducted in human subjects with tinnitus have shown altered patterns of resting-state oscillatory brain activity. However, to date this has not been extensively explored using animal models, which allow more invasive examination of changes in neural activity and their correlation with objective behavioural measures of tinnitus. Here, we describe the development of an awake preparation for examining tinnitus-related changes in resting-state and auditory-evoked brain activity. Guinea pigs were implanted with electrocorticography (ECoG) electrode arrays, with electrodes positioned on the surface of the dura over left and right auditory cortex and over the cerebellum, to monitor auditory brainstem responses. ECoG responses were subsequently compared before and two hours after tinnitus induction with sodium salicylate (350 mg kg^{-1}; i.p.), a drug which reliably induces tinnitus in both humans and animals. Subtle salicylate-induced changes in oscillatory activity were observed in all animals recorded from electrodes over auditory cortex, predominantly at low frequencies, i.e. delta, beta, and alpha bands. Click-evoked cortical field potentials were dramatically enhanced (~100% increase) following salicylate treatment, compared with vehicle treatment, potentially indicating increased sensitivity to sound (hyperacusis). These salicylate-induced changes in spontaneous and auditory-evoked cortical potentials may be due to direct effects on neuronal excitability. Furthermore, small increases in neural gap detection thresholds were observed, suggestive of a slight worsening in temporal processing. Tinnitus induction with salicylate is widely used as an acute tinnitus model. The next stage will involve a chronic tinnitus model (induced by acoustic over-exposure), that will allow us to track neural changes throughout tinnitus development in an awake-behaving model.

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Poster

137. Sensory Disorders: Auditory

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Topic: C.13. Sensory Disorders

Title: Circadian influence on the salicylate-induced changes in the acoustic startle responses of rats

Authors: *M. J. FERRAGAMO[1], T. C. SIGAFOOS[2], B. K. TITUS[2], J. M. WOTTON[2]; [1]Biol. and Psychologcal Sci., [2]Gustavus Adolphus Col., Saint Peter, MN

Abstract: Relatively high doses of sodium salicylate (aspirin) are used to induce temporary tinnitus in rodents. A common test of the effectiveness of this treatment is to compare the responses of the gap detection and pre-pulse inhibition (PPI) tasks in the acoustic startle paradigm. The assumption is that the induced tinnitus will partially fill in the gap and thus the signal will be less effective in inhibiting the response to the subsequent pulse. Typically this is reported as percentage suppression which is calculated by comparing the response to the gap-preceded pulse with that of the pulse alone. Circadian activity is known to influence the acoustic startle response and we examined whether time of day would differentially impact these responses to salicylate-induced tinnitus. Ten male Long-Evan rats were tested on gap and PPI (both in a single session) in a repeated measures experiment at four different times (8:00, 12:00, 16:00, 20:00) on different days established by a pseudo-random schedule. The rats were given IP injections of either saline or sodium salicylate (350 mg/kg) two hours prior to testing. Animals were maintained in 12 hours light (8:00 - 16:00) and 12 hours dark condition. As predicted, the salicylate condition caused less suppression of the response when compared to the saline control in the gap detection task but not in the PPI task. However, the change in suppression for the gap task was at least in part mediated by the change in the response to the pulse alone and to the time of day. In the gap detection task there was a decreased response to the pulse in the salicylate condition compared to control and this difference was greatest in the light phase. The gap trials also showed lower responses with salicylate but this varied with time of day with the light onset and offset times having the biggest difference. In the PPI task the response to pulse alone was greater in the dark than the light phase and with salicylate the pulse response increased especially in the dark. This change to pulse alone in the PPI task may indicate salicylate induced hyperacusis which is sensitive to time of day.

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Poster

137. Sensory Disorders: Auditory

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Title: Modulation of glucose transport by the lateral walls of cochlear OHCs

Authors: X.-T. CHENG[1,2], *N. YU[1], F.-B. YANG[1], C. LIU[1], X.-D. WANG[1], R. ZHANG[2], S.-Q. ZHAI[1], S.-M. YANG[1], N. WU[1], Q. SUN[3], T. CONG[1], C.-Q. LIU[1];
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Abstract: More clinical cases showed that the diabetic hearing loss is one of the common complaints of type II diabetic patients. It was known that glucose is a fundamental source of energy for mammalian cells; however, whether glucose is taken up through the lateral walls of cochlear outer hair cells (OHCs) is unknown. The OHC lateral wall is complex, composed of a plasma membrane, cortical lattice, and subsurface cisternae. This study assessed the uptake of glucose by OHCs using live cell microscopy and examined the distributions of glucose transporter isoforms by immunohistochemistry. We found that glucose transporter-4 was mostly expressed on the inner membrane of the OHC's lateral walls of guinea pig. Glucose crossed the lateral walls of OHCs via glucose transporters-4 mainly, and this process could be modulated. These results suggest that the lateral walls are involved in modulating energy transport into OHCs and may be useful to understand the mechanism of diabetic hearing loss.

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Poster

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Topic: C.13. Sensory Disorders

Title: Progressive hearing loss in mice carrying a mutation in the inactive deubiquitinating enzyme usp53

Authors: *M. SCHWANDER[1], M. KAZMIERCZAK[1], S. HARRIS[1], P. KAZMIERCZAK[2], P. SHAH[1], V. STAROVOYTOV[1], K. OHLEMILLER[3];
[1]Dept. of Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ; [2]Inst. for Neurosciences of Montpellier, Montpellier, France; [3]Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Disordered protein ubiquitination has been linked to neurodegenerative disease, yet its role in inner ear homeostasis and hearing loss is largely unknown. Here we show that progressive hearing loss in the ethylnitrosourea (ENU)-generated mambo mouse line is caused by a mutation in Usp53, a member of the deubiquitinating enzyme family. USP53 contains a catalytically inactive ubiquitin-specific protease (USP) domain and is expressed in cochlear hair cells and a subset of supporting cells. While hair cell differentiation is unaffected in mambo mice, outer hair cells rapidly degenerate after the first postnatal week. USP53 colocalizes and interacts with the tight junction scaffolding proteins TJP1 and TJP2 in polarized epithelial cells, suggesting that USP53 is part of the tight junction complex. The barrier properties of tight junctions of the stria vascularis appeared intact in a biotin tracer assay, but the endocochlear potential is reduced in adult mambo mice. Hair cell degeneration in mambo mice precedes endocochlear potential decline and is rescued in cochlear organotypic cultures in low potassium milieu, indicating that hair cell loss is triggered by extracellular factors. Remarkably, heterozygous mambo mice show increased susceptibility to noise injury at high frequencies. We conclude that USP53 is a novel tight junction-associated protein that is essential for the survival of auditory hair cells and normal hearing in mice, possibly by modulating the barrier properties and mechanical stability of tight junctions.

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Poster

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Title: A comparison of projections to primary (A1) and non-primary (PAF) fields of the cat auditory cortex following hearing loss

Authors: *B. BUTLER[1], N. CHABOT[1], S. LOMBER[2];
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Abstract: When a sensory modality is lost, compensatory advantages are often observed in remaining modalities. For example, both humans and animal models show behavioural advantages in peripheral acuity and motion perception following hearing loss. These advantages are thought to result from recruitment of cortical areas that typically process stimuli from the missing modality. While there remains some controversy regarding crossmodal reorganization of primary sensory cortices, there is growing evidence for age-dependent changes in higher-level cortical structures across modalities and species. Indeed, visually-evoked activity has been observed in the posterior auditory field (PAF) in the cat cortex using functional MRI. This current study examines how patterns of thalamo-cortical and cortico-cortical projections to PAF are altered following deafness in an effort to determine the anatomical basis of these functional changes. A retrograde neuronal tracer (biotinylated dextran amine; BDA) was deposited in the PAF of hearing and ototoxically-deafened cats. Coronal sections were taken and neurons showing a positive retrograde labeling were counted and assigned to cortical and thalamic areas according to published criteria. The proportion of labelled neurons in each area was determined in relation to the total number of labeled neurons in the entire brain. Group level analyses of the proportion of labels arising from auditory and non-auditory fields will be discussed to illustrate the intra- and inter-modal effects of hearing loss. Additionally, the results of cats deafened at the onset of hearing will be compared to those deafened following normal auditory development to examine those effects that are related to the age at the onset of deafness. Finally, these changes in high-level auditory cortex will be compared to changes in a primary field (A1) to illustrate differences in the capacity for reorganization between primary and non-primary areas.

Disclosures: **B. Butler:** None. **N. Chabot:** None. **S. Lomber:** None.

Poster

137. Sensory Disorders: Auditory

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 137.11/F40

Topic: C.13. Sensory Disorders

Title: Blast-induced hearing loss and differential gene expression in the inner ear and brainstem of rat

Authors: *Y. WANG, Y. WEI, S. OGUNTAYO, Y. SU, D. WILDER, I. GIST, P. ARUN, J. B. LONG;
Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Blast exposure may cause long term neurological and neurosensory deficits. Hearing loss, tinnitus and dizziness are disabilities commonly experienced by victims exposed to improvised explosive devices. However, the mechanisms underlying these injuries are largely undefined. In previous studies, we have demonstrated that closely coupled blast exposures caused motor coordination defects and axonal degeneration in rodents. The present research aimed to investigate the changes in auditory function and gene expression in the inner ear and brainstem structures involved in auditory signal processing. An air-driven shock tube was used to simulate primary blast on anesthetized SD rats (male, ~350 g). We have evaluated time-course effects of blast exposure on auditory function by assessing auditory brainstem response (ABR). The differentially expressed genes were screened using RT^2 Profiler PCR Array system (QIAGEN). Audiometry data revealed that blast exposure caused: (1) significant elevation of ABR threshold; (2) increased latency on ABR waves I, II and V; and (3) reduction in ABR amplitude immediately following insult which persisted over 7 days. These changes were over the entire acoustic frequency spectrum. Compared to lower frequency (2 KHz), ABR response to high frequency (32 KHz) tone stimulus displayed a stronger impairment after injury. Among the genes involved in inflammation, DNA repair and neural growth, we found IL-1β, IL-6, TNF, CCL2, CCL12 and Prok2 were significantly up-regulated, while Trpv3, Ngf and Ntf4 were down-regulated in the inner ear and brainstem at 6 h post injury. The results indicate that both of peripheral and central auditory systems are vulnerable to blast injuries. Neuroinflammation, which occurred during the early phase post injury, could be a major factor leading to secondary neuronal damage.

Disclosures: **Y. Wang:** None. **Y. Wei:** None. **S. Oguntayo:** None. **Y. Su:** None. **D. Wilder:** None. **I. Gist:** None. **P. Arun:** None. **J.B. Long:** None.

Poster

137. Sensory Disorders: Auditory

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Topic: C.13. Sensory Disorders

Support: NSERC Discovery Grant

CIHR Open Operating Grant

Title: Cortical crossmodal plasticity following adult-onset noise-induced hearing loss in multisensory and auditory areas

Authors: *A. L. SCHORMANS, M. TYPLT, B. L. ALLMAN;
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Abstract: Complete or partial hearing loss results in crossmodal plasticity, which is characterized by an increased responsiveness of neurons to other sensory modalities (e.g., auditory neurons become more responsive to visual and/or tactile stimuli). At present it is relatively unknown how adult-onset partial hearing loss affects areas of the cortex that are already capable of integrating multisensory information. Thus, we investigated the effect of noise-induced hearing loss on neurons in the dorsal auditory area (AuD) of the rat, as well as the neighboring lateral extrastriate visual cortex (V2L), an area known to integrate audiovisual stimuli. Adult male rats underwent an auditory brainstem response (ABR) test to determine their baseline hearing levels, followed by a bilateral noise exposure (0.8-20 kHz at 120dB for 2 hours). Two weeks later, hearing levels were reassessed, and an acute electrophysiological recording experiment was performed under ketamine/xylazine anesthesia. Using a 32-channel electrode, recordings were completed with either a dorsal/medial-to-ventral/lateral approach to record single neuron activity, or a laminar approach to simultaneously record local field potentials (LFPs) across the cortical layers. For each electrode penetration, computer generated auditory (noise burst), visual (light flash) and combined audiovisual stimuli were delivered, and the associated spiking activity and LFPs were recorded. In age-matched controls, the same ABR and acute electrophysiology experiments were performed. Noise exposure increased ABR threshold by 14.3 ± 4 dB and decreased wave 1 amplitude by 50%. In comparing noise exposed rats to controls, the proportion of visual neurons in V2L, increased 38%, and the proportion of multisensory neurons moderately decreased 17%. This reduction in multisensory neurons in V2L was inconsistent with the crossmodal plasticity observed in AuD, where the proportion of multisensory neurons nearly doubled. Current source density (CSD) analysis revealed altered laminar processing in AuD following noise exposure, in which there was a near doubling of sink amplitudes to auditory and audiovisual stimuli in most of the cortical layers. In contrast, no changes in sink amplitude were observed in V2L. Overall, noise-induced hearing loss resulted in an increase in the proportion of visually-responsive neurons; however, the degree and nature of crossmodal plasticity differed across cortical areas such that only the dorsal auditory area, AuD, showed altered laminar processing and an increased proportion of neurons capable of processing multisensory information following the adult-onset partial hearing loss.

Disclosures: **A.L. Schormans:** None. **M. Typlt:** None. **B.L. Allman:** None.

Poster

137. Sensory Disorders: Auditory

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Topic: C.13. Sensory Disorders

Support: NIH Grant R01DC013275

NIH grant MH43987

Title: Differentiation of spiral ganglion-derived neural stem cells into functional synaptogenetic neurons

Authors: *X. LI, A. ALEARDI, J. WANG, Y. ZHOU, R. ANDRADE, Z. HU;
Wayne State Univ., Detroit, MI

Abstract: Spiral ganglion neurons (SGNs) transmit sound signals from the inner ear to the brainstem, but they are usually damaged in sensorineural hearing loss. SGN-derived neural stem cells (NSCs) have been identified and proposed to differentiate into neurons to replace damaged SGNs. However, it remains unclear whether SGN-NSC-derived neurons are electrophysiologically functional and possess the capability to form neural connections. In this study, postnatal mouse spiral ganglia were harvested and cultured in suspension, followed by adherent cultures to study neuronal differentiation and function. It was found that SGN-derived cells demonstrated NSC characteristics, as they were able to proliferate to form neurospheres and express NSC markers Sox2 and nestin. These SGN-NSCs differentiated into bipolar SGN-like glutamatergic neurons expressing neuronal proteins (TUJ1, neurofilament, NeuN, and NeuroD) and the glutamatergic protein VGLUT1. Brain derived neurotrophic factor (BDNF) significantly increased neuronal differentiation and the neurite length of SGN-NSC derived neurons (ScNs). Patch clamp recording revealed that newly-generated ScNs possessed SGN-like NaV and HCN channels, suggesting that ScNs were electrophysiologically functional. Immunofluorescence showed positive synaptic protein staining in ScNs cultures, indicating that ScNs possess the capabilities to form neural connections. Moreover, astrocyte condition medium was found to be able to stimulate ScNs to express synaptic proteins. These data suggested that BDNF is able to stimulate postnatal mammalian SGN-NSCs to differentiate into glutamatergic, functional ScNs with the capability to form synaptic connections *in vitro*. This study opens avenues to develop stem cell-based replacement to substitute the function of damaged neurons in the peripheral sensory system.

Disclosures: **X. Li:** None. **A. Aleardi:** None. **J. Wang:** None. **Y. Zhou:** None. **R. Andrade:** None. **Z. Hu:** None.

Poster

137. Sensory Disorders: Auditory

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Program#/Poster#: 137.14/F43

Topic: C.13. Sensory Disorders

Title: Two amusic stroke patients: Behavior and imaging results

Authors: ***S. ROSEMANN**, P. ERHARD, M. FAHLE;
Univ. of Bremen, Bremen, Germany

Abstract: Music processing involves perception and cognitive analysis of melodic and temporal information and is accomplished by a distributed neuronal network including auditory cortices, frontal areas and basal ganglia. A stroke affecting one of these regions may impair music perception. The deficit termed amusia can affect both the melodic and rhythmic stream of music perception. The aim of this study was to find anatomical and functional correlates of amusia by means of functional MRI. Two amusic stroke patients and 16 healthy control subjects of the same age participated. Patient 1 had a right basal ganglia infarction and deficits in rhythm perception. Patient 2 had a left fronto-parietal lesion and severe deficits in melodic and moderate deficits in rhythmic perception. The functional MRI paradigm consisted of stimulation alternating with a rest condition in a 3 tesla scanner (Siemens). Stimulation sequences consisted of a section of a German musical and contained visual and/or auditory material without any task for the subjects. Our main aim was to identify brain regions associated with processing these stimuli in healthy subjects and to compare them with those of both of our patients. The rest condition was contrasted with activation associated with a) unimodal auditory, b) unimodal visual and c) bimodal visual and auditory perception. Group analysis of the healthy control subjects was based on a random effect analysis with separate subject predictors ($p<0.001$, uncorrected, cluster size > 25 voxels). Bainvoyager QX software served to analyze the data. The superior temporal cortex, occipital and occipito-temporal cortex, intraparietal sulcus, superior parietal lobule, parts of pre- and primary motor cortex, Wernicke and Broca areas were all activated in healthy subjects. Amusia-patients showed similar activation patterns for visual and auditory cortices. Parietal activation was missing completely in patient 2 for all contrasts. Patient 1 showed a disturbed activation pattern in parietal and frontal regions (ten patches of interest in somatosensory cortices) for auditory stimulation and no parietal activation for bimodal

stimulation. The lesions of both patients correspond to the network involved in healthy musical processing and suggest that basal ganglia (rhythmic) and frontal (melodic) regions are involved in the cognitive analysis of music. Both patients presented a disturbed activation pattern in the parietal cortex for both unimodal and bimodal auditory stimuli. This suggests that parietal regions play a major role in music perception.

Disclosures: S. Rosemann: None. **P. Erhard:** None. **M. Fahle:** None.

Poster

138. Sensory Disorders: Visual System

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Topic: C.13. Sensory Disorders

Support: NIH Grant EY022358

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Title: Three-dimensional ultrastructure of retinocollicular synapse mitochondria in early glaucoma

Authors: *B. R. SCHOFIELD[1], M. A. SMITH[2], C. Z. XIA[2], K. M. FENING[2], C. M. DENGLER-CRISH[2], D. M. INMAN[2], S. D. CRISH[2];
[1]Anat and Neurobiol, [2]Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH

Abstract: We have previously reported persistence of retinal ganglion cell terminals (RGC) in the superior colliculus after axon transport deficits are seen in the DBA/2J mouse model of high-pressure glaucoma. The vast majority of these synapses exhibited normal synaptic structures, including large mitochondria with sparse cristae, round, clear synaptic vesicles, and dense active zones. Paralleling morphometric changes in other RGC compartments in this model, RGC synapses and their mitochondria increase in volume early in the progression of pathology with later reductions in both measures. However, it is unclear how functional these hypertrophic mitochondria are at these stages, or their relationship to other aspects of synaptic changes. Using serial blockface scanning electron microscopy, we examined the ultrastructure of retinocollicular synapses in glaucomatous DBA/2J mice and age-matched controls. We found that, even though there were significant reductions in mitochondrial health score compared to controls, the overwhelming majority of mitochondria in our glaucoma models appeared healthy, a measure determined primarily by cristae appearance and density. Collicular areas exhibiting anterograde

axonal transport deficits had a greater number of unhealthy mitochondria and fewer active zones, suggesting reduced oxidative capacity and signaling capability. These findings suggest an early defect in synaptic metabolism and function in this model and may present useful therapeutic targets for glaucoma.

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Poster

138. Sensory Disorders: Visual System

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 138.02/G1

Topic: C.13. Sensory Disorders

Title: Relationship of retinal ganglion cell number and function in the glaucomatous murine eye

Authors: *L. L. COUGHLIN[1,2], D. M. INMAN[1];
[1]Dept. of Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [2]Sch. of Biomed. Sci., Kent State Univ., Kent, OH

Abstract: Glaucoma is an optic neuropathy that results in progressive and irreversible blindness due to retinal ganglion cell (RGC) axon degeneration. The exact mechanism that leads to RGC degeneration is not yet known. Neuroprotection has become a focus of therapeutic development in glaucoma, the rationale being that preserving RGCs will preserve vision. Inherent in this view is the notion that more RGCs translates to better vision. We decided to examine this assumption in the DBA/2J (D2) mouse, an inbred strain that develops glaucoma secondary to iris pigment dispersion disease-related increases in intraocular pressure (IOP). Visual acuities in young (5 month) and old (12 month) D2 mice were measured using a forced choice swim task. Mice were trained to associate a submerged platform with a vertical sinusoidal grating. By changing the cycles/degree of the sinusoidal grating, we could detect visual perceptual threshold based on the success of the mice to find the submerged platform. After visual acuities were established, RGCs were retrogradely labeled using FluoroGold (FG). We also injected Cholera Toxin B (CTB) into the vitreous chamber to anterogradely trace RGC axons. After quantifying FG-positive RGCs, we determined that overall visual acuity did not correlate well with RGC number in neither the young nor old age groups. Interestingly, 58 percent of the old D2 mice had a significant discrepancy in RGC number between the left and right eyes, suggesting that mice could compensate for extreme RGC loss by relying on remaining function in the more intact eye. The wide range of RGC number within an established visual acuity indicates that cell counts do not

capture function; therefore, functional testing should be an essential component of neuroprotective therapeutic development.

Disclosures: L.L. Coughlin: None. **D.M. Inman:** None.

Poster

138. Sensory Disorders: Visual System

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Topic: C.13. Sensory Disorders

Support: Project No.CityU111013

Title: Electrophysiology alterations in primary visual cortex neurons in the s334ter-line3 transgenic rat

Authors: *K. CHEN[1], Y. WANG[2], L. CHAN[2];
[1]Univ. of Electronic Sci. and Technol. of, Chengdu Sichuan, China; [2]City Univ. of HongKong, HongKong, China

Abstract: The role of the dynamic nature of the brain is critical in the success of treatments aimed at restoring visions at the retinal level, such as bioelectronic implants and stem-cell therapy. The success of these treatments highly relies on the functionality of the surviving neurons along the entire visual pathway. Hence, not only the retina but the visual cortex also plays an important role to allow patients suffering from retinal disease to perceive the fine details of a visual scene. The S334ter-line-3 rat is a transgenic model of retinal degeneration (RD) developed to express a rhodopsin mutation similar to that found in human retinitis pigmentosa (RP) patients. The electrophysiological properties on retina level during the progress of degeneration have been well investigated, however, little is known about the changes of electrophysiological properties in primary visual cortex (V1) during the course of retinal degeneration. By conducting the extracellular recording, we examined the electrophysiological properties of primary visual cortex in rat RD S334ter line-3 model. We have measured the orientation tuning, spatial and temporal frequency tuning and receptive field size from 18 S334ter line-3 rats and 16 long-Evans rat . Comparing to the control group (long-Evans rat), the V1 neurons in S334ter line-3 rats showed weaker orientation selectivity, lower values in optimal spatial and temporal frequency, but larger receptive field size. In addition, we found the V1 neurons in RD model have much stronger spontaneous neural firing than the control group.

These results suggest the visual cognitive ability significantly changed during the retinal degeneration.

Disclosures: **K. Chen:** None. **Y. Wang:** None. **L. Chan:** None.

Poster

138. Sensory Disorders: Visual System

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Support: Grant-in-Aid for Young Scientists (B) from JSPS (KAKENHI No. 26860150) to T. Ishii

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Title: Crizotinib disrupt visual processing in the mouse retina

Authors: T. ISHII[1], S. IWASAWA[2], R. KURIMOTO[2], A. MAEDA[1,3], Y. TAKIGUCHI[2], *M. KANEDA[1];
[1]Nippon Med. Sch., Tokyo, Japan; [2]Chiba Univ., Chiba, Japan; [3]Tokyo Med. Care and Welfare Vocational Sch., Tokyo, Japan

Abstract: Molecular target therapy for cancer is characterized by unique adverse effects that are not usually observed with cytotoxic chemotherapy. For example, the anaplastic lymphoma kinase (ALK)-tyrosine kinase inhibitor crizotinib causes characteristic visual disturbances, whereas such effects are rare when another ALK-tyrosine kinase inhibitor, alectinib, is used. To elucidate the mechanism responsible for these visual disturbances, the responses to light exhibited by retinal ganglion cells treated with these ALK-tyrosine kinase inhibitors were evaluated using a multi-electrode array system in the mouse retina. Both crizotinib and alectinib changed the firing rate of ON and OFF type retinal ganglion cells. Both an increase in firing rate and a decrease in firing rate were observed. However, the ratio of alectinib-affected cells (15.7%) was significantly lower than that of crizotinib-affected cells (38.6%). Furthermore, these drugs changed the response properties to light stimuli of retinal ganglion cells in some of the affected cells, i.e., OFF cells responded to both ON and OFF stimuli, etc. Finally, the expressions of ALK (a target receptor of both crizotinib and alectinib) and of MET and ROS1 (additional

target receptors of crizotinib) were observed at the mRNA level in the retina. Our findings suggest that these drugs might target retinal ganglion cells and that the potency of the drug actions on the light responses of retinal ganglion cells might be responsible for the difference in the frequencies of visual disturbances observed between patients treated with crizotinib and those treated with alectinib.

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Poster

138. Sensory Disorders: Visual System

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Topic: C.13. Sensory Disorders

Support: FNRS

ERC "facessvep 284025"

Title: Rapid and objective quantification of high-level visual impairment with fast periodic oddball stimulation in acquired prosopagnosia

Authors: ***J. LIU-SHUANG**[1], K. TORFS[1], A. NORCIA[2], B. ROSSION[1];
[1]IPSY, Louvain-la-Neuve, Belgium; [2]Stanford Univ., Stanford, CA

Abstract: Perceptual deficits are common in many neurological conditions, but their assessment can be hindered by many unrelated factors (e.g. attention, comprehension, motor impairments…). Hence, a method allowing for an objective, sensitive, and efficient quantification of perception without requiring explicit behavioural output would be highly valuable. In the recently developed fast periodic oddball paradigm, base stimuli appear at a fixed rate (F Hz) with oddball stimuli, differing on a dimension of interest, inserted at regular intervals (every nth stimulus, or F/n Hz; Liu-Shuang et al., 2014, Neuropsychologia, 52, 57-72). Periodic EEG responses at the F/n Hz oddball frequency and harmonics (2F/n Hz, 3F/n Hz…) reflect the perceptual discrimination between base and oddball stimuli. We tested this approach with PS, well-

described patient who is specifically impaired at face individualisation following brain damage (acquired prosopagnosia). PS was first presented with sequences of base "object" stimuli at 6 Hz with periodically interleaved oddball "face" stimuli at 1.2 Hz (every 5th stimulus). In line with her preserved ability to detect faces, PS showed periodic oddball responses within the normal range. However, when face identity discrimination was tested with "different" oddball face identities (B, C, D···) inserted into sequences containing a repeated "same" base face identity (A; sequence structure: AAAABAAAACAA···, Liu-Shuang et al., 2014), significant oddball responses were found in all control (young and age-matched) participants but were absent for patient PS. These observations were obtained within 8 and 12 min of recording, respectively. Overall, our findings underline the value of the fast periodic oddball paradigm as a diagnostic tool for the rapid and objective characterisation of visual discrimination in neuropsychology.

Disclosures: J. Liu-Shuang: None. **K. Torfs:** None. **A. Norcia:** None. **B. Rossion:** None.

Poster

138. Sensory Disorders: Visual System

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Support: NINDS Grant P01NS045260-01

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Title: Prevention of retinal ganglion neuron degeneration by a selective calpain-2 inhibitor in a mouse model of acute glaucoma

Authors: *Y. WANG, D. LOPEZ, K. NGUYEN, P. DAVEY, Y. LIU, E. MARQUEZ, X. BI, M. BAUDRY;
Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Primary angle-closure glaucoma (PACG) is a major cause of blindness in the world and is expected to become more prevalent as longevity increases. Angle-closure glaucoma produces a rapid rise in intraocular pressure (IOP), which results in retinal ischemia and retinal ganglion cell (RGC) death. However, the mechanisms by which increased IOP leads to RGC death are not currently understood. Previous studies have shown that NMDA receptor blockade as well as several pan-calpain inhibitors reduced RGC loss after retinal ischemia. However, none of the studies have specifically addressed the roles of the two major calpain isoforms in the

retina, calpain-1 and calpain-2. In this study, we first determined the time-course of calpain-1 and calpain-2 activation following increased IOP by analyzing changes in immunoreactivity for the spectrin breakdown product (SBDP) generated by either calpain-1 or calpain-2 and for PTEN, a calpain-2 selective substrate in the inner plexiform layer (IPL) containing dendrites of RGC. We also compared the time-courses in wild-type (wt) and calpain-1 knock-out (ko) mice. While calpain-1 was rapidly activated in the IPL at 2 h following increased IOP, calpain-2 activation was delayed and was only observed in the retina at 4 h after increased IOP. Increased IOP for 1 h resulted in about 42% decrease in the number of RGC in wt mice after 3 days. Calpain-1 ko mice exhibited more degenerating RGCs (about 63% decrease) than wt mice, supporting the notion that calpain-1 activation is neuroprotective. Systemic or intravitreal injection of a selective calpain-2 inhibitor (C2I) 2 h after increased IOP prevented calpain-2 but not calpain-1 activation, and promoted RGC survival (about 21% decrease after systemic injection and 19% decrease after intravitreal injection). Our data indicate that calpain-2 activation plays a critical role in acute glaucoma-induced RGN degeneration and support the idea that a selective calpain-2 inhibitor has the potential to prevent acute glaucoma-induced blindness.

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Poster

138. Sensory Disorders: Visual System

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Program of Introducing Talents of Discipline to Universities

Title: Brain-derived neurotrophic factor protects large retinal ganglion cells following acute optic nerve crush injury

Authors: *L. FENG[1], Z. PUYANG[4], H. CHEN[1], P. LIANG[4], J. B. TROY[2], X. LIU[1,3]; [1]Ophthalmology, [2]Biomed. Engin., [3]Neurobio., Northwestern Univ., Evanston, IL; [4]Sch. of Biomed. Engin., Shanghai Jiao Tong Univ., Shanghai, China

Abstract: Brain derived neurotrophic factor (BDNF) is known to regulate neuronal differentiation and survival in the central nervous system. For a number of neurodegenerative and psychiatric disorders, BDNF has been shown to play an important role in both their pathogenesis and treatment. Here we investigated whether BDNF preserves retinal ganglion cells (RGCs) against the acute optic nerve crush injury. We performed the partial optic nerve crush (pONC) following published procedures. Using a tamoxifen-induced Cre system, BDNF was over-expressed in the mouse retina following the pONC. We monitored the survival of RGCs expressing Thy-1-YFP transgene with the Micron III *in vivo* imaging system and RGC somas were counted using a customized Matlab program. Mice were sacrificed at different time points post pONC, confocal images were taken, and the numbers of axons were counted. First, we demonstrated that retinal BDNF was up-regulated in the BDNF overexpression (BDNF_OE) mice. *In vivo* retinal images were taken, and large RGC somas were identified, which was confirmed by double-immunostaining with RGC markers. A significant RGC and axon loss was found in control group following the pONC, consistent with previous findings. The numbers of RGC somas were 48 ± 8% and 16 ± 5% at 1- and 2- weeks post pONC, respectively. The number of axons was 25 ± 7% at 2-weeks post pONC. By contrast, more RGCs survived in BDNF_OE mice. The numbers of somas were 70 ± 8% and 36 ± 6% at 1- and 2- weeks post pONC, respectively. The number of axons was 61 ± 9% in BDNF_OE mice at 2-weeks post pONC. Our results demonstrated that the over-expression of BDNF delayed the RGC and axon loss following the partial optic nerve crush injury. Our studies provide new insights on how BDNF protects RGCs and vision against the optic nerve injury.

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Poster

138. Sensory Disorders: Visual System

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Beckman Initiative for Macular Research

Title: A potent, long-lasting photoswitch for restoring visual function to the blind retina

Authors: *I. TOCHITSKY[1], M. KIENZLER[2], V. MESEGUER[2], N. GALLERANI[2], J. TRAUTMAN[3], R. KRAMER[2];
[1]Harvard Med. Sch., Brookline, MA; [2]Univ. of California, Berkeley, Berkeley, CA; [3]Photoswitch Biosciences, Inc., Menlo Park, CA

Abstract: Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are blinding diseases caused by the degeneration of rod and cone photoreceptors, leaving the remainder of the visual system unable to respond to light. We have recently developed a novel pharmacological therapy that can potentially restore visual function in RP and AMD patients. Our strategy involves the use of photoswitches - light sensitive ion channel blockers - to generate visual responses in blind retinas. Here, we describe our most promising photoswitch compound, BENAQ, and evaluate its therapeutic potential. BENAQ restores retinal responses to white light with an intensity equivalent to ordinary daylight in animal models of RP. A single intraocular injection of BENAQ photosensitizes blind mouse retinas for weeks, with no apparent toxicity. BENAQ is the most potent photoswitch described to date, allowing for a larger potential therapeutic window. BENAQ restores visual function in retinas suffering from photoreceptor degeneration, while leaving healthy retinas unaffected. BENAQ's selective action on the blind retina suggests it may photosensitize regions of the retina undergoing degeneration, for example, in early stage RP patients or AMD patients with geographic atrophy, and not interfere with their remaining vision. BENAQ's potency, long lifetime, acceptable light sensitivity and selective targeting to diseased tissue make it a prime drug candidate for vision restoration in patients with RP and AMD.

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Poster

138. Sensory Disorders: Visual System

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 138.09/G8

Topic: C.13. Sensory Disorders

Support: NIH Grant EY018241

NIH Grant EY023937

Thome Foundation

Foundation Fighting Blindness Grant TA-GT-0614-0644

Beckman Initiative for Macular Research

Title: Cell-type-specific mechanism employed by photoswitch compounds to re-animate the blind retina

Authors: *R. H. KRAMER, I. TOCHITSKY, V. MESEGUER, Z. HELFT, N. GALLERANI; Univ. California Berkeley, Berkeley, CA

Abstract: Azobenzene photoswitches, such as AAQ, DENAQ, BENAQ, and QAQ, can confer light sensitivity onto retinal ganglion cells (RGCs) in blind mice. The high-sensitivity, fast kinetics, and low toxicity of these compounds make them interesting candidates as vision-restoring drugs in humans with degenerative blinding diseases, such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD). Remarkably, photosensitization is manifest only in photoreceptor-degenerated strains of mice, but absent from wild-type mice with intact rods and cones. Here we show that P2X receptors (ionotopic receptors for ATP) mediate the entry of photoswitches into RGCs where they associate with voltage-gated ion channels, enabling light to control action potential firing. While photoswitch compounds differ in which ion channels they affect, all of the compounds require membrane permeation through P2X receptors, whose gene expression is up-regulated in degenerated retina. Ordinarily membrane-impermeant fluorescent dyes also penetrate through P2X receptors to label a subset of RGCs in degenerated retina. Dendritic mapping and patch-clamp recording of dye-filled cells suggests that only the OFF-RGCs are labeled and re-animated; On-RGCs are unaffected. Hence P2X receptors are a natural

conduit for cell-type specific delivery of photoswitches to restore visual function in degenerative blinding disease, reinforcing their potential as drugs for treating advanced stages of RP and AMD.

Disclosures: **R.H. Kramer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Photoswitch Biosciences, Inc.. **I. Tochitsky:** None. **V. Meseguer:** None. **Z. Helft:** None. **N. Gallerani:** None.

Poster

138. Sensory Disorders: Visual System

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 138.10/G9

Topic: C.13. Sensory Disorders

Support: Lundbeck Foundation

Title: MEG reveals early activation of the occipital cortex by tactile stimulation in congenitally blind subjects

Authors: ***R. C. KUPERS**[1], F. MÜLLER[1], A. IOANNIDES[2], S. BAILLET[3], M. PTITO[1,4]; [1]Inst. of Neurosci. & Pharmacol. (INF), Copenhagen, Denmark; [2]Lab. for Human Brain Dynamics, AAI Scientific Cultural Services Ltd, Nicosia, Cyprus; [3]4McConnell Brain Imaging Ctr., McGill Univ., Montreal, QC, Canada; [4]Sch. of Optometry, Univ. of Montreal, Montreal, QC, Canada

Abstract: Following loss of vision from birth or later in life, the occipital cortex undergoes cross-modal neuroplastic changes and becomes involved in the processing of a variety of non-visual sensory inputs, and also in cognitive information processing (1). However, it remains unclear through which pathways non-visual information reaches the visual cortex in visually deprived individuals. Several studies suggested a strengthening of existing cortico-cortical pathways (2-5). Using magnetoencephalography (MEG), we recently provided evidence that in congenital blindness, tactile input is routed from somatosensory to occipital cortex via the posterior parietal cortex (6). However, the possibility that non-visual information is rerouted to the occipital cortex via an additional subcortical pathway cannot be ruled out and finds empirical support in some animal studies (7). In order to further elucidate this question, we used MEG to investigate tactile information flow in 8 congenitally blind, 3 late blind and 10 age and sex-matched sighted control subjects. We studied the spatio-temporal characteristics of occipital

cortex activations to electro-tactile stimulation of the index fingers. Our preliminary results show first cross-modal occipital cortex activations in the congenitally blind already at 37 and 55 ms. No such early occipital cortex activations were found in the sighted or late blind subjects. Source analysis revealed that these early occipital activations were located in BA17. In addition, we measured later occipital activations (> 85 ms) in both blind and sighted subjects. The timing of the early occipital responses in the congenital blind subjects suggests a direct rerouting of non-visual information from the thalamus to striate cortex. The later occipital activations may be mediated via a cortico- cortical pathway. Together, our data suggests that tactile information reaches the occipital cortex not only through a cortico-cortical pathway, but likely also through a direct route likely involving a thalamo-cortical pathway. Refs: 1. Kupers et al., Front Psychol. 2011;2:19; 2. Klinge et al., Brain 2010;133:1729-36; 3. Kupers et al., PNAS 2010;107:12716-21; 4. Ptito et al., Brain 2005;128:606-14; 5. Wittenberg et al., Eur J Neurosci 2004;20:1923-27; 6. Ioannides et al., Front Hum Neurosci 2013;7:4292013; 7. Chabot et al. Neurosci. Lett. 2008;433, 129-34.

Disclosures: **R.C. Kupers:** None. **F. Müller:** None. **A. Ioannides:** None. **S. Baillet:** None. **M. Ptito:** None.

Poster

139. Major Mental Disorders: Imaging

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Program#/Poster#: 139.01/G10

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant 1R01MH085667

Pat Rutherford, Jr Chair in Psychiatry at UTHealth

Title: White matter microstructural differences in bipolar disorder detected using ENIGMA-DTI protocols for data harmonization

Authors: ***S. M. KELLY**[1], N. JAHANSHAD[1], J. FASKOWITZ[1], C. CHING[1], D. P. HIBAR[1], G. ZUNTA-SOARES[2], B. MWANGI[2], J. C. SOARES[2], P. M. THOMPSON[1];
[1]Neuroimaging and Informatics, USC, Marina Del Rey, CA; [2]Dept. of Psychiatry and Behavioral Sci., Univ. of Texas, Houston, TX

Abstract: Introduction: Abnormal brain white matter (WM) structure has been reported in bipolar disorder (BP), but not consistently. Collaborative efforts in neuroimaging from the

ENIGMA Consortium have affirmed the importance of large-scale collaborations to increase statistical power and result in more robust estimates of effect size. To identify the most robust disease effects, we applied the ENIGMA-DTI data harmonization protocol to process DTI data from bipolar patients and controls scanned at multiple sites. Methods: We analyzed DTI data from 69 BP-1 patients and 53 healthy controls (HCs) with an age range of 18-64 years and an average age of 34 years. 45% of HCs were male while 42% of patients were male. Of the total sample, 92 participants were scanned using a 3T Siemens Allegra scanner and 30 participants were scanned using a 3T Philips Intera. Preprocessing of the DWI images, including motion/distortion correction and tensor calculation, was carried out using FSL. The ENIGMA-DTI protocols were run on the FA maps, as detailed online: http://enigma.ini.usc.edu/protocols/dti-protocols/. 24 bilateral WM regions of interest (ROIs) were extracted from the skeletonized images and the average FA within each ROI was determined. Cohen's d was calculated for the group difference between patients and controls for all ROIs after controlling for age and sex. Results: Compared to HCs, BP-1 patients showed lower FA in the anterior corona radiata (ACR) and corpus callosum (CC), including the body and genu, and the cingulum (CGC) ($p < 0.0022$, corrected for multiple comparisons). Conclusions: Using the ENIGMA-DTI protocol, we found significant FA differences between BP-1 patients and HCs. This illustrates the sensitivity of the ENIGMA-DTI protocol to detecting WM differences in BP-1 disorder. Using these harmonized protocols, future research will incorporate multi-site data in a large-scale collaboration for meta-analysis. With increased statistical power, subtle differences in WM FA between BP subtypes, and the factors that modulate them, may also be detected.

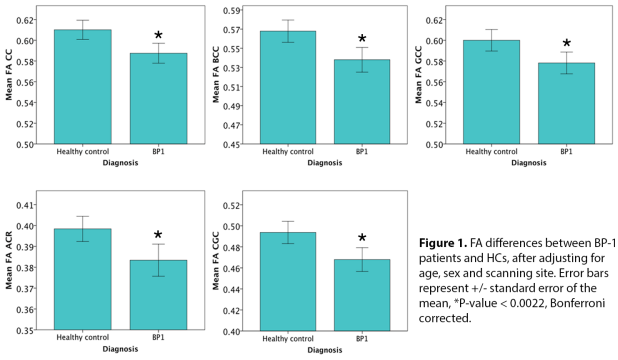

Figure 1. FA differences between BP-1 patients and HCs, after adjusting for age, sex and scanning site. Error bars represent +/- standard error of the mean, *P-value < 0.0022, Bonferroni corrected.

Disclosures: **S.M. Kelly:** None. **N. Jahanshad:** None. **J. Faskowitz:** None. **C. Ching:** None. **D.P. Hibar:** None. **G. Zunta-Soares:** None. **B. Mwangi:** None. **J.C. Soares:** None. **P.M. Thompson:** None.

Poster

139. Major Mental Disorders: Imaging

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 139.02/G11

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Cortico-striatal and amygdala-striatal structural connectivity uniquely relates to risk for bipolar disorder

Authors: *K. DAMME[1], C. YOUNG[2], R. NUSSLOCK[2];
[2]Psychology, [1]Northwestern Univ., Evanston, IL

Abstract: Risk for developing bipolar disorder is associated with particular traits; elevated reward sensitivity, increased goal-directed activity, etc. Elevated striatal activity in hypo/mania may result from differences in cortico-striatal reward connectivity, a synthesis of connections from both the subcortical and cortical-striatal input. The present study used DTI to examine the relationship between structural connectivity within the cortical-striatal and subcortical networks to the Hypomanic Personality Scale, and considers the specificity of these goal-oriented, mania relevant paths to paths generally related to depression, i.e. the uncinate fasciculus (UF). Methods- 49 healthy volunteers were recruited from the Chicago area to complete MRI scanning and the Hypomanic Personality Scale. Hypomanic Personality Scale is related to increased risk of developing bipolar disorder and is robust in subclinical populations. Individually defined masks, specifically the bilateral nucleus accumbens (NAcc), amygdala, and medial orbitofrontal cortex (mOFC), were seed regions for the diffusion weighted probabilistic tractography. Fractional anisotropy (FA) was extracted from the probabilistic tracts. A combined model compared mean FA for cortical and subcortical paths in predicting hypomanic personality traits. Results- Elevated structural connectivity between the Amygdala and NAcc was associated with elevated traits of hypomania ($F(1,47)=9.88$, $p=0.002$). Increased structural connectivity between the mOFC and NAcc were associated with higher symptoms of mania ($F(1,48)=6.945$, $p=0.011$). In a combined model, the structural integrity between Amygdala and NAcc (t-value= 2.55, $p=0.01$) demonstrates a unique contribution and the path between mOFC to NAcc (t-value= 1.92, $p=0.06$) is at a trending level, while the overall model is significant ($F(3, 46)=7.08$, $p=0.002$). This finding is unique to hypomania as the UF, which relates to negative emotionality, is not significantly related to hypomanic traits $F(1, 47)= 0.38$, $p= 0.5408$). Conclusion- These findings suggest that both cortical and subcortical paths account for variance in an overall model of risk for hypomania. Risk for hypomania is associated with increased integrity in white matter connectivity between NAcc and Amygdala, as well as the NAcc and mOFC, each conferring unique contributions in a combined model; this relationship does not hold equally well with all tracts related to emotion. As predicted the UF provides evidence of specificity of these pathways, demonstrating that not all emotion relevant tracts are equally effective at explaining variance in hypomanic risk.

Disclosures: **K. Damme:** None. **C. Young:** None. **R. Nusslock:** None.

Poster

139. Major Mental Disorders: Imaging

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 139.03/G12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: The dopaminergic response to acute stress in health and psychopathology: a systematic review

Authors: *T. VAESSEN, D. HERNAUS, I. MYIN-GERMEYS, T. VAN AMELSVOORT; Maastricht Univ., Maastricht, Netherlands

Abstract: Previous work in animals has shown that dopamine (DA) in cortex and striatum plays an essential role in stress processing. For the first time, we systematically reviewed the *in vivo* evidence for DAergic stress processing in health and psychopathology in humans. All studies included (n studies=25, n observations=324) utilized positron emission tomography and measured DAergic activity during an acute stress challenge. The evidence in healthy volunteers (HV) suggests that physiological, but not psychological, stress consistently increases striatal DA release. Instead, increased medial prefrontal cortex (mPFC) DAergic activity in HV was observed during psychological stress. Across brain regions, stress-related DAergic activity was correlated with the physiological and psychological intensity of the stressor. The magnitude of stress-induced DA release was dependent on rearing conditions, personality traits and genetic variations in several SNPs. In psychopathology, preliminary evidence was found for stress-related dorsal striatal DAergic hyperactivity in psychosis spectrum and a blunted response in chronic cannabis use and pain-related disorders, but results were inconsistent. Physiological stress-induced DAergic activity in striatum in HV may reflect somatosensory properties of the stressor and readiness for active *fight-or-flight* behavior. DAergic activity in HV in the ventral striatum and mPFC may be more related to expectations about the stressor and threat evaluation, respectively. Future studies with increased sample size in HV and psychopathology assessing the function of stress-induced DAergic activity, the association between cortical and subcortical DAergic activity and the direct comparison of different stressors are necessary to conclusively elucidate the role of DAergic activity during stress.

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Poster

139. Major Mental Disorders: Imaging

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 139.04/G13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH094172

Title: Resting state oscillatory activation associated with schizophrenia and high and low cognitive control

Authors: *R. V. HART[1], A. M. BOBILEV[2], W. T. OLIVER[2], M. E. HUDGENS-HANEY[2], L. K. HAYRYNEN[2], D. A. PARKER[2], P. BUCKLEY[3], J. E. MCDOWELL[2], B. A. CLEMENTZ[2]; [1]psychology, [2]Univ. of Georgia, Athens, GA; [3]Georgia Regents Univ., Augusta, GA

Abstract: Poor performance on tasks requiring cognitive control (CC) is a hallmark of schizophrenia (SZ). A substantial portion of the healthy population, however, exhibit similar performance deficits, while manifesting neither signs nor symptoms of SZ. Contrasting these healthy individuals with low CC, individuals with SZ, and also healthy individuals with particularly high CC allows for differentiation of aberrations specific to the pathology of SZ from those merely associated with poor CC. We examine the differences amongst the three groups by utilizing a resting state paradigm, which is a visual stimulus that allows us to see differences in oscillatory power at baseline level activation. This study examines the difference in resting state neural activity between person's with SZ and healthy subjects with high and low CC. Cognitive control was assessed via working memory capacity, measures of which have high reliability and high correlation with performance on CC tasks. In the current study, working memory capacity was determined by a conglomerate score based on performance on computerized operation, symmetry, and reading span tasks. Participants completed a five-minute eyes-open resting state paradigm while whole-head magnetoencephalography (MEG) data were collected. Time-frequency analysis of resting state activity demonstrated differences in oscillatory power between all groups (SZ, High CC, and Low CC) across frequency bands. The Low CC group exhibited commonalities with both the High CC and SZ groups, though these commonalities were found at distinct frequencies. Schizophrenia-specific oscillatory patterns were found in lower frequency bands such as delta and theta. Conversely, the higher frequency bands, including low and high-gamma, show cognitive control-specific activation that differentiates individuals with high CC from those with low CC regardless of DSM diagnosis. Power in alpha band activity is dissociable between all three groups. Results from this study suggest that frequency-specific intrinsic activity may markedly contribute to differences in

cognitive control in both healthy persons and persons with schizophrenia. Further, resting state oscillatory activity in different frequency bands may differentially characterize variation in cognitive control in the healthy population as well as deficits which are exclusive to clinical populations. In this way, resting state neural activity could be a candidate endophenotype for elucidating cognitive control deficits in schizophrenia

Disclosures: R.V. Hart: None. **A.M. Bobilev:** None. **W.T. Oliver:** None. **M.E. Hudgens-Haney:** None. **L.K. Hayrynen:** None. **D.A. Parker:** None. **P. Buckley:** None. **J.E. McDowell:** None. **B.A. Clementz:** None.

Poster

139. Major Mental Disorders: Imaging

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Topic: C.15. Schizophrenia and Bi-polar Disorder

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Grants-in-Aid for Young Scientists B (23791329) from the Japan Society for the Promotion of Science

Title: Cognitive impairments and brain morphological alterations in schizophrenia

Authors: N. MATSUKAWA, *J. MIYATA, H. SASAKI, J. FUJINO, H. TAKAHASHI, T. ASO, S. URAYAMA, H. FUKUYAMA, T. MURAI;
Kyoto Univ., Kyoto, Japan

Abstract: Introduction: It is well known that cognitive deficits affect quality of life and social functions in individuals with schizophrenia. To elucidate the neural basis of cognitive impairments in schizophrenia, we explored associations of cognitive functions with gray matter volume as well as white matter integrity. Methods: Fifty-four individuals with schizophrenia and 45 healthy controls underwent structural magnetic resonance imaging. Cognitive functions were assessed using the brief assessment of cognition in schizophrenia Japanese version (BACS-J), comprising six subscales measuring various dimensions of cognition: verbal memory, working memory, motor speed, verbal fluency, attention and processing speed of information, and executive function. Voxel-based morphometry and tract-based spatial statistics were applied to investigate the correlation of BACS-J scores and regional gray matter volume as well as white matter fractional anisotropy (FA). Results: The schizophrenia group exhibited significant gray matter volume reductions and FA reductions in several regions relative to controls. In the schizophrenia group, the composite score of BACS-J was positively correlated with gray matter volume in inferior and middle frontal gyrus, parahippocampal gyrus as well as anterior cingulate gyrus. The score was also positively correlated with FA in the body of corpus callosum. In some brain regions, morphological change was correlated with multiple subscores of BACS-J, while in others, it was correlated with a specific subscore. Conclusion: Our findings demonstrate that different aspects of cognitive impairments have common as well as specific neural bases in schizophrenia. Comprehensive cognitive assessments are suggested to be important to uncover complex cognition-anatomy correlations in schizophrenia.

Disclosures: N. Matsukawa: None. **J. Miyata:** None. **H. Sasaki:** None. **J. Fujino:** None. **H. Takahashi:** None. **T. Aso:** None. **S. Urayama:** None. **H. Fukuyama:** None. **T. Murai:** None.

Poster

139. Major Mental Disorders: Imaging

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Program#/Poster#: 139.06/G15

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Royal Society Wolfson Research Merit Award

EU Grant BION

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National Centre for Mathematics and Interdisciplinary Sciences (NCMIS) in Chinese Academy of Sciences

Wellcome Trust (Research Training Fellowship WT096002/Z/11)

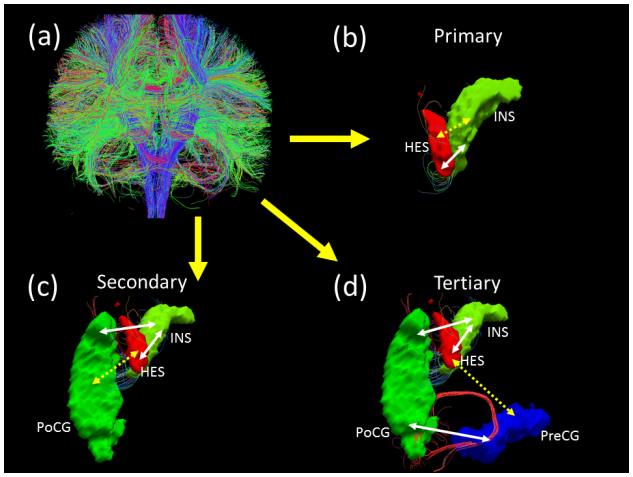
China Scholarship Council (CSC).

National Science Foundation of China NSFC 61104143

Title: Variability of structurally constrained and unconstrained functional connectivity in schizophrenia

Authors: *Y. YAO[1], L. PALANIYAPPAN[2], P. LIDDLE[2], J. ZHANG[3], S. FRANCIS[4], J. FENG[1];
[1]Warwick Univ., Coventry, United Kingdom; [2]Ctr. for Translational Neuroimaging in Mental Health, Inst. of Mental Hlth., Nottingham, United Kingdom; [3]Fudan Univ., Shanghai, China; [4]Sir Peter Mansfield Magnetic Resonance Centre, Sch. of Physics and Astronomy, Univ. of Nottingham, Nottingham, United Kingdom

Abstract: Spatial variation in connectivity is an integral aspect of the brain's architecture. Without this variability, the brain may act as a single homogenous entity without regional specialization. In this study, we investigate the variability in functional links categorized on the basis of the presence of direct structural paths (primary) or indirect paths mediated by one (secondary) or more (tertiary) brain regions ascertained by diffusion tensor imaging. We quantified the variability in functional connectivity using an unbiased estimate of unpredictability (functional connectivity entropy) in a neuropsychiatric disorder where structure-function relationship is considered to be abnormal. 34 patients and 32 healthy controls underwent DTI and resting state functional MRI scans. Less than one-third (27.4% in patients, 27.85% in controls) of functional links between brain regions were regarded as direct primary links on the basis of DTI tractography, while the rest were secondary or tertiary. The most significant changes in the distribution of functional connectivity in schizophrenia occur in indirect tertiary paths with no direct axonal linkage in both early ($p=0.0002$, $d=1.46$) and late ($p=1x10-17$, $d=4.66$) stages of schizophrenia, and are not altered by the severity of symptoms, suggesting that this is an invariant feature of this illness. Unlike those with early stage illness, patients with chronic illness show some additional reduction in the distribution of connectivity among functional links that have direct structural paths ($p=0.08$, $d=0.44$). Our findings address a critical gap in the literature linking structure and function in schizophrenia, and demonstrate for the first time that the abnormal state of functional connectivity preferentially affects structurally unconstrained links in schizophrenia. It also raises the question of a continuum of dysconnectivity ranging from less direct (structurally unconstrained) to more direct (structurally constrained) brain pathways underlying the clinical severity and persistence of schizophrenia.



Disclosures: Y. Yao: None. **L. Palaniyappan:** None. **P. Liddle:** None. **J. Zhang:** None. **S. Francis:** None. **J. Feng:** None.

Poster

139. Major Mental Disorders: Imaging

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 139.07/G16

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Strategic Research Program for Brain Science

JSPS KAKENHI 24591716

JSPS KAKENHI 15K09832

Title: Distinction of brain morphometric abnormalities in bipolar and unipolar depression: A voxel-based multi-center study

Authors: *K. MATSUO[1], K. HARADA[2], Y. OKAMOTO[4], M. OHTA[5], H. NARITA[6], Y. FUJITA[3], G. OKADA[4], M. TAKAMURA[4], H. YAMAGATA[2], I. KUSUMI[6], T. INOUE[6,7], H. KUNUGI[5], S. YAMAWAKI[4], Y. WATANABE[2];
[2]Neuropsychiatry, [3]Biomolecular Engin. Applied Mol. Biosci., [1]Yamaguchi Univ. Grad. Sch. of Med., Ube/Yamaguchi, Japan; [4]Psychiatry and Neurosciences, Hiroshima Univ., Hiroshima, Japan; [5]Mental Disorder Res., Natl. Inst. of Neuroscience, Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Japan; [6]Psychiatry, Hokkaido Univ., Sapporo, Japan; [7]Psychiatry, Tokyo Med. Sch., Tokyo, Japan

Abstract: Diagnostic differentiation of depressed patients with bipolar (BD) and major depressive disorder (MDD) is clinically a crucial issue because the two disorders show similar depressive symptoms and have different strategy for pharmacological treatment. The misdiagnosis of them leads to make the outcomes solemn. However biological markers of the differentiation has been unknown. The aim of this study was to determine whether depressed patients with BD and MDD show different morphometric abnormalities involved in the pathophysiology of these disorders using voxel-based morphometry (VBM) in multi-centers. This study was a part of the Strategic Research Program for Brain Science. Subjects consisted of 140 patients with BD and 569 those with MDD and 717 healthy subjects in 8 scanners of 6 sites. All patients were depressed. Distribution of sex and age were statistically matched between the three groups. Image preprocessing was performed using VBM8 in SPM8 software. We analyzed the gray matter (GM) image implemented a General Linear Model. The analysis was applied to a full-factorial model with two factors with diagnosis with 2 levels (patients and healthy subjects) and scanners with 8 levels and with age, sex and intracranial volume as covariates in SPM8. The whole brain analysis showed significant main effect of diagnosis in GM volume of hippocampus/amygdala complex, insular and medial and orbitofrontal cortex, significant main effect of scanners in GM volume of extra-nuclear and significant interaction of diagnosis with scanner in GM volume of striatal and temporal areas. The GM volume of anterior cingulate, insula and middle frontal gyrus statistically survived by the analysis of the main effect of diagnosis masked exclusively the main effect of scanner. The significant voxels obtained from the analyses were compared among patients with BD and MDD and healthy subjects by analysis of covariance. The GM volumes of the 3 regions showed significantly small in patients with BD, MDD and healthy subjects in the order. The results suggest that depressed patients with the two disorder are distinctive morphometric abnormalities in the brain regions relevant to emotion processing, which abnormalities are observed more pronounced in BD.

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Poster

139. Major Mental Disorders: Imaging

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 139.08/G17

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Wellcome Trust Senior Fellowship (#100227)

JSPS KAKENHI Grant Number 25240019

Title: Volume reduction in insula and frontal lobe in schizophrenia patients: a confirmatory VBM study

Authors: *F. MEGUMI[1], H. KUGA[2], S. MORITA[2], K. KAWAKAMI[2], H. YAO[2], M. HASHIMOTO[2], Y. TAKASHIMA[2], N. ORIBE[2], T. ONITSUKA[2], S. KANBA[2], T. YUZURIHA[2], H. MIZUHARA[3], G. REES[1,4], R. KANAI[5,6], T. UENO[2];
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Abstract: Introduction: Recent studies reported that schizophrenic patients showed focal brain volume reduction in temporal lobe, and frontal lobe (e.g. Borgwardt et al., 2008). In this confirmatory study, we asked if we could obtain similar results reported in previous studies in middle-aged (over 40) chronic schizophrenia patients in Japan. Method: We collected brain structure data with 35 schizophrenia patients (aged 40 - 79) met to DSM-IV criteria from Hizen psychiatric hospital and the ratio of age- and gender-matched 35 normal control from Sefuri town, a rural town in Saga prefecture in Japan. 1.5T MRI machine (Philips Achieva) was used to acquire T1 weighted high-resolution images with 1mm × 1mm × 1mm resolution, with 160 slices covering the whole brain (slice repetition time, 9.30ms; echo time, 4.60ms; flip angle, 10°). We analyzed the structural images with DARTEL tools (Ashburner et al., 2007) in Statistical Parametric Mapping software (SPM12). Results: We asked which area showed gray matter reduction in schizophrenia patients comparing to healthy controls and found significant gray-matter reduction in the frontal lobe and anterior insula cortex and orbitofrontal cortex ($p < 0.05$, FWE corrected). The central functional roles of the insula cortex is to mediate self-consciousness and process pain sensation, and orbitofrontal cortex is known as a part of the pathway of emotion. The current results support the previous studies that patients with schizophrenia show volume reduction in specific brain regions involved in impaired functions in schizophrenia (Hirao et al. 2008; Segall et al., 2009) Conclusion: This study showed that schizophrenia patients had volume reduction in the insula, and orbitofrontal cortex. These results corroborate the previous VBM findings that frontal lobe volume reduction is a characteristic feature of schizophrenia and focal brain structure could potentially be a biomarker to assess the condition of schizophrenic patients.

Disclosures: **F. Megumi:** None. **H. Kuga:** None. **S. Morita:** None. **K. Kawakami:** None. **H. Yao:** None. **M. Hashimoto:** None. **Y. Takashima:** None. **N. Oribe:** None. **T. Onitsuka:** None. **S. Kanba:** None. **T. Yuzuriha:** None. **H. Mizuhara:** None. **G. Rees:** None. **R. Kanai:** A.

Employment/Salary (full or part-time):; ARAYA Brain Imaging. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ARAYA Brain Imaging. **T. Ueno:** None.

Poster

139. Major Mental Disorders: Imaging

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 139.09/G18

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: R01MH094520

R01MH085646

P50MH103222

R01DA027680

T32MH067533

Title: The relationship between glutamate, gaba, and mismatch negativity in schizophrenia

Authors: *S. A. KORENIC, A. SUMMERFELT, S. A. WIJTENBURG, X. DU, J. CHIAPPELLI, F. MUELLERKLEIN, P. KOCHUNOV, L. E. HONG, L. M. ROWLAND; Univ. of Maryland Sch. of Med. (MPRC), Baltimore, MD

Abstract: Auditory mismatch negativity (MMN) is a replicated biomarker for schizophrenia and is thought to reflect glutamate NMDA receptor function and excitatory-inhibitory neurotransmission balance. However, the glutamate-MMN association has not been directly examined. A GABAergic link to MMN is even less clear. This study investigated the relationship between glutamate, GABA, and MMN in schizophrenia. Fifty-three controls and 45 persons with schizophrenia completed this study. Participants completed an EEG session for MMN and a magnetic resonance spectroscopy (MRS) session for glutamate and GABA. The ERP paradigm was a duration-deviant oddball task. Subjects were presented with 1000 auditory stimuli, of which 800 (80%) were standard tones presented at 75 dB, 60-ms, 1000 Hz; 200 (20%) were duration-deviant tones at 75 dB, 150-ms, 1000 Hz. FZ was used for MMN measurement. The center of the MRS voxel was positioned below the electrode FZ and covered the anterior cingulate. For detection of glutamate, spectra were acquired with a phase rotation STEAM: TR/TM/TE = 2000/10/6.5-ms, VOI ~ 24-cm3, NEX=128, 2.5-kHz spectral width, 2048 complex

points. For detection of GABA, spectra were acquired using a macromolecule-suppressed MEGA-PRESS sequence: TR=2000, TE=68 ms, 20.36 ms length and 44 Hz bandwidth full width at half maximum (FWHM) editing pulses applied at 1.9 (ON) and 1.5 (OFF) ppm, and 256 averages. Metabolites were referenced to water and corrected for proportion of CSF, white and gray matter tissue concentrations. The schizophrenia group showed significantly reduced MMN amplitude ($p = 0.039$) but not latency ($p = 0.28$) compared with controls. Glutamate levels were significantly lower in the schizophrenia group compared to the control group ($p = 0.002$) but GABA levels and glutamine/glutamate ratio were not significantly different between groups (p's > 0.05). The relationship between glutamate and MMN amplitude was significant in schizophrenia, such that higher glutamate levels were associated with greater (more negative) MMN amplitude ($r = -0.29$, $p = 0.05$). The smaller the glutamine/glutamate ratio, which could be interpreted as more glutamine to glutamate conversion, was related to greater (more negative) MMN amplitude in schizophrenia ($r= 0.45$, $p =003$). Higher GABA levels were also associated with greater MMN amplitude ($r= -0.39$, $p =008$). There were no significant correlations observed in the control group. This is the first study to show a strong association between *in vivo* glutamate, GABA, and MMN in schizophrenia. These data provide strong support for the involvement of glutamatergic and GABAergic systems in the generation of MMN in schizophrenia.

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Poster

139. Major Mental Disorders: Imaging

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Support: CIHR Grant CBG-101827, MOP-137103

BC Mental Health and Substance Use Services

Title: Characterization of white matter integrity deficits in cocaine-dependent individuals with substance-induced psychosis compared to non-psychotic cocaine users

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Abstract: With sufficient drug exposure, some individuals develop transient psychotic symptoms referred to as "substance induced psychosis" (SIP), which closely resemble the symptoms observed in schizophrenia spectrum disorders. The comparability in psychotic presentation between SIP and the schizophrenias suggests that similar underlying neural deficits may contribute to the emergence of psychosis across these disorders. Only a small number of studies have investigated structural alterations in SIP, and all have been limited to volumetric imaging methods, with none controlling for the effects of chronic drug exposure. To investigate white matter abnormalities associated with SIP, diffusion tensor imaging was employed in a group of individuals with cocaine-associated psychosis (CAP; n=24) and a cocaine dependent nonpsychotic group (CDN; n=43). Tract based spatial statistics (TBSS) was used to investigate group-differences in white matter diffusion parameters. The cocaine-associated psychosis group showed significantly lower fractional anisotropy values than cocaine dependent nonpsychotic group ($p<0.05$) in voxels within white matter tracts of fronto-temporal, fronto-thalamic, and interhemispheric pathways. The greatest magnitudes of difference in white matter integrity were present in the corpus callosum, corona radiata, bilateral superior longitudinal fasciculi, and bilateral inferior longitudinal fasciculi. Additionally, the cocaine-associated psychosis group had voxels of significantly higher radial diffusivity in a subset of the above mentioned pathways. These results are the first description of white matter integrity abnormalities in a SIP sample, and indicate that differences in these pathways may be a shared factor in the expression of different forms of psychosis.

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Poster

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Support: NIH Fellowship F31-MH102879

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Blowitz-Ridgeway Foundation

Brain and Behavior Foundation

Title: Nicotine reduces hippocampal hyperactivity in schizophrenia

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Abstract: Although intrinsic hippocampal hyperactivity and deficits in nicotinic signaling are increasingly recognized as core features of schizophrenia, no studies have yet examined the effects of nicotinic agonists on hippocampal activity in the illness. To that end, in this study we examined the effects of an acute dose (7 mg patch) of nicotine vs. placebo on hippocampal activity during the resting state as well as during a selective attention task in nonsmoking patients with schizophrenia and healthy subjects using functional magnetic resonance imaging. We found that nicotine, relative to placebo, reduced hyperactivity in patients during both the resting state and the attention task. Nicotine also reduced hyperactivity of the ventral parietal cortex in schizophrenia. These results suggest that hippocampal hyperactivity is reduced by nicotine in schizophrenia, and that hippocampal response may be targeted by nicotinic agonists and other investigational compounds in the illness.

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Poster

139. Major Mental Disorders: Imaging

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: The Mental Health Okamoto Memorial Foundation

Title: Gamma band auditory response with fMRI BOLD in schizophrenia

Authors: *H. KUGA[1,2], T. UENO[1], N. ORIBE[1], H. OKAMOTO[2], I. NAKAMURA[2], Y. HIRANO[2], H. MIZUHARA[3], T. ONITSUKA[2];
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Abstract: INTRODUCTION Schizophrenia has been conceptualized by dysfunctional cognition and behavior related to abnormalities in neural circuitry. The functioning of the neural circuitry can be assessed using the auditory steady state response (ASSR). Moreover, in recent years, research on high (> 60-Hz) gamma band oscillations has become of increasing interest. The current study used 1.5 Tesla Magnetic Resonance Imaging(MRI) and investigated low and high gamma band oscillations with the ASSR. METHODS 24 patients with schizophrenia and 26 normal controls participated in this study. ASSR to 20, 30, 40 and 80-Hz binaural click trains were measured with 1.5 T MRI (Achieva, Philips). The duration of one click was 1 msec, and the duration of click trains was 500 msec(5 scans). The intensity of the click trains was 80 dB sound pressure level. The interval between each click trains was 500 msec. The experiments were designed as "block design". Each block task (15 sec, 5 scans) contained 15 click trains and intervals, which was followed by resting state without any stimuli (15 sec, 5 scans). In total, we performed 240 sec (80 scans) on each subject. SPM8 was used in order to analyze BOLD fMRI data within each subject, as well as a group. The mean ASSR BOLD intensity were analyzed using repeated measures analysis of variance (rmANOVA) with group (SZ or NC) as a between-subjects factor; and frequency (20, 30, 40 or 80-Hz) and hemisphere (left or right) as within-subjects factors. RESULTS Schizophrenia showed bilaterally increased ASSR BOLD to 80-Hz frequency, compared to healthy subjects. CONCLUSIONS The current study indicated that patients with schizophrenia showed increased ASSR BOLD to the 80-Hz frequency. The BOLD-ASSR activation found in broadman 41 and 42 area might reflect GABAergic system dysfunction in schizophrenia, as ASSR is linked to GABAergic inhibitory interneuron activity. This study highlighted the abnormal 80-Hz ASSR and provided clear evidence that schizophrenia is characterized by abnormalities in neural circuitry.

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Poster

139. Major Mental Disorders: Imaging

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Psychosis chronicity and fMRI responses during basic visuo-motor function

Authors: *K. RAMASESHAN[1], F. BADOUI[1], S. TOLIA[1], M. BELLANI[2], G. RAMBALDELLI[2], V. A. DIWADKAR[1], P. BRAMBILLA[3];
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Abstract: Background: The chronicity of psychosis may exert accumulative effects on the integrity of brain function. For example, patients with a diagnosis of psychosis may transition to chronic schizophrenia, yet differences in brain function are relatively understudied across populations. Inter-hemispheric transfer (IT) which relies on the integrity of the macroscopic brain networks, is a useful domain in which to examine this question. We assessed fMRI activations during IT in three age- and gender matched group of participants: Typical controls, first-episode psychosis patients (FEP) and chronic schizophrenia patients (SCZ). IT was assessed using the Poffenberger paradigm (Poffenberger, 1912): A probe is briefly presented to one visual hemi-field and subjects detect it signally with the hand that is contra- or ipsilateral to the hemi-field thus generating inter- or intra-hemispheric visuo-motor transfer. **Methods:** Data from eighteen HC subjects were age and gender matched to FEP and SCZ patient groups (14 male, 4 female, mean age = 33 years). Whole brain fMRI scans were acquired (3T, Siemens Trio) and data were modeled in SPM8 using typical methods. IT was assessed for right-handed responses by contrasting inter- with intra-hemispheric transfer at the first level. A one-way (Group as single facto) random-effects analysis of variance was used to assess comparative changes in activation as a function of illness chronicity. A contrast was used to assess changes in activation across groups (representing effects of psychosis chronicity). **Results:** Three regions in the core and extended motor circuit showed increases in activation associated with chronicity: Left M1 (i.e., responding M1), the supplementary motor area (SMA), and the basal ganglia. Increased chronicity was associated with decreased brain activation in the right (i.e., stimulated) visual cortex (lingual gyrus). **Conclusion:** In this cross-sectional analysis, illness chronicity appeared to exert complementary effects in the visual and motor regions. Chronicity was associated with decreased responses in the primary visual cortex, but increase responses in responding motor regions. These effects may constitute complex effects of psychosis at the level of regional brain function, detectable using even basic tasks.

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Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH(5R01MH059883)

Title: The contribution of cognitive-affective interactions to motivational deficits in schizophrenia: an fMRI study

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Abstract: Background: Recent research suggests that individuals with schizophrenia (SZ) show unimpaired in-the-moment emotional reactivity, and that symptoms traditionally attributed to impaired affective processing may be in fact closely related to deficits in higher order cognitive control consistently associated with this disorder. Similarly, it has been proposed that the motivational deficits characterizing schizophrenia may also reflect impaired cortical-striatal interactions rather than a primary alteration of affective states (Barch & Dowd, 2010). The goal of the work presented was to examine the neural substrates of motivational and control processes in schizophrenia and to analyze the engagement of networks of brain activity to incentives and cognitive control load in both schizophrenia patients and healthy controls. A secondary aim was to examine the influence of treatment with antipsychotic medications when processing cues that represent reward or punishment. Methods: Twenty-three patients and twenty-one demographically matched controls were scanned using Functional Magnetic Resonance Imaging (fMRI) while performing a computerized task that orthogonally manipulates monetary incentives (potential monetary reward, avoiding monetary loss, and no monetary consequence) and cognitive control demands (task of responding consistent or against a pre-potent response bias (Ursu and Carter, 2005). A 3-way repeated measures ANOVA (Group x Incentive x Control Load) of response accuracy showed main effects of task demands, incentives, and group ($P < 0.05$), but no 3-way interaction, $F(1.7, 72.2) = 0.79$, n.s. The Group x Task interaction was significant, $F(1,42)=4.25$, $p = 0.04$, and reflected a greater change in accuracy between the low and high cognitive control load in the patient group than in controls. In contrast, the effect of incentives on accuracy was similar across the two groups, (Group X Incentives, $F(2,84) =1.58$, n.s). In analyses of fMRI data, the patients showed a deficit in prefrontal and striatal activation in response to task demands, but similar activity in response to the prospect of monetary gain. Furthermore, performing responses resulting in monetary gains was associated with robust activation of reward-related brain structures in both groups. Conclusions: These results provide additional novel evidence that primary affective experience is relatively spared in schizophrenia, and emphasize the role of impaired cognition in dysfunctions of motivated behavior in this disorder.

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Poster

139. Major Mental Disorders: Imaging

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Resting state functional connectivity of the hippocampus in psychosis

Authors: ***I. NWABUDIKE**, S. KEEDY;
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Abstract: CONTEXT: Psychotic disorders like schizophrenia are associated with temporal lobe abnormalities. These may be from neurodevelopmental problems that result in altered synaptic plasticity mechanisms and impact network function. These mechanisms are key for the hippocampus given its role in memory formation, and hippocampal abnormality could account for psychosis symptoms such as auditory hallucinations. We tested hippocampal integrity via resting state functional connectivity in 13 psychosis patients with auditory hallucination (AH) histories and 18 matched healthy individuals. METHODS: Resting state fMRI was acquired with a Philips 3.0T scanner (8 channel headcoil, TR=2.5s, TE=25ms, 3.5x3.5x3.3mm voxel size, 43 oblique axial slices, flip angle=90deg, 144 volumes). A high-resolution T1-weighted image was also acquired to aid spatial standardization. Data was preprocessed using AFNI, including regressing motion estimates, white matter and CSF –related signal, and drift parameters. Separate right and left hippocampus masks served as seed regions for correlation with other voxels for each participant to characterize connectivity. These connectivity maps were submitted to group comparison t tests, and abnormal connectivity was correlated with symptom severity measures including detailed AH assessment. RESULTS: Psychotic subjects had relatively decreased connectivity between the left hippocampus and bilateral ventrolateral prefrontal cortex as well as left lentiform nuclei, but increased connectivity between left hippocampus and left thalamus. For the right hippocampus, patients had decreased connectivity to left insula. There was a strong negative correlation between connectivity of left hippocampus to ventral prefrontal cortex and severity of past AH, and not general psychosis symptom severity or current AH severity. CONCLUSIONS: The right and left hippocampus may have differentially altered connectivity, with overall greater extent of abnormality for left hippocampus. Of the alterations, reduced left hippocampal connectivity with prefrontal cortex

was associated with past AH severity. This is consistent with the notion that AH is dominantly verbal in nature and thus may be more associated with alterations in the left hemisphere where language functions are mediated. The findings also support a research strategy of parsing psychosis symptoms and seeking specific associations to neural system abnormalities to address disease heterogeneity.

Disclosures: I. Nwabudike: None. **S. Keedy:** A. Employment/Salary (full or part-time):; University of Chicago.

Poster

139. Major Mental Disorders: Imaging

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Program#/Poster#: 139.16/G25

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Cerebellar dysconnectivity in schizophrenia

Authors: *J. JI[1], C. DIEHL[1], M. GLASSER[2], N. SANTAMAURO[1], C. SCHLEIFER[1], V. SRIHARI[1], G. REPOVS[3], J. KRYSTAL[1], A. ANTICEVIC[1];
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Abstract: Distributed aberrant neural connectivity is thought to be involved in the pathophysiology of schizophrenia. Disturbances in functional connectivity within different regions of the cerebral cortex as well as between cortex and other major nodes of neural processing (such as the thalamus) have been shown in schizophrenic patients. These findings highlight that perturbations in connectivity occur at a whole-brain level and that distributed neural networks are likely implicated in schizophrenia. The cerebellum shares dense reciprocal connections with the thalamus and cerebral cortex and contains approximately 60% of all neurons in the central nervous system. This makes the cerebellum a critical target for pathogenic brain-wide dysconnectivity. Furthermore, a growing body of literature implicates the role of the cerebellum in higher-order cognitive functions that are notably affected in schizophrenia. Yet, brain-wide disturbances in cerebellar connectivity in schizophrenia remain unknown. Here, we characterized whole-brain cerebellar connectivity using resting-state functional magnetic resonance imaging in a population of patient diagnosed with schizophrenia and matched controls. Data were collected using the acquisition methods harmonized with the Human Connectome Project (Glasser et al., 2013). Using an a priori functionally-defined parcellations of the human cerebellum (Buckner et al. 2011), we generated a map of functionally correlated

regions for each of 7 major cerebellar functional subdivisions. Analyses revealed hyper-connectivity between cerebellum and bilateral sensory-motor cortices in patients relative to controls, specifically prominent for executive cerebellar subdivisions. Additionally, patients showed hypo-connectivity between cerebellum and regions of prefrontal and parietal association cortex as well as striatum, also prominent for executive cerebellar subdivisions. Previous findings have shown a highly similar pattern of whole-brain dysconnectivity in schizophrenic patients for associative thalamic nuclei. This disturbance may reflect weakened top-down gating of sensory information in schizophrenia, and adds to our basic understanding of cerebellar dysconnectivity in psychiatric disease.

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Poster

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MH43775

MH077945

MH074797

2P50AA012870-11

R01-MH062349

Title: Cortical hierarchy underlies preferential connectivity disturbances in schizophrenia

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Abstract: Schizophrenia (SCZ) is a neuropsychiatric illness associated with abnormal neural connectivity. In particular, patients show prefrontal cortex (PFC) hypo-connectivity, assessed by computing blood-oxygen level-dependent (BOLD) signal correlations. However, recent studies reveal elevated BOLD signal variance in SCZ, which may impact correlations, computed as covariance normalized by variance. We hypothesized that functional connectivity (using covariance) may be elevated in SCZ, but that this may occur in the context of elevated signal variance. Further, we hypothesized that preferential PFC effects may intrinsically arise from the information-processing hierarchy and corresponding physiological consequences, which we tested via biophysically grounded computational modeling. We conducted resting-state fMRI in 161 SCZ and 164 matched healthy subjects, assessing group differences in connectivity and BOLD variance. Both voxel-wise and network-level analyses were performed. To mechanistically inform fMRI findings, we used a large scale neural network model to simulate a well-known synaptic hypothesis of SCZ pathology—namely excitation/inhibition (E/I) imbalance, and analyzed resulting *in silico* 'BOLD' signals. Empirically, we observed hyper-connectivity in PFC and other associative regions in SCZ, with concurrent increases in BOLD variance. These effects were absent in a comparison group of bipolar patients (N=73). In initial simulations of E/I imbalance, we observed global elevations in covariance and variance of model-generated BOLD signals. To investigate our empirical associative effects, we extended our model to reflect known differences in associative vs. sensory neuronal dynamics. This extended model reproduced preferential associative effects, and predicted that covariance and variance elevations would be positively correlated, which we confirmed empirically. Collectively, we show that elevations in BOLD covariance and variance in chronic SCZ co-occur and are strongly related phenomena. Thus, some connectivity elevations may not be fully captured by correlation measures that normalize connectivity by variance. Hypo-connectivity seen in previous studies may be reconciled with our findings by considering the effect of elevated variance in reducing correlation-based measures. We also computationally demonstrate how a common cellular-level mechanism can produce elevations in covariance and variance of BOLD signals. Further, we show how this global perturbation can produce preferential effects in associative regions due to neural differences arising from the intrinsic functional cortical hierarchy.

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Poster

139. Major Mental Disorders: Imaging

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Health and Labour Sciences Research Grants, Comprehensive Research on Disability Health and Welfare 2012-002

Title: Brain activity associated with intrinsic motivation in patients with schizophrenia

Authors: *K. TAKEDA[1], M. MATSUMOTO[2], Y. OGATA[3], K. MAIDA[4], H. MURAKAMI[3], K. MURAYAMA[5], K. SHIMOJI[6], T. HANAKAWA[3], K. MATSUMOTO[7], K. NAKAGOME[8]; [1]Dept. of Psychiatry, Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Tokyo, Japan; [2]Dept. of Psychiatry, Univ. of Tokyo, Tokyo, Japan; [3]Integrative Brain Imaging Center, Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [4]Dept. of Psychiatry, Natl. Ctr. Hospital, Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan; [5]Fac. of Life Sciences, Univ. of Reading, Reading, United Kingdom; [6]Dept. of Radiology, Tokyo Metropolitan Geriatric Hosp. and Inst. of Gerontology, Tokyo, Japan; [7]Brain Sci. Institute, Tamagawa Univ., Tokyo, Japan; [8]Natl. Ctr. Hospital, Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan

Abstract: In search for the biological marker of intrinsic motivation in patients with schizophrenia (SCZ), we adopted a stopwatch (SW) task developed by Murayama et al. (2010). In the task, participants were presented with a SW that starts automatically, and the goal was to press a button with the right thumb within 50 ms around the 5-s time point. The control task was a watch-stop (WS) task, in which participants passively viewed a SW and were asked to simply press a button when it automatically stopped. Both tasks were pseudorandomly intermixed and preceded by a cue that indicated which task to perform. Because the accumulated number of successful trials in SW task was continuously presented at the top right corner of the screen and was updated only after every success in SW task, the SW task was considered more interesting than the WS task. In an fMRI study, Murayama et al. (2010) demonstrated that in response to the task cue the lateral prefrontal cortex (PFC) showed a larger activation in the SW task as compared to the WS task, suggesting that the healthy subjects were more engaged in mental preparation for tasks with higher value. Therefore we hypothesized the difference activation induced by the task cue between the SW and WS tasks in PFC may act as a biological marker for intrinsic motivation in SCZ. Sixteen patients and 17 healthy control subjects (HC), age and sex matched, participated in the study. The study was approved by the ethical committee in National Center of Neurology and Psychiatry. The subjects all gave informed consent before participating in the study. In addition to fMRI data acquisition, we evaluated intrinsic motivation inventory (IMI) (Choi, et al. 2010). First, difference scores of IMI obtained by subtracting the scores for the SW task from the WS task were considered to represent the intrinsic motivation to this task in general. The difference scores of IMI showed a significant between-group difference suggesting that intrinsic motivation to the task in SCZ was lower than that in HC. Correspondingly, the difference striatal activation in response to the task cue between SW and

WS tasks was significantly lower in SCZ than in HC. Interestingly, difference PFC activation in response to the task cue between the SW and WS tasks was positively and significantly correlated with difference scores of IMI in HC, whereas this was not true for SCZ. Although highly speculative as it is, the lack of significant correlation between the PFC activation and IMI in SCZ may reflect the anomalous neural activity in the PFC which may have been necessary to adequately integrate intrinsic motivation in the cognitive process supporting the correct performance based on intrinsic motivation.

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Poster

139. Major Mental Disorders: Imaging

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NARSAD

NIH K23MH079216

Title: Resting state connectivity in prodromal schizophrenia

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Abstract: Introduction: Schizophrenia is a debilitating mental illness affecting between 0.5-1% of the world population. The prodromal stage of the illness is characterized by subtle perceptual, social, and behavioral dysfunctions. rsfMRI describes the temporal correlation of activity between spatially separate brain regions at rest. Hypothesis: Prodromal schizophrenics (PRO) will display hyperconnectivity between the amygdala and sensory cortices compared to controls (CTRL). Methods: 11 PRO and 11 CTRL subjects completed phenotypic characterization using the Structured Interview for Prodromal Syndromes (SIPS) and rsfMRI data collection (6 minutes). Data analysis used a seed-based approach using an anatomically defined amygdala mask. Results: PRO subjects showed increased functional connectivity between the amygdala and the right postcentral gyrus, the left precentral gyrus, the orbitofrontal cortex, and the left

superior temporal gyrus. Conclusion: This study found hyperconnectivity in PRO subjects between the amygdala and brain regions associated with processing and interpreting emotional information.

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Poster

139. Major Mental Disorders: Imaging

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MRC Fellowship (grant MR/J008915/1) to MK

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Title: Gray matter changes in major depression and bipolar disorder: Evidence from voxel-based meta-analysis

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Abstract: Major depression (MDD) and bipolar disorder (BD) are serious affective disorders with overlapping symptomatology. Many studies have shown both conditions to be associated with reduced gray matter volume, suggesting that structural brain alterations may be involved in these disorders. However it is unclear whether the two conditions show common or distinct

patterns of structural changes. We conducted a meta-analysis of voxel-based morphometry (VBM) studies in MDD and BD to identify patterns of gray matter changes that are common and distinct in these conditions. Literature was searched up to January 2015 for studies comparing whole brain gray matter volume in patients with MDD and BD vs. healthy individuals. We conducted voxel-based meta-analysis on the data using Anisotropic Effect Size Signed Differential Mapping. Original statistical maps were used when available to improve the sensitivity of the analysis, but peak coordinates, as reported in articles, were used if these were not available. We used meta-analytic comparisons of effect sizes and conjunction analyses to identify regions showing shared and distinct gray matter changes in the two conditions.41 studies comparing MDD vs. healthy individuals and 32 studies comparing BD vs. healthy controls were included, with statistical maps included for 14 of these. Medial prefrontal volumetric reduction, specifically in the bilateral medial superior frontal gyri, ventromedial prefrontal cortex, and anterior cingulate cortex, was common to both conditions, as was reduction in the bilateral insula. When groups were contrasted, volume reduction was greater in MDD in the left hippocampus, right inferior temporal gyrus, right middle frontal gyrus and left inferior parietal gyrus. BD showed greater reductions in the left orbitofrontal cortex.These results indicate that both disorders are associated with gray matter volume decreases in the medial prefrontal cortex and insula, regions involved in affective processing. Findings suggest that common morphometric alterations in key neurobiological circuits may underlie the aberrant mood states that characterize both conditions. Robust differences between MDD and BD were identified in a number of regions. The left hippocampus and small areas in the frontal, temporal and parietal lobes showed more substantial reductions in MDD than BD, we found greater reductions in BD in the left orbitofrontal cortex. This might reflect differences in the affective and cognitive profiles of the two disorders, although it is possibly that confounding effects of mood state or medication use in the included studies may influence our results.

Disclosures: **T. Wise:** None. **J. Radua:** A. Employment/Salary (full or part-time):; Clinical rater in Roche clinical trials. **E. Via:** None. **N. Cardoner:** None. **A.J. Cleare:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Received research grant support from Lundbeck. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Received honoraria for speaking from Astra Zeneca and Pfizer. **T. Adams:** None. **J.H. Cole:** None. **M. Kempton:** None. **S. Pezzoli:** None. **C.H. Fu:** None. **A.H. Young:** 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.; Is lead Investigator for the Embolden Study (AZ), BCI Neuroplasticity study and Aripiprazole Mania Study and investigator initiated studies from AZ, Eli Lilly, Lundbeck, Wyeth. F. Consulting Fees (e.g., advisory boards); Has given paid lectures and sits on advisory boards for all major pharmaceutical companies with drugs used in affective and related disorders. **D. Arnone:** D. Fees for Non-CME Services

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Poster

139. Major Mental Disorders: Imaging

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 139.21/G30

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH MH092702

Title: Auditory hallucination severity and abnormal auditory cortex connectivity in psychotic disorders

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Abstract: Background: Psychotic disordered individuals show numerous neural system abnormalities, such as in the default mode network when at rest, but these findings have been poorly linked to symptoms. A hallmark symptom of psychotic disorders is auditory hallucinations (AH), which involve the auditory cortex (AUD) when occurring. However, it is not determined whether auditory networks have general functional alterations and whether any alterations may be associated with AH severity. Methods: Patients with psychosis (Pts) with AH histories and healthy controls (HC) were instructed to lie still with eyes open during a 6-minute fMRI resting-state task. Data was preprocessed using AFNI, including regressing out motion estimates, white matter and CSF-related signal and drift parameters. Each participant's bilateral AUD was identified via response to sounds in a separate fMRI run and used as a seed region to correlate with the rest of the brain to characterize AUD connectivity. Pts and HC connectivity maps were compared with t tests, cluster corrected at $p < .05$ (familywise). Average connectivity values were then extracted from each patient for each significant cluster and were correlated with hallucination symptom measurements. Results: Pts had significantly lower auditory cortex connectivity in the left fusiform, right and left insula, left lenticular formation, and cerebellum. Pts with greater insula-AUD connectivity reported worse past AH symptom severity overall, and with specific Emotional and Cognitive dimensions of past AH severity. Conclusion: The analysis of fMRI resting-state data revealed lower auditory cortex connectivity with the insula cortex for Pts with AH histories relative to Ctls, as well as with basal ganglia and cerebellar regions. Together, these findings are consistent with hypotheses of cortical-subcortical-cerebellar loop dysfunction in psychosis. Further, past AH symptom severity was uniquely related to

connectivity of AUD to insula, an area believed to be responsible for emotional regulation and general salience, used for monitoring the environment. This study provides insight that connectivity between AUD and insula could be a neural system mechanism of vulnerability to hallucinations. It also highlights the utility of symptom-based hypothesis testing and detailed symptom assessment, allowing for clearer links between neural system abnormalities and clinical presentation.

Disclosures: V. Ramirez: None. **S. Keedy:** None.

Poster

139. Major Mental Disorders: Imaging

Location: Hall A

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Program#/Poster#: 139.22/G31

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIMH R21MH092430

Title: Multivariate classification algorithm effectively identifies individuals with MDD using whole brain white matter microstructure calculated from diffusion tensor MRI

Authors: *D. M. SCHNYER[1], P. C. CLASEN[2], C. GONZALEZ[3], C. G. BEEVERS[4]; [1]Univ. of Texas, Austin, Austin, TX; [2]Dept. of Psychology, Stanford Univ., Palo Alto, CA; [3]Natl. Inst. of Hlth., Baltimore, MD; [4]Psychology, Inst. for Mental Hlth. Res., Univ. of Texas, Austin, TX

Abstract: Considerable research has examined the relationship between white matter microstructure and major depressive disorder (MDD) using diffusion tensor MRI (DTI). A recent meta-analysis (Liao Y, Huang X, Wu Q, et al., 2013) examined 11 studies that utilized whole brain univariate approaches for revealing important voxel-based DTI differences between MDD and non-MDD controls - revealing 4 consistent locations of decreased fractional anisotropy (FA) in patients with MDD - the right frontal lobe, right fusiform gyrus, left frontal lobe and right occipital lobe. While univariate approaches are useful in identifying localized differences between patients and controls, they don't tell us how well these differences perform in identifying who has MDD and who doesn't. Here we examine the potential value of applying multivariate classification algorithms in order to identify individuals with MDD based on the totality of their brain white matter. DTI data from forty individuals, 20 each MDD and non-MDD matched on gender and age were examined. Whole brain DTI was acquired for 25 directions using a dual shot echo planar imaging and a twice-refocused spin echo pulse sequence,

optimized to minimize eddy current-induced distortions (GE 3T, TR/TE = 12000/71.1, B = 1000, 128 × 128 matrix, 3-mm (0 mm gap) slice thickness, 0.94 × 0.94 mm in plane resolution, 1 T2 + 25 DWI). Forty-one slices were acquired and diffusion tensors and multiple scalar measures, including FA values were calculated on a voxel-by-voxel basis using conventional reconstruction methods. A support vector machine-learning algorithm, C-classification with a linear kernel and 5-fold cross validation was applied for multivariate classification. Average classifier training accuracy using FA across each of the sets with 1/5th of the participants randomly removed was 73.4% - corresponding to a Kappa 0.40. After removing regions that showed univariate between group differences, the classification accuracy remained statistically unchanged. Moreover, of the multiple scalar measures examined - mean, radial and axial diffusivity, FA resulted in the most accurate classification. Finally, a searchlight procedure will be applied to understand the possible predictors of response to an attention training treatment regime in the patients with MDD. These results support the contention that distributed microstructural differences across the entire white matter network are important for understanding the brain basis of MDD.

Disclosures: D.M. Schnyer: None. **P.C. Clasen:** None. **C. Gonzalez:** None. **C.G. Beevers:** None.

Poster

139. Major Mental Disorders: Imaging

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH R21MH098668

Title: Decreased social connectedness in women with Premenstrual Dysphoric Disorder

Authors: *L. LIANG[1], N. PETERSEN[2], D. GHAHREMANI[2], R. GERARDS[2], A. CHOUDHARY[2], L. GOLDMAN[2], A. RAPKIN[2], E. LONDON[2];
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Abstract: Premenstrual Dysphoric Disorder (PMDD) is a disorder that afflicts approximately 5% of women in their reproductive years. Women with PMDD experience psychological, physical, and behavioral symptoms in the days that precede the onset of menses, and symptoms abate as the menstrual cycle begins. PMDD can cause extreme mood disturbances that decrease quality of life and daily functioning. Based on evidence that social ties are significantly related to mental health outcomes, we hypothesized that women with PMDD may experience deficits in

social connectedness. To test this hypothesis, 16 women with PMDD and 22 healthy controls were recruited. Social connectedness was assessed using the Social Connectedness Scale, Revised (SCSR), a well-validated 20-item questionnaire evaluating the degree to which participants feel connected to others and their environments, and neural correlates of social connectedness were evaluated using resting state functional magnetic resonance imaging in an exploratory analysis. Participants completed the resting-state scan and SCSR once during the follicular phase and once during the luteal phase. Consistent with our hypothesis, women with PMDD had significantly lower social connectedness scores than healthy controls ($p=0.0179$). Menstrual phase did not significantly influence social connectedness scores in PMDD participants or healthy controls. Our findings suggest that women with PMDD experience significant deficits in social connectedness regardless of cycle phase, suggesting that therapies to increase social connectedness may represent an effective clinical target for therapeutic investigations.

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Poster

139. Major Mental Disorders: Imaging

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: RO1 MH069942

Title: Alterations of cortical activity at local and network levels during visual working memory updating in major depressive disorder

Authors: ***T. M. LE**[1], J. KANG[2], D. TYSZ[2], D. KLEIN[2], H.-C. LEUNG[2];
[2]Psychology, [1]Stony Brook Univ., Stony Brook, NY

Abstract: Major depressive disorder (MDD) is associated with deficits in cognitive control including the ability to efficiently update working memory in the face of irrelevant or obsolete information. While prefrontal dysfunction is strongly implicated in cognitive control deficits in MDD, the few studies examined visual areas (e.g., V4) have also found reduced activity and abnormal functional connectivity during visual perception and attention. To determine whether working memory updating impairment in MDD involves alterations in regional and network activity, we examined visual association regions involved in selective maintenance and

processing as well as their functional connectivity with the prefrontal cortex (PFC). We acquired functional magnetic resonance imaging data from 17 unmedicated participants with MDD and 21 healthy controls (CTL) while they performed a visual delayed recognition task with an updating cue inserted in the delay period. Three cues, Remember Face (Ignore Scene), Remember Scene (Ignore Face) and Remember Both, were used to indicate which one or both of the two memorized stimuli (a neutral face and a neutral scene) would remain relevant for the recognition test. Regions of interest (ROIs) were functionally defined by face versus scene contrasts using data from a localizer task. Compared to the CTL group, the MDD group showed lower accuracy on the Remember Scene condition and reduced category selectivity in the scene-selective areas during postcue delay. Intriguingly, activity in the fusiform face area during Remember Scene positively correlated with rumination across the MDD subjects. At the network level, beta series analysis revealed a significant increase in coupling between the left lateral PFC and scene-selective areas in the parahippocampal gyrus and posterior parietal cortex during Remember Scene compared to Remember Both in the CTL group. No such enhancement in functional connectivity was observed in the MDD group even at very lenient threshold ($p<0.01$, uncorrected). In sum, our findings suggest impairments in working memory updating performance and rumination are associated with alterations in category-specific activity in visual association areas in MDD. Such local changes may be a result of changes at the network level affecting modulatory top-down control from the PFC, producing dysfunctions in working memory updating. Such results provide new evidence of the role of visual association areas in working memory updating deficits in depression while supporting the role of prefrontal dysfunction in the disorder.

Disclosures: **T.M. Le:** None. **J. Kang:** None. **D. Tysz:** None. **D. Klein:** None. **H. Leung:** None.

Poster

139. Major Mental Disorders: Imaging

Location: Hall A

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Program#/Poster#: 139.25/G34

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Effects of behavioral activation therapy on the neural correlates of subthreshold depression with a monetary incentive delay task

Authors: A. MORI, Y. OKAMOTO, M. TAKAMURA, *G. OKADA, R. JINNIN, K. TAKAGAKI, M. KOBAYAKAWA, A. MACHINO, S. YAMAWAKI;
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Abstract: Behavioral activation is known to be an effective treatment for subthreshold depression to prevent development from major depressive disorder. Behavioral activation is equally effective to cognitive behavioral therapy for the treatment of depression and has been a growing interest for its feasibility. To date, a few studies have examined the neural effects of psychotherapy on depression, mainly cognitive behavioral therapy. However, neural correlates of behavioral activation has not been clearly understood. Here, we investigated neural response using functional magnetic resonance imaging during reward processing to study neural mechanisms of behavioral activation in subthreshold depression. 16 subthreshold depression (age 18-20) with high BDI score and 16 age-matched controls with low BDI score participated in two identical functional magnetic resonance imaging scans with monetary incentive delay task, frequently used for MDD to detect changes in neural activity in response to the processing of reward and punishment. Between scans, subthreshold depression group received a 5 week behavioral activation therapy without medication. We demonstrated that behavioral activation significantly reduced depressive symptoms. Relative to changes in brain function in controls, behavioral activation resulted in functional changes in structures that mediate cognitive emotion regulation, including left ventrolateral prefrontal cortex and angular gyrus during loss anticipation in subthreshold depression. In conclusion, the present study revealed neural effects of brief behavioral activation treatment to subthreshold depression , that is, improved functioning of fronto-parietal region during loss anticipation. These results provide further evidence for understanding mechanisms underlying specific psychotherapy.

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Poster

140. Role of the Habenula in Drug Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH AA021657

NIH AA022292

Title: Increased excitability of lateral habenula neurons via down regulation of m-type potassium channel in rats withdrawn from repeated ethanol exposure

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Abstract: Accumulating evidence suggests the lateral habenula (LHb), an epithalamic structure, plays a pivotal role in neuropsychiatric diseases, including drug addiction; neuronal changes in the LHb after chronic drug exposure may therefore represent a critical mechanism leading to addiction. However, the effects of ethanol exposure on LHb neurons have not been well investigated. In this study, we investigated how LHb neuronal activity is changed by repeated ethanol exposure. Since many studies have shown that potassium channels, including the m-channel (KCNQ/Kv7 family), are importantly involved in alcohol abuse, and histological evidence indicates the abundance of m-channel expression in the LHb, we investigated whether m-channel is involved in the ethanol-induced changes of LHb neurons. Using patch-clamp techniques and the Western blot approaches, we found that in rats at 24 h withdrawal from repeated ethanol exposure (2 g/kg, i.p., twice/d for 7 d), the excitability of the LHb neurons was increased. In addition, the m-channel current as well as the protein expression of m-channel subunit, KCNQ2, were decreased in the LHb of ethanol-treated rats, compared to the ethanol-naïve counterparts. These observations suggest that m-channel has an important role in ethanol-induced neuronal adaptation on LHb and imply that m-channel could serve as a target for treatment of ethanol dependence.

Disclosures: **S. Kang:** None. **J. Ye:** None.

Poster

140. Role of the Habenula in Drug Addiction

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 140.02/G36

Topic: C.17. Drugs of Abuse and Addiction

Support: MH094870

Title: Role of afferents and efferents of the lateral habenula in ethanol-directed behaviors

Authors: *C. SHETH[1], T. M. FURLONG[1], K. A. KEEFE[1], S. A. TAHA[2];
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Abstract: Alcohol has both rewarding and aversive properties, each of which play a critical role in drug intake. The aversive properties of alcohol (e.g. nausea, sedation) are important in limiting alcohol intake and implicated in determining vulnerability to developing an alcohol use disorder. The lateral habenula (LHb) is an epithalamic brain region that plays an important role in processing aversive stimuli and aversion learning. In previous work, we demonstrated that LHb lesions increase voluntary ethanol consumption, increase operant ethanol self-administration; block yohimbine-induced reinstatement of ethanol-seeking and attenuate conditioned-taste aversion (CTA) to ethanol. However, the anatomical circuit through which the LHb mediates ethanol-directed behaviors is unknown. Given that the LHb inhibits the midbrain dopaminergic system through a disynaptic pathway involving the rostromedial tegmental nucleus (RMTg) and that the RMTg itself has been implicated in mediating aversion-driven behavior, we hypothesized a role for RMTg in mediating ethanol-directed behaviors. To test this hypothesis, male Long-Evans rats received bilateral excitotoxic RMTg or control lesions. After recovery from surgery, rats were subjected to intermittent alcohol access (IAA) for 8 weeks to induce voluntary ethanol consumption. Rats were then trained to lever press for ethanol, extinguished and tested for yohimbine-induced reinstatement. Finally, ethanol-induced conditioned taste aversion (CTA) was measured in a group of rats with RMTg or control lesions. We found that RMTg- lesioned rats consumed significantly more ethanol in the IAA paradigm than the controls. There were no differences between lesioned and control rats in ethanol-seeking and yohimbine-induced reinstatement of ethanol-seeking. Finally RMTg-lesioned rats showed robust CTA comparable to control levels in our paradigm, but extinguished this conditioned aversion significantly faster than control animals. Thus the RMTg, like the LHb, plays a role in regulating voluntary alcohol consumption potentially by mediating aversion to alcohol. Along similar lines, we investigated whether afferent input to LHb from reward-relevant structures including ventral pallidum (VP) and lateral hypothalamus (LH) is critical for regulating ethanol-directed behaviors. We found that LH to LHb input regulates voluntary ethanol consumption but is not critical in other ethanol-directed behaviors. We conclude that LH and RMTg are potential afferent and efferent targets in mediating LHb effects on voluntary ethanol consumption.

Disclosures: C. Sheth: None. **T.M. Furlong:** None. **K.A. Keefe:** None. **S.A. Taha:** None.

Poster

140. Role of the Habenula in Drug Addiction

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 140.03/G37

Topic: C.17. Drugs of Abuse and Addiction

Support: MH094870

Title: Excitatory activity in lateral habenula neurons during expression of ethanol-induced conditioned taste aversion

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Abstract: Ethanol, similar to other drugs of abuse, has potent rewarding and aversive properties. Clinical and preclinical data suggest that sensitivity to ethanol's aversive effects negatively modulates voluntary alcohol intake and thus may play a role in vulnerability to develop alcohol use disorders. We previously showed that bilateral lesions of the lateral habenula (LHb) in rats attenuate ethanol-induced conditioned taste aversion (CTA) (Haack et al., 2014). To understand the role of neural encoding in the LHb in contributing to learned aversion induced by ethanol injection, we recorded neuronal activity in the LHb of freely behaving, water-deprived rats (n=6) during an operant task to obtain a saccharin solution (0.125%) both before ("Pre-CTA" session after control saline injection) and after an ethanol-induced CTA ("CTA" session after injection of 1.5 g/kg 20% ethanol). Ethanol-induced CTA caused robust behavioral effects: relative to pre-CTA sessions, rats responded less frequently and at longer latencies in the operant task to obtain saccharin. Four major differences in LHb neural firing properties were apparent in comparing pre-CTA vs. CTA sessions. First, baseline firing rates in CTA sessions were significantly higher. Second, firing evoked by cues predicting saccharin availability shifted from a pattern of primarily inhibition during pre-CTA sessions to primarily excitation during CTA sessions. Third, lever press-evoked firing rates were higher in CTA sessions. Finally, LHb neurons were significantly more excited during consumption of the devalued saccharin solution in CTA sessions. Together, these results suggest that a shift from inhibition to excitation of LHb neurons contributes to expression of ethanol-induced CTA. To directly evaluate the role of LHb activity in ethanol-induced aversion, we next compared behavior in sham vs. LHb-lesioned rats in an identical operant CTA paradigm. LHb lesion attenuated CTA effects: LHb-lesioned rats initiated more trials and completed trials faster as compared to controls. Our data thus show that LHb excitatory activity is important in regulating expression of alcohol-induced aversion and point towards the LHb as a major node in modulating alcohol-seeking behaviors.

Disclosures: S. Tandon: None. **K.A. Keefe:** None. **S. Taha:** None.

Poster

140. Role of the Habenula in Drug Addiction

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Program#/Poster#: 140.04/G38

Topic: C.17. Drugs of Abuse and Addiction

Support: AA007474

AA010983

AA019967

AA022836

DA016511

Title: The role of the rostromedial tegmental nucleus in ethanol-induced conditioned taste aversion in male and female rats

Authors: *E. J. BURNETT[1], T. C. JHOU[2], L. J. CHANDLER[2];
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Abstract: The past several decades have witnessed a closing of the gender gap in the prevalence of alcohol use disorders (AUDs). In addition, recent work suggests the presence of a telescoping phenomenon, with women developing dependence over a shorter drinking duration and fewer total drinks compared to men. Current theories suggest that a competitive balance between a drug's rewarding and aversive properties changes as drug use progresses from recreational use to addiction. While the involvement of alcohol's rewarding properties in the path to dependence continues to be extensively investigated, the role of alcohol's aversive characteristics has been less well studied, particularly as it relates to differences between the sexes. The rostromedial tegmental nucleus (RMTg) exerts inhibitory control over midbrain dopamine neurons and is involved in encoding aversive stimuli and the behavioral responses to those stimuli. Recent work has also implicated activity within the RMTg in mediating the aversive effects of stimulants. To begin to investigate the role of the RMTg in signaling the aversive properties of alcohol, we measured cFos induction in this brain region following conditioned taste aversion (CTA). Adult male and female Long-Evans rats were exposed to three pairings of a novel 0.1% saccharin solution followed by an i.p. injection of 20 ml/kg 0.15 M lithium chloride (LiCl), 1.5 g/kg 20% ethanol (EtOH) or saline. Ninety minutes after a fourth exposure to saccharin only, the rats were sacrificed and brains processed for cFos immunohistochemistry. Both LiCl and EtOH produced significant CTA of equal magnitude between males and females compared to saline ($p<0.01$). LiCl-induced CTA was significantly stronger than EtOH-induced CTA in both sexes ($p<0.01$). Both LiCl- and EtOH-induced CTA significantly enhanced cFos expression in the RMTg compared to saline ($p<0.05$). cFos expression was similarly significantly enhanced in the lateral

habenula (LHb; $p<0.05$) - a source of prominent glutamatergic input to the RMTg. No significant sex differences in cFos expression were observed. Of note, cFos expression in both the RMTg and LHb were significantly positively correlated with CTA magnitude ($p<0.05$). In addition, RMTg cFos expression was significantly positively correlated with LHb cFos expression ($p<0.01$). Together, these data suggest that activity within the RMTg, possibly driven by activity within the LHb, plays a role in the aversive properties of drugs, including alcohol, in both male and female rats.

Disclosures: E.J. Burnett: None. **T.C. Jhou:** None. **L.J. Chandler:** None.

Poster

140. Role of the Habenula in Drug Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Title: The medial habenula and interpeduncular nucleus differentially modulate nicotine sensitization

Authors: B. L. EGGAN, *S. E. MCCALLUM;
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Abstract: The habenulo-interpeduncular pathway has previously been shown to modulate both rewarding and aversive effects of nicotine. The medial habenula (MHb) and interpeduncular nucleus (IPN) communicate directly via the fasciculus retroflexus. Further downstream these nuclei influence the mesolimbic reward pathway which extends from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Previously, we have shown that blocking α3β4 nicotinic acetylcholine receptors (nAChR) in the MHb decreases nicotine self-administration and blocks nicotine induced dopamine release in the NAc. In this study we sought to fully investigate both cholinergic and peptidergic signaling in the habenulo-interpeduncular pathway and elucidate the role of these two transmitter systems in nicotine sensitization, a phenomenon believed to be mediated by the VTA. Rats were sensitized to nicotine via 5 days of nicotine administration (0.4 mg/kg, s.c.) and underwent either locomotor activity testing (to quantitate behavioral sensitization) or *in vivo* microdialysis (to quantitate neurochemical sensitization in the NAc). Treatment drugs were administered directly to the MHb or IPN via indwelling cannulae. Intra-MHb administration of the α3β4 nAChR antagonists α-conotoxin AuIB and 18-methoxycoronaridine significantly reduced behavioral and neurochemical sensitization to nicotine. Sensitization was not blocked in the MHb by the α4β2 nAChR antagonist dihydro-β-

erythroidine, the partial α4β2 nAChR agonist varenicline, or the neurokinin-1 receptor antagonist, ezlopitant. Somewhat surprisingly, intra-IPN drug administration did not parallel that of the intra-MHb injections. While intra-IPN administration of α3β4 nAChR antagonists had no effect, behavioral sensitization was blocked by ezlopitant administered in the IPN. Further analysis of the IPN's role in neurochemical and behavioral sensitization is currently underway. Our results suggest that α3β4 nicotinic receptors in the MHb are important for the expression of behavioral and neurochemical sensitization to nicotine, which is in agreement with recent findings implicating habenular α3, β4 and α5 subunits in nicotine reward and dependence. In contrast, in the IPN, substance P signaling appears to modulate nicotine sensitization, while nicotinic receptors in the IPN do not contribute to this phenomenon.

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Poster

140. Role of the Habenula in Drug Addiction

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MCST EPILEFREE R&I-2013-014

Title: Lateral habenula in nicotine addiction: an electrophysiological *in vivo* study in rats

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Abstract: Tobacco smoking represents a well-known risk factor for health. So far, existing smoking cessation therapies have not been proven very successful at stopping this habit completely. Consequently, there remains a need for a better understanding of the neurobiology of tobacco dependence. Nicotine, the neuro-active compound in tobacco, is responsible for its rewarding and reinforcing properties through its action on the midbrain dopaminergic (DA) system. The lateral habenula (LHb), an epithalamic structure involved in pain, stress, depression and in encoding aversive stimuli, is known to inhibit the DA system through activation of the rostromedial tegmental nucleus (RMTg), a GABA-ergic area located caudally to the ventral

tegmental area (VTA). The RMTg receives a strong glutamatergic input from the LHb and is activated by systemic injection of nicotine in rats. Thus, the LHb might represent a possible target for the action of nicotine. Our data show that systemic administration of nicotine increases LHb neuronal activity *in vivo* in rats. Following chronic nicotine treatment this response is drastically decreased, while after 1 day of withdrawal only low doses of nicotine are able to significantly affect the firing rate of the LHb neurons compared to controls. To further elucidate the role of the LHb in central nicotine effects, we recorded the activity of VTA putative-DA neurons following electrolytic LHb lesion after acute and chronic nicotine administration. Acute systemic administration of nicotine induced a significant increase of VTA DA neuronal activity. Moreover, splitting the neurons on the basis of their localization within the VTA, revealed a significant effect of nicotine only on paranigral nucleus (PN) neurons compared to those of parabrachial pigmented nucleus (PBP). The electrolytic LHb lesion did not modify the nicotine acute effect on overall DA neurons. Strikingly, LHb lesion completely abolished the effect of nicotine on PN neurons and produced a significant excitation of PBP neurons. Following chronic nicotine treatment, an acute challenge with nicotine in LHb-lesioned rats failed to increase VTA DA cells neuronal activity compared to sham-lesioned and control rats. Our evidences strongly suggest that the LHb may play an important role in mediating the effects of nicotine on the midbrain DA system, thus participating to the mechanism of addiction to this drug.

Disclosures: **G. Deidda:** None. **M. Pierucci:** None. **R. Colangeli:** None. **G. Di Giovanni:** None.

Poster

140. Role of the Habenula in Drug Addiction

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 140.07/G41

Topic: C.17. Drugs of Abuse and Addiction

Support: 1R01DA036978

Title: Cellular and synaptic mechanisms of nicotine aversion

Authors: ***S. L. WOLFMAN**[1], F. BOGDANIC[2], D. F. GILL[1], R. AL-HASANI[3], J. G. MCCALL[3], M. R. BRUCHAS[3], D. S. MCGEHEE[2];
[1]Committee on Neurobio., [2]Dept. of Anesthesia and Critical Care, Univ. of Chicago, Chicago, IL; [3]Basic Res. Division, Dept. of Anesthesiol., Washington Univ., St. Louis, MO

Abstract: Nicotine addiction remains a major health problem in the US and throughout the world. Nicotine has rewarding effects at relatively low doses and intensely aversive effects at higher doses. The projection from the medial habenula (MHb) to the interpeduncular nucleus (IPN) contributes to these aversive effects. This “aversive” pathway may influence the development and maintenance of nicotine dependence and also contributes to withdrawal effects. Thus, improved understanding of the mechanisms that underlie the aversive effects of nicotine may be important in developing more effective therapies for smoking cessation. Excitation of the MHb-IPN projections enhances aversion to nicotine, while inhibition of this pathway increases appetitive responding for high doses of nicotine that were previously aversive. Although these results implicate the MHb-IPN circuitry, the downstream post-synaptic targets of the IPN that mediate these effects remain largely uncharacterized. Our hypothesis is that the aversive effects of nicotine occur through an indirect suppression of the excitability of VTA dopamine (DA) neurons. Burst activity in DA neurons is important for reward-associated behaviors, and aversive experiences can suppress DA neuron activity. While the IPN projects to several brain areas, it strongly innervates the lateral dorsal tegmental nucleus (LDTg), a brainstem cholinergic center that controls burst firing of VTA DA neurons. To test the nature of synaptic transmission between IPN projections and LDTg, we expressed Channelrhodopsin (ChR2) in IPN neurons and stimulated the terminals with light while recording specifically from LDTg neurons that project to the VTA. We found that light-evoked synaptic inputs were blocked by the GABAA receptor antagonist bicuculline. Additionally, optogenetic stimulation of either the IPN directly, or the IPN terminals in the LDTg specifically, results in aversion. We are testing the modulation of these inhibitory inputs by high and low concentrations of nicotine, and preliminary evidence suggests that high concentrations of nicotine selectively enhance light-evoked GABAergic currents from the IPN onto LDTg neurons that project to the VTA. We also have evidence that optogenetic inhibition of IPN terminals in the LDTg not only reduces aversion to a high dose of nicotine, but actually shifts the aversion to reward. These findings highlight the importance of the IPN-LDTg connection in mediating the aversive effects of nicotine and reveal interactions between reward and aversive circuitries that influence overall affective state.

Disclosures: **S.L. Wolfman:** None. **F. Bogdanic:** None. **D.F. Gill:** None. **R. Al-Hasani:** None. **J.G. McCall:** None. **M.R. Bruchas:** None. **D.S. McGehee:** None.

Poster

140. Role of the Habenula in Drug Addiction

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Program#/Poster#: 140.08/G42

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA DA020686

Title: Septohabenular regulation of nicotine consumption

Authors: *G. VOREN[1], P. J. KENNY[2];
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Abstract: The medial habenula (MHb) projects almost exclusively to the interpeduncular nucleus (IPN) via the fasciculus retroflexus. This major descending projection serves to connect the limbic forebrain and midbrain monoaminergic centers. Recently, our laboratory has shown that the MHb-IPN system, which densely expresses nicotinic acetylcholine receptors (nAChRs), plays an important role in regulating aversive properties of nicotine that limit consumption of the drug. Genetic evidence suggests that deficits in the sensitivity of this system to nicotine increases vulnerability to tobacco dependence. The MHb receives prominent excitatory input almost exclusively from the triangular nucleus of the septum (TNS) and inhibitory input from medial septum (MS) and diagonal band nucleus (NDB). The role of septohabenular pathways in regulating nicotine intake had not yet been explored. Here, we report that TNS neurons are highly sensitive to nicotine in a dose-dependent manner, as measured using *in vivo* single-unit recordings in freely behaving rats. DREADD-mediated excitation of TNS neurons decreased intravenous nicotine self-administration in rats. Conversely, pharmacological blockade of nicotinic signaling in TNS increased nicotine intake. We hypothesized that nicotine stimulates TNS projections to MHb, and that the TNS-MHb pathway inhibits nicotine intake. Surprisingly, DREADD-controlled activation or inhibition of TNS projections to the MHb did not alter nicotine intake. Similarly, modulation of direct TNS projections to the IPN that bypass the MHb did not alter nicotine intake. These data suggest the TNS functions to regulate nicotine consumption independent of its projections to the MHb or IPN. These data reveal the TNS region of the posterior septum as an important neuroanatomical substrate that regulates nicotine intake, but the circuit-level mechanisms through which TNS acts are unknown.

Disclosures: G. Voren: None. **P.J. Kenny:** None.

Poster

140. Role of the Habenula in Drug Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA 034192 (S.G.N)

Brain and Behavior NARSAD Young Investigator Award 65-6076 (S.G.N)

Title: Effect of DREADD-mediated transient activation of Gq-coupled signaling in lateral habenula neurons on cocaine and food self-administration in rats

Authors: *S. G. NAIR[1], D. SMIRNOV[2], J. F. NEUMAIER[2];
[1]Psychiatry and Behavioral Sci., [2]Univ. of Washington, Seattle, WA

Abstract: The lateral habenula (LHb), an epithalamic nucleus located in the dorsal diencephalon is an important regulator of midbrain dopaminergic systems that are known to be involved in the reinforcing properties of cocaine. We previously examined the effect of DREADD (hM4Di) - induced transient activation of Gi/o-coupled signaling in LHb neurons and found that it significantly increased operant cocaine self-administration. Here, we firstly examined the effect of DREADD (hM3Dq)-induced transient activation of Gq coupled signaling on cocaine reinforced operant responding. Male Long-Evans rats were injected with hM3Dq into the LHb and implanted with jugular venous catheters. Approximately 10-12 days after viral infusions, rats were trained to self-administer cocaine (0.75 mg/kg/infusion) on a fixed ratio 1 (FR1) reinforcement schedule. Initial results indicate that activation of hM3Dq by the pharmacologically inert synthetic ligand clozapine-N-oxide CNO (1 and 3 mg/kg, i.p) decreases cocaine reinforced operant responding. Secondly, a distinct cohort of rats was infused with viral vectors as described above and trained to self-administer cocaine (0.75 mg/kg/infusion) on a progressive ratio reinforcement schedule. CNO-induced activation of hM3Dq significantly decreased operant responding on a progressive ratio reinforcement schedule. Thirdly, rats infused with hM3Dq into the LHb were trained to self-administer 45 mg food pellets on either a FR 1 or a progressive ratio reinforcement schedule. Interestingly, our initial results indicate that this manipulation decreases food reinforced operant responding on both FR1 and progressive ratio reinforcement schedules. To determine if the observed effects are due to locomotor depression, we measured locomotor activity in rats previously infused with hM3Dq into the LHb. Following CNO (1 and 3 mg/kg) injections we found a decrease in locomotor activity at baseline levels as well as upon challenge with an acute injection of cocaine (10 mg/kg). Taken together, our results suggest that DREADD-mediated transient activation of Gq-coupled signaling in the LHb decreases operant cocaine and food self-administration and that these effects may, in part, be due to deficits in locomotor activity.

Disclosures: **S.G. Nair:** None. **D. Smirnov:** None. **J.F. Neumaier:** None.

Poster

140. Role of the Habenula in Drug Addiction

Location: Hall A

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Program#/Poster#: 140.10/G44

Topic: F.03. Motivation and Emotion

Support: NIDA Grant R01DA037327

Title: Cocaine and cue encoding in the rodent entopeduncular nucleus

Authors: *H. LI, J. L. THOMPSON, T. C. JHOU;
Med. Univ. of South Carolina, Charleston, SC

Abstract: Previous studies have shown that lateral habenula (LHb) neurons are activated by aversive stimuli and their predictors. We recently showed also that LHb neurons show a biphasic response to cocaine, with an initial inhibition followed by a delayed excitation that parallels the rewarding and aversive effects of cocaine. We sought to test whether these patterns of responses are also present in the entopeduncular nucleus (EPN), which provides a major afferent to the LHb. We found using retrograde labeling and cFos that rostral parvalbumine negative EPN neurons projecting to the LHb were activated by 30x footshocks and by 0.75 mg/kg intravenous cocaine infusion. Furthermore, the animals were conditioned with three different auditory cues: an appetitive cue paired with food pellet delivery, an aversive cue paired with a footshock, and a neutral cue paired with neither food nor footshock. Electrophysiological recordings from awake behaving animals further showed that most of neurons in the rostral EPN showed excitations to the footshock, and that cue-responsive EPN neurons exhibited relatively higher firing after aversive and neutral cues than after appetitive cues. Further experiments are underway to determine whether these neurons encode motivational properties of cocaine.

Disclosures: H. Li: None. **J.L. Thompson:** None. **T.C. Jhou:** None.

Poster

140. Role of the Habenula in Drug Addiction

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Program#/Poster#: 140.11/H1

Topic: C.17. Drugs of Abuse and Addiction

Support: Fondation Fyssen

ERC

Avenir

Title: The projection from the entopeduncular nucleus to the lateral habenula encodes aspects of cocaine withdrawal

Authors: *F. MEYE[1], T. SMIT[2], M. MAMELI[1];
[1]Inst. Du Fer A Moulin, UMR-S 839 INSERM/UPMC, Paris, France; [2]Master Neurosci. and Cognition, Utrecht Univ., Utrecht, Netherlands

Abstract: The lateral habenula (LHb) plays a pivotal role in the processing of negative emotional states, including those that occur after the intake of addictive drugs. The negative withdrawal state is an important contributor to relapse behavior and the maintenance of drug addiction. Therefore it is critical to understand the neural underpinnings of aversive drug withdrawal. Previously, we found that cocaine induces synaptic and intrinsic plasticity in the LHb, which results in hyperexcitability of this region, and which promotes the development of a negative emotional state. However, it is currently unknown how specific afferent regions that project to the LHb play a role in this. In the current study we investigate the role of the projection from the entopeduncular (EP) nucleus, the output structure of the basal ganglia, to the LHb in cocaine withdrawal. To this end we make use of a combination of *in vitro* electrophysiology and optogenetics. After stereotactic injection of viral vectors encoding for channelrhodopsin-2 into the EP, we treated mice with chronic saline or cocaine. After 1.5 days or 2 weeks of withdrawal, whole-cell voltage clamp recordings in LHb were made and EP-LHb connectivity was assessed. We show that abstinence after cocaine exposure induces persistent GABAergic synaptic plasticity in the EP-LHb projection. We also demonstrate the functional relevance of these modifications in controlling LHb neuronal output, as GABAergic masking of the excitatory transmission from EP to LHb neurons was reduced after cocaine exposure. To determine the neural circuits affected by these cocaine-evoked modifications, we use rabies viral strategies to determine which downstream neuronal subpopulation in the midbrain is mostly affected by EP-LHb signaling. Overall, our findings show that the connection between the basal ganglia and lateral habenula can be relevant in encoding aspects of drug withdrawal.

Disclosures: **F. Meye:** None. **T. Smit:** None. **M. Mameli:** None.

Poster

140. Role of the Habenula in Drug Addiction

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Topic: F.02. Animal Cognition and Behavior

Support: NIH grant NS076416

UW RRF grant

NIDA T32 DA07278-20

Title: A role for the lateral habenula and downstream efferent pathways in repeated probabilistic reversal learning

Authors: *K. S. KIDDER[1], P. M. BAKER[2], S. J. Y. MIZUMORI[2];
[2]Psychology, [1]Univ. of Washington, Seattle, WA

Abstract: A number of neuropsychiatric disorders including depression, autism spectrum disorder, and Parkinson's disease are characterized by deficits in the ability to rapidly switch behaviors under changing reward contingencies. This ability to change responses when outcomes change is an executive function commonly termed cognitive flexibility. A common task used to test cognitive flexibility across species is known as reversal learning. Previous studies have shown that manipulations of serotonin (5-HT) and dopamine (DA) affect cognitive flexibility in tasks such as reversal learning. Importantly, these two neurotransmitters are known to play a role in a variety of neuropsychiatric conditions, including the ones mentioned, raising the possibility that a common mechanism may play a role across diseases. The lateral habenula (LHb) may be a key structure in mediating reversal learning as it is known to affect transmission of 5-HT and DA. Behaviorally, the LHb is thought to provide an error signal during decision making tasks making it likely that it is critically involved in tasks requiring learning from errors such as reversal learning. To test this hypothesis, a maze based probabilistic reversal learning task (PRL) with male Long-Evans rats was used to examine the role of the LHb via neurotransmitter inactivation with the gamma-aminobutyric acid (GABA) agonists baclofen and muscimol (50ng/0.2 μL).Prior to behavioral testing rats were implanted with guide cannula aimed at the LHb for subsequent injections. The PRL task took place on a t-shaped maze with return arms. The correct arm resulted in reward on 80% of choices while the incorrect arm was never reinforced. Rats ran 200 trials per daily session. If an animal chose the correct arm over 10 consecutive trials, the reward contingences were reversed. Once animals were able to complete at least 3 reversals per session over consecutive days, injections began. Results revealed that inactivation of the LHb led to fewer overall reversals than rats injected with a saline control. Error analysis revealed a slight increase in perseverative errors and a large increase in regressive errors. Additionally deceases in stay/win behavior and increases in shift/loose behavior indicated a generalized reward sensitivity impairment after LHb inactivation. Current work is utilizing designer receptors engineered and activated by designer drugs (DREADDs) to target LHb projections to dopamine systems to further understand it's involvement in cognitive flexibility. Overall, these findings suggest that the LHb is important for learning and/or implementing switches in behavior when reward contingencies change.

Disclosures: **K.S. Kidder:** None. **P.M. Baker:** None. **S.J.Y. Mizumori:** None.

Poster

140. Role of the Habenula in Drug Addiction

Location: Hall A

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Program#/Poster#: 140.13/H3

Topic: F.03. Motivation and Emotion

Title: Microarray analysis of transcripts with elevated expressions in the rat medial or lateral habenula indicate GABAergic excitation in the medial habenula and habenular involvement in the regulation of circadian rhythm and energy balance

Authors: F. WAGNER[1], L. FRENCH[2], C. DERST[3], R. BERNARD[3], *R. W. VEH[3];
[1]Clin. for Neurol., Univ. of Jena, Jena, Germany; [2]Rotman Res. Inst. at Baycrest, Toronto, ON, Canada; [3]Universitaetsklinikum Charite, Berlin, Germany

Abstract: In vertebrates the "anti-reward-system" mainly is represented by the habenula and its medial (MHb) and especially lateral (LHb) complexes. So far, considerable knowledge has accumulated especially concerning subnuclear structures and the connectivities of MHb and LHb subnuclei. The present investigation aimed to obtain novel information, whether MHb or LHb or their subnuclei display characteristic gene products, which may shed more light on biological functions of these areas. Using microarray analysis of mRNAs expressed in microdissected habenular and thalamic control areas yielded expression values of 17,745 RNAs representing protein-coding genes, to which annotated gene names could be assigned. High relative values of genes with known expression in MHb, LHb or thalamus in the corresponding areas indicated a high precision of the microdissection procedure. The present investigation focused on gene transcripts related to major transmitter systems, catecholamines and neuropeptides. Quite surprisingly, our data indicate potentially inhibitory effects of acetylcholine and glutamate in the habenula and support a largely excitatory role of GABAergic transmission especially in the MHb. The high expression of the prokineticin 2 receptor is in line with a suspected role of the LHb during the circadian rhythm. Furthermore, several G-protein related receptors (Grp83, Grp139, Grp149, Grp151, Grp158) and many neuropeptides related to feeding are differentially expressed in the habenular region, indicating that the involvement of MHb and LHb in the regulation of food consumption and energy expenditure may have been underestimated so far.

Disclosures: F. Wagner: None. **L. French:** None. **C. Derst:** None. **R. Bernard:** None. **R.W. Veh:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA022862

NIH Grant AA022445

Title: Functional adaptations of mesolimbic dopamine neurons underlying individual alcohol drinking behaviors

Authors: *B. JUAREZ[1], A. K. FRIEDMAN[1], E. S. CALIPARI[2], J. T. YORGASON[3], M. CRUMILLER[4], S. M. KU[1], H. ZHANG[1], C. MOREL[1], D. CHAUDHURY[5], M.-H. HAN[1]; [1]Pharmacol. and Systems Therapeut., [2]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Oregon Hlth. & Sci. Univ., Portland, OR; [4]Rockefeller Univ., New York, NY; [5]New York University- Abu Dhabi, Abu Dhabi, United Arab Emirates

Abstract: Most individuals are known to consume alcohol in a controlled manner throughout their lifetimes, while others succumb to pathological, compulsive drinking behaviors. In order to understand the emergence of individual alcohol drinking behaviors, there is a need to explore the neural substrates involved in mediating the different phases of alcohol-use. The initial rewarding phase of alcohol-use is known to recruit brain regions involved in dopamine release and signaling, particularly the ventral tegmental area (VTA) sending dopaminergic projections to the nucleus accumbens (NAc). To determine if there are any neuroadaptations in this circuit (VTA-NAc) that result in individual alcohol drinking behaviors, we used a continuous access, two-bottle choice alcohol drinking paradigm in C57BL/6J mice to establish individual alcohol drinking behaviors based on alcohol preference and consumption. We discovered that low alcohol drinking mice display increased *in vivo* VTA dopaminergic firing activity and bursting parameters, while high alcohol drinking mice surprisingly maintained control-like *in vivo* firing properties. Utilizing a circuit-dissecting approach to label VTA-NAc dopamine neurons with retrograding viral eYFP tags, we performed *in vitro* electrophysiological recordings and discovered that the VTA-NAc dopamine cells of low alcohol drinking mice consistently show increased firing activity, while high alcohol drinking mice continue to display control-like levels of firing. We then used fast-scan cyclic voltammetry to determine if there were any differences in dopamine release in the NAc. We have discovered distinct differences in dopamine release and uptake in the NAc core and shell between the alcohol drinking groups. These results implicate an adapted response to alcohol's rewarding effects on the VTA-NAc pathway.

Disclosures: **B. Juarez:** None. **A.K. Friedman:** None. **E.S. Calipari:** None. **J.T. Yorgason:** None. **M. Crumiller:** None. **S.M. Ku:** None. **H. Zhang:** None. **C. Morel:** None. **D. Chaudhury:** None. **M. Han:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA R01AA021505

Title: Optogenetic-induced corticostriatal synaptic plasticity in the dorsomedial striatum of adult rodents

Authors: ***T. MA**, C.-Y. HUANG, X. WANG, J. WANG, J. WANG;
Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: The dorsomedial striatum (DMS) receives glutamatergic cortical input, which form a critical circuit controlling the learning of goal-directed instrumental actions. Long-term potentiation (LTP) in the DMS is thought to be the cellular substrate underlying the strengthening of instrumental actions in this circuit. However, large is unknown about this synaptic plasticity, due to the unreliable induction of LTP in striatum. Here, we used optogenetics to induce LTP in adult DMS slice. We found that tetanic electrical stimulation pairing with optogenetic-mediated postsynaptic depolarization, which efficiently removes Mg2+ blockade of postsynaptic NMDA receptors, induces robust LTP in DMS slices from adult rats. Importantly, activation of NMDA receptors, but not of dopamine D1 receptors (D1Rs), is required for the LTP induction. The LTP was observed in both D1R- and D2R-expressing DMS neurons of mice. In addition, using dual-channel optogenetics, we found that corticostriatal LTP was reliably induced by pairing optogenetic stimulation of prefrontal inputs with optogenetic-mediated postsynaptic depolarization of DMS neurons. Lastly, we discovered that the NMDA receptor in the distal dendrites of DMS neurons is effectively unblocked by optogenetic-induced postsynaptic depolarization. Together, these results suggest that postsynaptic optogenetic depolarization facilitates the induction of NMDAR-dependent corticostriatal LTP in the adult DMS, which help the investigation of corticostriatal LTP in instrumental learning and memory.

Disclosures: **T. Ma:** None. **C. Huang:** None. **X. Wang:** None. **J. Wang:** None. **J. Wang:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA R01AA021505

TRSA

Title: D1r-msns of the dorsomedial striatum positively control excessive alcohol intake via the nmda receptor

Authors: *Y. CHENG, T. MA, C.-Y. HUANG, X. WANG, J. WANG;
Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Dopamine D1 receptor (D1R)- and D2 receptor (D2R)-expressing medium spiny neurons (MSNs) in the striatum give rise to the direct and indirect pathways that contribute to "Go" and "NoGo" actions in rewarding behaviors, respectively. It was recently found that excessive alcohol intake enhances synaptic strength in D1R-MSNs of the dorsomedial striatum (DMS), which may contribute to the maintenance of excessive alcohol intake. Here, we examined whether and how D1R-MSNs in the DMS control alcohol consumption. We infused into the DMS of Drd1a-Cre mice with a viral vector containing Cre-dependent hM4Di. Mice were then trained to consume high levels of alcohol using the intermittent access 2-bottle choice drinking procedure. We found that chemicogenetic downregulation of D1R-MSNs activity in the DMS of mice significantly reduced excessive alcohol intake accompanying by a decrease in alcohol preference. However, neither locomotion activity nor saccharin intake was altered following chemicogenetic manipulation. In addition, we observed that excessive alcohol consumption caused an increase in NMDA receptor activity in D1R-MSNs of the DMS. Together, our results suggest that D1R-MSNs play a positive role in alcohol consumption, which is specific mediated by glutamatergic receptors.

Disclosures: Y. Cheng: None. **T. Ma:** None. **C. Huang:** None. **X. Wang:** None. **J. Wang:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA R01AA021505 (JW)

Title: Input-specific aberrant synaptic plasticity in the dorsomedial striatum of alcohol-drinking rats

Authors: *J. WANG, T. MA, E. HELLARD, C.-Y. HUANG, X. WANG;
Dept. of Neurosci. & Exptl. Therapeut., Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX

Abstract: Accumulated evidence suggests that the dorsomedial striatum (DMS) of the basal ganglia plays an essential role in pathological excessive alcohol consumption. The DMS receives multiple glutamatergic inputs. However, whether and how alcohol consumption distinctly affects these excitatory afferents in the DMS remains unknown. Here we used optogenetics to selectively activate the prefrontal cortex (PFC) and basolateral amygdala (BLA) inputs in the DMS slices from alcohol-drinking rats and measured glutamatergic transmission in corticostriatal and amygdalostriatal circuits. We found that excessive alcohol consumption leads to increases in activities of NMDA and AMPA receptors at the corticostriatal input in the DMS. Furthermore, we found that the NR2B/NMDA ratio was increased in the corticostriatal input and the NMDA/AMPA ratio remained unchanged in both inputs. We are currently examining whether *in vivo* optogenetic reversal of alcohol-evoked plasticity at the corticostriatal input abolishes alcohol seeking. Taken together, we describe how the glutamatergic input changes in the DMS of alcohol-drinking adult rats and these results suggest that excessive alcohol intake enhances corticostriatal input plasticity, which may in turn contribute to pathological alcohol consumption.

Disclosures: J. Wang: None. **T. Ma:** None. **E. Hellard:** None. **C. Huang:** None. **X. Wang:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA R01AA021505

Title: Inhibition of D1R-MSNs in the dorsomedial striatum decreases operant alcohol consumption

Authors: *E. R. HELLARD, C. HUANG, Y. CHENG, J. WANG;
Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: The striatum of the basal ganglia contains two types of principle medium spiny neurons (MSNs), dopamine D1 receptor (D1R)- and D2 receptor (D2R)-expressing MSNs, both of which have been reported as dysfunctional in neuropsychiatric diseases, including alcohol use disorder. Currently, identification and manipulation of these neuronal populations are largely dependent on the use of transgenic mice. Unfortunately, many alcohol addictive behaviors are measured better in rats and rat genetics is not nearly as advanced as mouse genetics. To target specific cell populations in the rat brain, we have developed a method called "Dual Virus Infusion" in which two viruses, a retrograde containing a Cre-recombinase and an anterograde Cre-dependent, are infused at different locations along the neural pathway of interest. Using this method, we targeted the striatonigral pathway in order to suppress D1R-MSN activity with an inhibitory light-activated channel, halorhodopsin. Our results show that using this technology we are able to label and manipulate striatonigral neurons, originating in the dorsomedial striatum (DMS) and synapsing in the substantia nigra pars reticulate. Light stimulation from optic fibers implanted into the DMS inhibited D1R-MSN firing and significantly decreased operant self-administration of alcohol. Importantly, inhibition of D1R-MSN firing did not decrease locomotion activity in an open-field test. In addition, D1R-MSN inhibition decreased cue-induced reinstatement of operant alcohol intake. These data illustrate a potential role of DMS D1R-MSNs in alcohol consumption and relapse.

Disclosures: **E.R. Hellard:** None. **C. Huang:** None. **Y. Cheng:** None. **J. Wang:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Support: NIAAA Division of Intramural Clinical and Biological Research

CNPq 2496/13-5

International Brain Research Organization (IBRO)

Title: Ethanol decreases the spontaneous firing rate of a specific subset of Globus Pallidus neurons: role of the Large-Conductance Ca^{2+}-activated potassium channel

Authors: *D. M. LOVINGER[1], K. P. ABRAHAO[2];
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Abstract: The Globus Pallidus external segment (GPe) is a large nucleus located in the core of the basal ganglia, caudomedial to the striatum. The GPe receives strong GABAergic innervation from the striatal indirect pathway. GPe neurons generate autonomous pacemaker activity which helps to control basal ganglia output, placing GPe as a central pacemaker of the basal ganglia. Little is known about ethanol effects on neuronal pacemaker activity, and especially on GPe neurons. We performed whole-cell patch-clamp recordings from GPe neurons in brain slices from wildtype C57BL/6J mice and mice expressing GFP under control of the promoter for the Lhx6 gene (Lhx6-GFP mice) to examine acute ethanol actions. Recent work has shown that the GPe is comprised of different GABAergic neuronal subtypes and a small percentage of cholinergic neurons. Each neuronal subpopulation shows specific electrophysiological properties. We have developed a cluster analysis-based classification model to identify GPe neuron subpopulations, to facilitate the study of drug effects in different neuronal subtypes. Bath application of 40mM ethanol decreased the firing rate of low-frequency-firing GPe neurons by ~25% but it did not alter the firing rate of high frequency-firing neurons. The effect was not blocked by GABAergic or glutamatergic antagonists, indicating that ethanol may affect specific channels involved in the intrinsic control of the pacemaker activity. The application of a specific BK channel antagonist, Penitrem-A (500nM), blocked the ethanol-induced decrease in firing rate, suggesting that BK channel activity is affected by ethanol in the low-frequency-firing GPe neurons. The Lhx6-GFP mouse has been used to label a subpopulation of GPe neurons with low-frequency firing rate. In Lhx6-GFP positive neurons, ethanol also decreased firing rate and this effect was blocked by the BK channel antagonist. Ongoing studies are examining the effect of *in vivo* chronic ethanol exposure on the firing rate of Lhx6-GFP GPe neurons. This knowledge may provide better understanding of the mechanisms contributing to ethanol effects on the Basal Ganglia and associated behaviors.

Disclosures: D.M. Lovinger: None. **K.P. Abrahao:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

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NIH Grant AA021618

Title: A role for Kv7 channels in alcohol drinking: Genetics, pharmacology, and neuroadaptations

Authors: *N. M. STRAIGHT MCGUIER[1], W. C. GRIFFIN, III[1], J. T. GASS[1], A. E. PADULA[1], E. J. CHESLER[2], P. J. MULHOLLAND[1];
[1]Med. Univ. of South Carolina, Charleston, SC; [2]The Jackson Lab., Bar Harbor, ME

Abstract: Emerging evidence suggests that anticonvulsants are a promising class of drugs for treating individuals with AUDs. Retigabine (RTG), a unique anticonvulsant in that it opens Kv7 (Kcnq) channels was recently approved for the treatment of partial onset seizures. Using an integrated functional genomics approach (GeneWeaver), we examined the genetic relationship between Kcnq genes and alcohol-related behaviors in rodents including alcohol consumption, preference, and acceptance. This suggested that Kv7 channels regulate drinking. We next used the 2-bottle choice intermittent access (IAA) drinking model to investigate the ability of RTG to modulate drinking both when systemically administered (0 - 7.5 mg/kg) and when microinjected (0 - 10 ng) to key nodes of the addiction neurocircuitry of high drinking (HD) and low drinking (LD) Wistar rats. 7.5 mg/kg IP RTG reduced alcohol consumption and preference in HD and LD rats, however 5.0 mg/kg IP RTG increased consumption in LD rats, suggesting a genetic component to the ability of RTG to alter drinking. We then microinjected RTG directly to the nucleus accumbens (NAc) core and the ventral tegmental area (VTA). 10 ng of RTG in the NAc reduced alcohol drinking in HD rats, but had no effect on LD rats. Similarly, 5 and 10 ng of RTG in the VTA reduced consumption in HD rats, but increased alcohol preference in LD rats, suggesting that Kv7 channels in both the NAc and VTA regulate drinking behaviors, but individual differences in drinking are mediated through the VTA. Finally, a history of alcohol consumption increased sensitivity to Kv7 antagonist-induced seizure activity, indicating a drinking-induced alteration in Kv7 channel expression and/or functionality. Given the large number of alcohol-induced neuroadaptations in the NAc, we used western blots to investigate changes in Kv7.2 expression in the NAc after IAA and 3 d withdrawal. Kv7.2 expression was increased in triton X-100 insoluble membranes (i.e. postsynaptic density, axon initial segment) and decreased in triton X-100 soluble membranes. Furthermore, the expression of SUMOylated Kv7.2 was decreased in insoluble membranes, suggesting that Kv7 channels are de-SUMOylated and recruited to scaffolded signaling domains during withdrawal. To our knowledge, these data are the first to show evidence for post-translational modification by SUMOylation in a model of

alcohol or drug exposure. Altogether this work indicates that retigabine may be a promising treatment for AUDs, and that Kv7 channels are a target of alcohol-induced neuroadaptations.

Disclosures: **N.M. Straight McGuier:** None. **W.C. Griffin:** None. **J.T. Gass:** None. **A.E. Padula:** None. **E.J. Chesler:** None. **P.J. Mulholland:** None.

Poster

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AA023288

Title: Gene regulation as a basis for investigating pharmacological therapies that target Kv7 channels to reduce drinking

Authors: J. A. RINKER, D. B. FULMER, *P. J. MULHOLLAND;
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Abstract: Alcohol (ethanol) dependence is a chronic relapsing brain disorder largely influenced by genetics and characterized by an inability to regulate harmful levels of drinking. Emerging evidence suggests that Kcnq2/3 genes that encode Kv7 channels are included in the support interval for replicated QTLs for alcohol consumption and alcohol-related behaviors in rodents. Recent data from our lab has shown that Kv7 channel positive modulators are more effective at reducing consumption in high-drinking Wistar rats. This led us to further explore the relationship between Kcnq genes and escalation of drinking in a mouse chronic intermittent ethanol (CIE) exposure model of dependence. Using the GeneNetwork.org database, we found that the CIE-induced change in transcript levels for Kcnq1 (Kv7.1 subunit) in the prefrontal cortex and both Kcnq1 and Kcnq5 (Kv7.5 subunit) in the nucleus accumbens significantly correlated ($p < 0.05$) with the change in ethanol consumption after CIE-induced dependence in BXD mice. To determine if retigabine, an FDA-approved anticonvulsant and Kv7 channel opener, reduces consumption in a high drinking strain, we utilized an intermittent access to alcohol (IAA) model where C57BL/6J mice were given 24-hour access to 20% ethanol and tap water every other day. After establishing a stable baseline of intake (approximately 8 weeks), mice were injected with retigabine (5, 10 or 15 mg/kg, IP) or vehicle (0.9% saline) 15 min prior to ethanol access, and

consumption was assessed at 4 and 24 hour. Retigabine dose dependently reduced ethanol consumption at the 4-hour time point ($F_{3,15} = 3.904$, $p = 0.03$), but the effect of retigabine on drinking was no longer evident at the 24-hour time point. Taken together, these data suggest that dependence-induced regulation of Kcnq1/5 genes correlated with post-dependence ethanol consumption. Additionally, these data in mice showing that retigabine reduces drinking provide additional evidence that Kv7 channel openers, such as retigabine, could be useful pharmacotherapeutics for regulating heavy alcohol drinking in individuals with an alcohol use disorder.

Disclosures: J.A. Rinker: None. **D.B. Fulmer:** None. **P.J. Mulholland:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: AA020930

AA020929

AA014095

Title: KCa2 channels modulate forced swim test behavior and voluntary alcohol consumption in nondependent C57BL/6J mice

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Abstract: A large body of evidence indicates that alcohol dependence produces altered responses to stress, which may enhance relapse vulnerability as well as further exacerbate excessive levels of drinking. In an animal model of alcohol dependence and relapse drinking, repeated cycles of chronic intermittent ethanol (CIE) vapor exposure produce escalated voluntary alcohol drinking. CIE exposure was also shown to produce aberrant coping behavior to a stress challenge - the forced swim test (FST). That is, CIE-exposed mice compared to controls persisted in struggling in the face of an inescapable stress situation (Lopez and Becker,

unpublished observations). Previous studies also have shown that pharmacological manipulation or genetic depletion of small-conductance Ca2+-activated K+ (KCa2) channels can influence FST behavior. KCa2 channels regulate neuronal excitability and synaptic plasticity and have recently been implicated in alcohol and drug addiction. KCa2 channels in the nucleus accumbens (NAc) regulate heavy alcohol drinking, and we have shown that ethanol dependence reduced expression and function of KCa2 channels in hippocampus and NAc. The purpose of this study was to determine if CIE-induced modulation of KCa2 channels influences behavior on the FST. Treatment of ethanol-naïve C57BL/6J mice with systemic injections of the KCa2 channel allosteric inhibitor apamin (0.1 and 0.2 mg/kg) significantly decreased immobility during the FST (n=8/group), producing the same behavioral phenotype of mice exposed to CIE exposure. A separate cohort of mice was exposed to CIE treatment and received the KCa2 channel positive modulator 1-EBIO (5, 10 and 20 mg/kg, IP, n=10/group) 30 min prior to the FST. In contrast to our expectations, 1-EBIO did not prevent the CIE-induced change in coping behavior on the FST. In another study using a two-bottle (15% ethanol (v/v) vs. water), long intermittent (22hr) access design, our preliminary results in non-dependent mice show that systemic administration of 1-EBIO (2.5, 5 and 10mg/kg) dose-dependently reduced alcohol consumption in C57BL/6J mice two hours into their drinking cycle. In summary, while blockade of KCa2 channels in naïve mice produced a similar behavioral phenotype as dependent mice, positive modulation of KCa2 channels did not reverse the CIE-induced deficit on coping behavior in the FST. However, 1-EBIO markedly reduced drinking in naïve mice, suggesting that KCa2 channels may be a target to prevent escalation of drinking in dependent mice exposed to chronic FST.

Disclosures: **A.E. Padula:** None. **M.F. Lopez:** None. **W.C. Griffin:** None. **H.C. Becker:** None. **P.J. Mulholland:** None.

Poster

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Title: Ethanol-associated social avoidance in male sprague-dawley rats and the involvement of the endogenous dynorphin/kappa opioid receptor system

Authors: ***T. L. DOREMUS-FITZWATER**, E. I. VARLINSKAYA;
Psychology, Binghamton Univ., Binghamton, NY

Abstract: Adolescence is an ontogenetic period characterized by a unique responsivity to ethanol, with younger animals demonstrating attenuated sensitivity to a number of ethanol effects relative to adults. More specifically, adolescents are less sensitive to social avoidance induced by acute ethanol, thus requiring higher doses of ethanol in order to observe comparable ethanol-induced reductions in social preference (and enhancement of social avoidance) that are exhibited by adult animals at lower doses. Given the aversive effects (e.g., anxiogenesis, dysphoria) evident following activation of the dynorphin/kappa opioid receptor (DYN/KOR) system, the present study was designed to assess whether attenuated sensitivity of adolescents to ethanol-induced social avoidance was associated with ontogenetically decreased responsiveness of the DYN/KOR system to acute ethanol. Adolescent and adult male Sprague-Dawley rats were first pretreated systemically with a selective long-lasting KOR antagonist, nor-binaltorphimine (nor-BNI; 0, 2.5, or 5.0 mg/kg), 24 hr prior to an acute ethanol challenge (0, 0.5, 1.0, 1.5, or 2.0 g/kg). Whereas acute ethanol alone induced social avoidance with as low as 1.0 g/kg in adults, adolescents demonstrated ethanol-related social avoidance only after a dose of 1.5 g/kg ethanol or higher. Furthermore, nor-BNI was more effective in adults at preventing the occurrence of ethanol-induced social avoidance following the 1.0, 1.5, or 2.0 g/kg doses: although the 2.5 mg/kg dose of nor-BNI significantly reversed ethanol-induced social avoidance in adults, nor-BNI had similar effects in adolescents only following the 5.0 mg/kg dose. By far the most striking age-related difference in sensitivity to social inhibitory effects of acute ethanol_and its reversal by nor-BNI_was observed at the 1.0 g/kg dose of ethanol. Thus, to further examine the possible involvement of the DYN/KOR system to these ethanol effects, a separate group of adolescent and adult male rats were given an acute 1.0 g/kg ethanol challenge and mRNA expression of several genes associated with the opioid system were assessed 30 min later in the amygdala and anterior cingulate cortex (ACC). Results showed that acute ethanol did not significantly impact expression of opioid-related genes in the amygdala. In contrast, acute ethanol was associated with modest increases in expression of prodynorphin and KOR mRNA in adults but not adolescents in the ACC. Taken together, these results suggest that age-related differences in social consequences of ethanol may be related, at least in part, to an adolescent-typical decrease in sensitivity of the DYN/KOR system to acute ethanol challenge.

Disclosures: **T.L. Doremus-Fitzwater:** None. **E.I. Varlinskaya:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01 AA013983

Title: NMDA receptor channel blockers and alcohol-escalated aggression in mice

Authors: *E. L. NEWMAN[1], T. WANG[1], M. TERUNUMA[2], J. F. DEBOLD[1], K. A. MICZEK[1];
[1]Psychology, Tufts Univ., Medford, MA; [2]Dept. of Cell Physiol. and Pharmacol., Univ. of Leicester, Leicester, United Kingdom

Abstract: NMDA receptor antagonists including memantine and ketamine may possess therapeutic potential for the treatment of major depressive disorder and alcohol use disorder. However, in mice that self-administer a moderate dose of alcohol, memantine can promote aggression. The present investigation aimed to determine whether individual differences in aggressive phenotype could predict the effects of ketamine and memantine on alcohol-heightened aggression. First, breeding resident males were conditioned to rapidly self-administer 1.0 g/kg of ethanol (EtOH, 6% w/v) or water. Stable aggressive behavior was then evaluated via five-minute, resident-intruder confrontations. To identify mice as alcohol-heightened (AHA) or alcohol non-heightened aggressors (ANA), agonistic behavior was assessed every other day ten minutes after EtOH or water self-administration. Subsequently, mice were administered doses of memantine (i.p., 0.0-30.0 mg/kg) or ketamine (i.p. 0.0-30.0 mg/kg) after consuming either 1.0 g/kg of 6% EtOH (w/v) or water. Following water intake and treatment with a moderate dose of memantine (3.0 mg/kg), AHAs showed a significant increase in attack bite frequency. ANAs expressed an equally pronounced increase in aggression following EtOH intake and administration of memantine (3.0 mg/kg). After water self-administration, treatment with a moderate dose of ketamine (5.6 mg/kg) increased aggression in AHA mice; however, unlike memantine, ketamine had no effect on aggression in ANAs. These findings suggest that moderate doses of alcohol, ketamine and memantine can escalate aggression in AHAs, possibly due to similar mechanisms of action on NMDA receptors. Interestingly, memantine interacted with alcohol to increase aggression in alcohol non-heightened aggressors. Studies suggest that memantine may have some preference for receptors with weaker Mg2+ blocks while alcohol may selectively phosphorylate NR2B-containing NMDA receptors. Therefore, we speculate that Western blot analysis may reveal differential expression of NR2D and phosphorylated NR2B in AHA versus ANA mice within the prefrontal cortex and hypothalamus.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: SDSU Research Foundation

Title: Decreased hippocampal cell proliferation following chronic ethanol exposure: reversal by cholinergic mechanisms

Authors: *S. RAHMAN, M. RONI;
Pharmaceut. Sci., South Dakota State Univ., Brookings, SD

Abstract: We have shown that brain nicotinic cholinergic signaling plays a critical role in ethanol exposure-induced depression-like behavior. Cholinergic mechanisms might be associated with changes in hippocampal cell proliferation that are characteristic of major depression following chronic ethanol exposure. Adult C57BL/6J male mice were allowed to drink 10% ethanol or water for 28 days using a two-bottle choice procedure. Twenty-four hour after removal of ethanol, mice received once daily injection of saline or 1 mg/kg of lobeline (a nicotinic acetylcholine receptor ligand) during a 14 day abstinence period. Mice were tested after the last injection using the forced swim test (FST), a measure to test depression-like behavior, and brain samples were collected for immunohistochemistry. Chronic ethanol exposure significantly increased immobility time in the FST. Repeated lobeline treatment decreased immobility time in ethanol-exposed mice (control: 163±12 sec, lobeline: 84±10 sec, $p<0.05$). Chronic ethanol exposure also significantly ($p<0.05$) reduced the number of BrdU-positive cells in the dentate gyrus of hippocampus, indicating decreased hippocampal cell proliferation. Similarly, chronic ethanol exposure significantly ($p<0.05$) reduced brain-derived neurotrophic factor (BDNF)-positive cells in the dentate gyrus of hippocampus. In contrast, repeated lobeline treatment significantly ($p<0.05$) increased both BrdU and BDNF-positive cells in the dentate gyrus, indicating the antidepressant-like effects of lobeline associated with changes in hippocampal cell proliferation. These results suggest that nicotinic cholinergic signaling mechanisms play important role in hippocampal cell proliferation following chronic ethanol exposure. (Supported in part by SDSU Research Foundation).

Disclosures: S. Rahman: None. **M. Roni:** None.

Poster

141. Alcohol: Molecular Mechanisms

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ISCIII MINECO Grant PI13/02261

Title: Exposure to ethanol during adolescence alters CB_1-dependent synaptic plasticity in hippocampal dentate gyrus of adult mice

Authors: *P. GRANDES[1,2], S. PEÑASCO[1,2], N. PUENTE[1,2], A. RAMOS[1,2], N. ROYO[1,2], A. GUTIÉRREZ[1,2], I. BONILLA[1,2], L. REGUERO[1,2], M.-J. CANDUELA[1,2], J. MENDIZABAL-ZUBIAGA[1,2], F. RODRÍGUEZ DE FONSECA[3], J. SUÁREZ[3], I. ELEZGARAI[1,2];
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Abstract: Alcohol dependence affects millions of people worldwide, and alcohol consumption in adolescence has reached alarming rates. Ethanol interacts with the endocannabinoid system (ECS) and that the function of this system may be altered in ethanol dependence. Here we investigated the effect of ethanol consumption on excitatory synaptic transmission and plasticity mediated by the cannabinoid CB_1 receptor in hippocampal dentate gyrus (DG). Male C57BL6 mice were exposed to intermittent ethanol intake (20% (v/v) in tap water) using a 4 days drinking-in-the-dark (DID) procedure during adolescence (PD 30 ± 2 to 54 ± 2). Animals were given access to ethanol (or water) for 2-h sessions during three days, and for an additional 4-h session on the 4th day. At 18 - 21 days withdrawal from DID procedure, ethanol and control adult mice were sacrificed. Electrophysiological, immunohistochemical, and molecular techniques were applied. Excitatory postsynaptic potentials (fEPSPs) were evoked after stimulation of the medial perforant path and recordings were made in the supragranular zone of the dentate molecular layer (ML) in the presence of the $GABA_A$ antagonist picrotoxin. CB_1 receptor activation by CP55,940 (10 μM) inhibited fEPSPs in controls (26.43 ± 2.77% of baseline, ** $P < 0.01$, Mann Whitney test), as was previously described. However, this effect was not observed in ethanol-exposed mice (4.9 ± 7.47% of baseline, ns). Furthermore, ML

synaptic stimulation (10 min, 10 Hz) triggered a long term depression (LTD) of the excitatory synaptic transmission (about 20% of inhibition) that was absent in adult mice after ethanol consumption during adolescence (2.7 ± 3.12% of inhibition, ** $P < 0.0001$, Mann Whitney test). This excitatory plasticity was CB_1 dependent as the CB_1 receptor antagonist AM251 (4 μM) abolished LTD (8 ± 6.6% of inhibition, *** $P < 0.0001$, Mann Whitney test). CB_1 immunoreactivity decreased in the dentate ML of ethanol-exposed (87.47 ± 0.58%) versus control (100 ± 0.77%) mice (*** $P < 0.0001$, Mann Whitney test). Also, the relative mRNA and CB_1 protein significantly decreased, while a significant increase in MAGL (mRNA and protein) was detected after ethanol exposure. Altogether, repetitive exposure to ethanol during adolescence leads to a deficit of endocannabinoid-dependent LTD in adult DG excitatory synapses, probably due to a down-regulation of CB_1 receptors and a reduction of the endocannabinoid tone by an increase of MAGL. Funded by RETICS, ISCIII (RD12/0028/0001; RD12/0028/0004); MINECO (BFU2012-33334); Basque Government (IT764-13); UPV/EHU (UFI11/41); ISCIII (MINECO) (CP12/03109, PI13/02261); JA (SAS111224); JA UE/ERDF (PI45403, CTS-8221).

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Poster

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Support: NIAAA 5T32AA007468-28

Title: Effects of alcohol preference, exposure, and withdrawal on c-Myc protein levels in the brain

Authors: T. AKINYEKE[1], *J. RABER[2];
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Abstract: Alcoholism is a use disorder categorized by significant impairments that are directly related to persistent and extreme intake of alcohol. Alcoholism can affect multiple organs. With regard to the brain, many brain regions and a range of cellular and molecular processes in those

regions are involved in alcoholism. The CNS impairments involve physiological, psychological, and/or social dysfunction. c-Myc is a multifunctional, nuclear phosphoprotein that plays a role in regulation of cell cycle progression, apoptosis, and cellular transformation. Previous studies in rats exposed to ethanol for less than 24 h have suggested that increased c-myc levels may be instrumental in skeletal muscle, possibly via activating transcriptional pathways. The effects of alcohol exposure and withdrawal on c-Myc protein expression in the brain are not clear. In a 2008 study, in which a proteomics analysis was used to determine protein levels in frontal cortices of chronic alcoholics and controls, chronic and excessive alcohol consumption lead to increases in c-Myc protein levels. To study the effects of alcohol preference, exposure, and withdrawal on c-Myc protein levels in the brain, we started to analyze by Western blot c-Myc protein levels in the hypothalamus and amygdala in mouse lines selected for alcohol preference or dislike (SOT/NOT) and mouse lines selectively bred for severe or mild ethanol withdrawal handling-induced convulsions (HICs) after cessation of three days of ethanol vapor inhalation (WSR/WSP). In addition, we started to analyze the effects of acute alcohol exposure on c-Myc protein levels in the brain. In addition to c-Myc levels, Western blot analysis is used to determine protein levels of β-catenin (upstream target) and p21 (downstream target). The results of these studies will be presented at the meeting. This work was supported by the NIAAA Training Grant 5T32AA007468-28 and the development account of Dr Raber.

Disclosures: **T. Akinyeke:** None. **J. Raber:** None.

Poster

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CONICYT DPI20140008

Title: Low concentrations of ethanol potentiates non-synaptic glycine receptors in D1 medium spiny neurons

Authors: *B. MUÑOZ[1], D. M. LOVINGER[2], L. G. AGUAYO[1];
[1]Dept. of Physiol., Univ. of Concepcion, Concepcion, Chile; [2]Natl. Inst. on Alcohol Abuse and Alcoholism, NIH, Rockville, MD

Abstract: Introduction Alcohol, like other drugs of abuse, activates the mesolimbic dopaminergic system increasing dopamine in the nucleus accumbens (nAc) and producing reinforcement and abuse. Glycine receptors (GlyRs) in the nAc were reported to regulate the release of dopamine in the presence of ethanol. Here, we examined the effects of ethanol on dissociated medium spiny neurons (MSN) and nAc slices from D1-GFP+ mice. Results MSNs presented phasic and tonic GlyR activated currents. We found different GlyR properties between D1-GFP+ and D1-GFP- neurons. For example, the EC50 for D1-GFP+ neurons was 37±5 μM, whereas the EC50 for D1-GFP- neurons was 61±22 μM. Interestingly, D1-GFP+ neurons were strongly potentiated by 1-100 mM ethanol. On the other hand, D1-GFP- were only sensitive to high ethanol concentrations (100 mM). In addition, ethanol increased the tonic current in D1 MSN, which was sensitive to Org24598, and this effect was inhibited by strychnine (STN). However, phasic synaptic currents (mIPSC and eIPSC) were insensitive to ethanol modulation, but sensitive to STN. Finally, we evaluated the role of GlyR in neuronal excitability finding that STN increased the action potential (AP) firing. Ethanol slightly decreased the frequency of AP firing and when co-applied with STN, this effect was blocked. Conclusion This study shows that the GlyR-mediated tonic current is potentiated by ethanol. Finally, these data show that ethanol sensitive GlyRs are expressed in the mesolimbic dopamine system, specifically in D1 MSNs, which possibly regulate the addictive properties of alcohol. Supported by NIH AA17875 and CONICYT DPI20140008

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Poster

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Support: NIH P50 AA017072 (DR)

Title: Excessive alcohol drinking in rodents induces increased mTORC1-dependent Prosapip1 translation in the nucleus accumbens

Authors: *S. LAGUESSE, F. LIU, N. MORISOT, D. RON;
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Abstract: We previously found that excessive alcohol consumption activates a PI3K/AKT signaling pathway that ultimately lead to the activation of the mechanistic target of rapamycin

complex 1 (mTORC1) in the nucleus accumbens (Nac) of rodents (1, 2). mTORC1 is localized in dendrites, promotes the translation of synaptic proteins and plays an important role in synaptic plasticity (3). Together, these findings suggest that mTORC1-dependent mRNA to protein translation could play a major role in neuroadaptations induced by alcohol drinking that underlie the development and/or maintenance of excessive consumption. To identify novel gene products whose translation is induced in the Nac in response to the alcohol-dependent mTORC1 activation, we utilized the high throughput RNA sequencing (RNA seq) approach. Specifically, mice underwent an intermittent access to 20% alcohol or water only using 2-bottle choice paradigm for 8 weeks, and were then treated with rapamycin or vehicle. The NAc was dissected, polysomes (e.g. RNA undergoing translation) were isolated, and RNA seq was performed. Among the 12 identified candidates whose translation was dependent of mTORC1 was ProSAP-interacting protein 1 (Prosapip1), a synaptic protein that interacts with the scaffolding protein ProSAP2/Shank3 and the Rap GAP SPAR (4). Using quantitative Real-Time PCR (qRT-PCR), we first confirmed the RNAseq data and showed that the translation of Prosapip1 was induced in response to excessive alcohol drinking in an mTORC1-dependent manner. We further obtained data suggesting that the increase in mRNA of Prosapip1 was due solely to a translation and not a transcription event, as Prosapip1mRNA expression was only increased in the polysomal mRNA fraction and not in the total mRNA fraction. By western blot analysis, we found that Prosapip1 protein expression is significantly increased in the Nac of mice after a 4 hours binge drinking session and was maintained even after 24 hours of withdrawal. Prosapip1 levels were not altered in other brain regions where mTORC1 is not activated by alcohol. Finally, similar findings were obtained in rats undergoing an intermittent 20% alcohol 2-bottle choice paradigm. Together our data suggest that Propapip1 is a novel mTORC1-dependent gene product whose levels are induced by voluntary alcohol intake in the Nac. Functional consequences of the molecular findings will also be described. 1.Neasta,J., Proc Natl Acad Sci U S A (2010). 2.Neasta,J., Biological psychiatry (2011). 3.Lipton, J.O. and Sahin, M., Neuron (2014). 4.Wendholt, D., The Journal of biological chemistry (2006).

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Title: GABAA signaling mediates the locomotor stimulant response to ethanol in *Drosophila* melanogaster

Authors: C. W. DAACK, *C. L. KLIETHERMES;
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Abstract: The fruit fly *Drosophila melanogaster* shows a repertoire of behavioral responses to ethanol that is remarkably similar to that of rodents. These homologous behaviors are believed to result from ethanol's effects on conserved neurotransmitter systems, but few studies have directly tested this assumption. In the current experiments, we used genetic and pharmacological approaches to determine whether a conserved behavioral response to ethanol, low dose locomotor stimulation, might be mediated by GABAergic signaling in *D melanogaster.* We first used pan-neuronal expression of RNAis to downregulate expression of the three known $GABA_A$ subunits in *D melanogaster,* GRD, RDL, and LCCH3. Decreased expression of GRD or RDL resulted in a non-selective decrease in basal and ethanol-stimulated locomotion, while LCCH3 downregulation resulted in a relatively selective attenuation of the stimulant response. Feeding flies the $GABA_A$ antagonist picrotoxin prior to ethanol exposure resulted in a biphasic effect on the locomotor stimulant response, with low doses increasing and high doses attenuating the response to ethanol. These results suggest that $GABA_A$ receptors are involved in the locomotor stimulant response to ethanol in flies, a finding that further validates their usefulness in studying ethanol's conserved neurobiological effects. We are currently examining the effects of adult-specific receptor downregulation on the stimulant response, and have also begun studies aimed at localizing the GABAergic neurons that mediate this effect.

Disclosures: C.W. Daack: None. **C.L. Kliethermes:** None.

Poster

141. Alcohol: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 141.18/H21

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA-019971 (NADIA project; to S.C.P)

VA Senior Career Scientist Award (to S.C.P)

VA Merit Grant I01BX000143 (to S.C.P)

Title: Decreased lysine demethylase 1 (Lsd1) and increased H3K9 dimethylation in the adult amygdala after adolescent alcohol exposure

Authors: *E. J. KYZAR[1,2], H. ZHANG[2], A. SAKHARKAR[2], S. C. PANDEY[2,3];
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Abstract: Alcohol exposure in adolescence predisposes to the development of alcoholism and psychiatric disturbances in adulthood. Epigenetic processes in the amygdala have been implicated in the persistence of alcohol-related behavioral and physiological changes to adulthood. Rats were exposed to 2g/kg ethanol (2 days on/2 days off) on post-natal days (PND) 28-41, corresponding to adolescence. Animals were sacrificed 1 hr and 24 hr after last ethanol exposure for measurement of enzymes involved in histone methylation mechanisms in the amygdala immediately following adolescent alcohol exposure. A cohort of animals was allowed to mature to PND 92 to measure the lasting effects of adolescent ethanol exposure on histone methylation mechanisms. Adolescent intermittent ethanol (AIE) exposure increased Lsd1 mRNA expression in the amygdala 1 hr after last alcohol exposure. When measured at adulthood, AIE rats displayed increased anxiety-like behaviors in the elevated plus maze (EPM) and a decrease in mRNA levels of Lsd1 and Lsd1+8a, a neuron-specific splice variant, in the amygdala. Decreased LSD1 immunostaining was observed only in the central nucleus of amygdala (CeA) but not the medial nucleus of amygdala (MeA) or basolateral amygdala (BLA), while Lsd1+8a mRNA was decreased in both the CeA and MeA as measured by *in situ* PCR. Interestingly, AIE increased H3K9 dimethylation (H3K9me2) in the CeA and MeA, but not the BLA, in adult rats, while no change was found in H3K4me2 protein levels. We also observed a decrease in mRNA levels of the H3K9 demethylase Kdm4c. Our results indicate that AIE specifically modulates epigenetic enzymes involved in histone methylation at particular residues in the amygdaloid structures, and that some of these effects persist into adulthood and are possibly involved in AIE-induced anxiety-like behaviors as observed here and reported earlier by our laboratory. These results also highlight the importance of developmental plasticity by splice variants of Lsd1 in the amygdala during adolescent alcohol exposure.

Disclosures: **E.J. Kyzar:** None. **H. Zhang:** None. **A. Sakharkar:** None. **S.C. Pandey:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA grant P50 AA010761

NIAAA grant U01 AA014095

VA Medical Research

Title: Repeated cycles of chronic intermittent ethanol exposure alters BDNF signaling in the prefrontal cortex of C57BL/6J mice

Authors: *H. HAUN[1], W. GRIFFIN[2], L. LUDERMAN[2], K. KOCH[3], H. BECKER[2]; [1]Med. Univ. of South Carolina, Charleston, SC; [2]Charleston Alcohol Res. Ctr., Med. Univ. of South Carolina & VAMC, Charleston, SC; [3]Col. of Charleston, Charleston, SC

Abstract: Repeated cycles of chronic intermittent ethanol (CIE) exposure significantly decreases expression of brain-derived neurotropic factor (BDNF) in the medial prefrontal cortex (mPFC), which is associated with escalation of voluntary ethanol drinking in C57BL/6J mice. Additionally, we found BDNF microinfusion into the mPFC decreased CIE-induced elevated drinking to control (nondependent) levels of intake. The present study examined expression and phosphorylation of the BDNF receptor, TrkB, and downstream intracellular ERK1/2 expression and phosphorylation in the prelimbic (PRL) subregion of the mPFC in ethanol dependent and nondependent mice. Adult male C57BL/6J mice were first trained to drink ethanol in a limited access (2 hr/day) 2-bottle choice (15% ethanol vs. water) situation. Once stable baseline intake was established (2.3±0.2 g/kg), mice were separated into two groups and exposed to 4 weekly cycles of CIE vapor (EtOH group) or control air (CTL group) exposure (16 hr/day x 4 days/week), which alternated with weekly limited access 2-bottle choice drinking sessions (2 hr/day x 5 days/week). As expected, following four cycles of CIE exposure, voluntary ethanol intake escalated in the EtOH group (3.7±0.2 g/kg) while remaining relatively stable in the CTL group (2.7±0.2 g/kg) ($p< 0.05$). Tissue was collected from the PRL prior to the last scheduled drinking test session after the 4th cycle of CIE exposure. Western blot analysis revealed no change in TrkB expression, but a significant decrease (40%) in TrkB phosphorylation (Tyr-706) in EtOH mice compared to CTL mice ($p< 0.05$). Similarly, there was no change in total ERK1/2 expression in the PRL, but a significant decrease (32%) in ERK1/2 phosphorylation in EtOH mice compared to CTL mice ($p< 0.05$). Ongoing studies are exploring TrkB and ERK1/2 expression and phosphorylation within the infralimbic subregion of the mPFC under the same conditions as above. Together, these data support our earlier findings and suggest long lasting adaptations in BDNF signaling within discrete subregions of the mPFC after chronic ethanol exposure that may play a role in mediating CIE-related escalated drinking. Supported by NIAAA grants P50 AA010761, U01 AA014095, and VA Medical Research.

Disclosures: **H. Haun:** None. **W. Griffin:** None. **L. Luderman:** None. **K. Koch:** None. **H. Becker:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant U01 AA020912

NIH grant U01 AA016654

Title: Ethanol activates midkine and anaplastic lymphoma kinase signaling in neuroblastoma cells and in the brain

Authors: D. HE[1], H. CHEN[1], H. MURAMATSU[2], *A. W. LASEK[1];
[1]Psychiatry, Univ. of Illinois At Chicago, Chicago, IL; [2]Psychological and Physical Sci., Aichi Gakuin Univ., Aichi, Japan

Abstract: Alcohol engages signaling pathways in the brain. Midkine (MDK) is a neurotrophic factor that is overexpressed in the prefrontal cortex of alcoholics. MDK and one of its receptors, anaplastic lymphoma kinase (ALK), also regulate behavioral responses to ethanol in mice. The goal of this study was to determine whether MDK and ALK expression and signaling are activated by ethanol. We found that ethanol treatment of neuroblastoma cells increased MDK and ALK expression. We also assessed activation of ALK by ethanol in cells and found that ALK and ALK-dependent extracellular signal-regulated kinase (ERK) and signal transducer and activator of transcription 3 (STAT3) phosphorylation increased rapidly with ethanol exposure. Similarly, treatment of cells with recombinant MDK protein increased ALK, ERK and STAT3 phosphorylation, suggesting that ethanol may utilize MDK to activate ALK signaling. In support of this, transfection of cells with MDK siRNAs attenuated ALK signaling in response to ethanol. Ethanol also activates ERK signaling in the brain. We found that inhibition of ALK or knockout of MDK attenuated ethanol-induced ERK phosphorylation in mouse amygdala. These results demonstrate that ethanol engages MDK and ALK signaling, which has important consequences for alcohol-induced neurotoxicity and the regulation of behaviors related to alcohol abuse.

Disclosures: D. He: None. **H. Chen:** None. **H. Muramatsu:** None. **A.W. Lasek:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant 2G12-RR003051

NIH Grant 8G12-MD007600

NIH Grant HRD-1137725-03

Title: Time-dependent Wnt/β-catenin signaling in response to 25mM ethanol exposure

Authors: *A. BURGOS[1], R. APONTE[2], S. N. TREISTMAN[2], C. VELAZQUEZ-MARRERO[1]; [1]Inst. of Neurobio. Univ. of Puerto Ric, San Juan, PR; [2]Inst. of Neurobio. Univ. of Puerto Ric, San Juan, Puerto Rico

Abstract: Molecular alcohol tolerance includes and important persistent component characterized by the redistribution of the high conductance voltage- and calcium-dependent potassium channel (BK) plasma membrane surface expression. This form of alcohol tolerance has been shown to be protein synthesis-dependent after 6 hr ethanol exposure (Velázquez-Marrero et al., 2014 - In Progress). Redistribution of the BK channel only occurs after 6hr exposure and not 1 or 3 hrs and further persists after 24 hr withdrawal. Protein synthesis is required for certain forms of learning and memory. It has been characterized in the development of long-term memory and eventually maintenance of long-term plasticity (Sutton and Schuman, 2006). As has been shown, neuronal plasticity, specifically related to neurotransmission, is critical to the development and maintenance of an addiction and directly correlated to the reward-related learning process (Mulholland et al., 2009). We have characterized global changes in *de novo* protein synthesis in response to 6hr ethanol treatment with 25mM EtOH in HEK 293 cells using Click-iT technology coupled to Tandem Mass Spectrometry and Western Blot analysis. HEK293 cells show one hundred and forty five proteins with significant increase in spectral counts after ethanol incubation when exposed to 25mM EtOH for 6hrs. The highest increase was observed for β-catenin which more than doubles. This suggests a possible role in the protein synthesis-dependent regulation of BK channel redistribution at the plasma membrane. Furthermore, prior to 6hr EtOH exposure, the increase of β-catenin during 1 and 3hr treatment is not IWP-2 dependent. This suggests that the activation of the Wnt receptor during ethanol exposure only occurs after 6hr exposure. The current study allows an overview of how synthesis of specific proteins, particularly β-catenin, is correlated to EtOH exposure and presents a possible regulatory mechanism mediating protein synthesis-dependent BK channel alcohol tolerance.

Disclosures: **A. Burgos:** None. **R. Aponte:** None. **S.N. Treistman:** None. **C. Velazquez-Marrero:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIGMS COBRE: The Delaware Center for Neuroscience Research Grant 1P20GM103653 - 01A1

Title: Increase in apoptosis following a single binge ethanol exposure is followed by subsequent compensatory events in hippocampal CA1 in rats

Authors: *Z. GURSKY[1], K. J. CRISS[1], R. M. A. NAPPER[2], A. Y. KLINTSOVA[1];
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Abstract: Ethanol (EtOH) exposure in early development can lead to deleterious effects on the central nervous system. Significant increases in apoptosis are observed in many brain structures, an effect that is dependent on time and pattern of exposure (Bonthius & West, 1900; Ikonomidou et. al., 2000; Smith et al., 2015). The CA1 region of hippocampus is particularly vulnerable to EtOH exposure during the early postnatal period in rat pups (Tran & Kelly, 2003). We utilized a one-day binge model to characterize the effects of a single-day EtOH exposure on hippocampal CA1. Apoptosis in the CA1 was evaluated at three time-points following EtOH exposure on PD4. In examining rates of apoptosis following PD4 EtOH exposure, we hypothesized an increase in apoptosis of pyramidal cells in CA1 24 hours after exposure, which would gradually return to baseline over the week following the insult. On PD4, rats were assigned to one of three treatments: moderate binge exposure of EtOH via intragastric intubation (AE; 4.5 g/kg/day, two doses 2 hours apart, in milk substitute), intubated without EtOH administration (sham intubated, SI), or left undisturbed (suckle control, SC). On PD5, 8 or 11 the pups were perfused. Horizontal 40 um sections of the entire hippocampus were mounted on slides and stained with cresyl violet; using unbiased stereology, apoptotic bodies in hippocampal CA1 were quantified. A dramatic increase (by 300%) in apoptosis was found on PD5 in AE compared to SI pups. On PD8 the number of apoptotic cells in AE was significantly less than in AE on PD5 but also, AE apoptosis was significantly lower than in SC. There were no significant effects at PD11. This shows a single-day binge-like EtOH exposure on PD4 can significantly increase apoptosis which may be

compensated for by a decline in normal apoptosis on subsequent days. In addition, we observed numerous proliferating cells (labeled with BrdU) in CA1 layer at PD8 and PD11; the phenotyping of these cells is underway. Based on other models of CNS damage (e.g., asphyxiation, ischemia) (Bendel et. al., 2005; Morales et. al. 2008), a certain percentage of newly proliferated cells in CA1 appearing beyond the time of normal development could be neuronal. We conclude that a single-day binge EtOH exposure first produces an increase of apoptosis in hippocampus 24 hours after the insult; however, the brain partially compensates for initial EtOH effect by decreases in apoptosis.

Disclosures: **Z. Gursky:** None. **K.J. Criss:** None. **R.M.A. Napper:** None. **A.Y. Klintsova:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: Molecular Basis of Disease Program, Menzies Health Institute Queensland, Griffith University

Title: Regulation of α-synuclein splice variants in human alcoholic brain by miRNAs

Authors: *P. JANECZEK, J. M. LEWOHL;
Griffith Univ., Menzies Hlth. Inst. Queensland, Griffith Univ., Gold Coast, Australia

Abstract: Chronic alcohol abuse results in alterations in gene expression in brain regions susceptible to the neurotoxic effects of alcohol. α-Synuclein exists in a number of different splice variants and its expression is influenced by genetic factors and microRNAs. Here we investigate the influence of ethanol on the expression of the SNCA-140, SNCA-112 and SNCA-115 variants following exposure of HEK293T cells to 75 mM ethanol. Real-time PCR was used to measure α-synuclein splice variant mRNA levels. Comparisons were made between chronic and chronic-intermittent treatment with and without a withdrawal period to determine if the variants are differentially expressed in response to ethanol. Two transfection methods were used to investigate the miRNA-mediated regulation of miR-7, -153, -144 and -203 on α-synuclein. Results show that the expression of SNCA-140 and SNCA-112 was down-regulated following chronic ethanol exposure with ($P < 0.001$, $n = 16$) and without withdrawal ($P < 0.001$, $n = 8$), whereas the expression of SNCA-115 was up-regulated ($P < 0.001$, $n = 16$ and $P < 0.001$, $n = 8$).

HEK293T cells transfected with miR-203 mimic showed no change in SNCA-140 expression ($P = 0.988$, $n = 8$), a significant decrease in expression of the SNCA-115 ($P < 0.001$, $n = 8$) and a marginal decrease for the SNCA-112 variant ($P = 0.084$, $n = 8$). The same was seen in cells transfected with miR-144 mimic ($P = 1.000$, $n = 8$; $P = 0.002$, $n = 8$ and $P = 0.098$, $n = 8$ respectively). Preliminary studies where HEK293T cells were transfected with SNCA 3′UTR and miRNA precursor clones showed a small decrease in luciferase activity in those cells transfected with the miR-203 precursor however this was not significant ($P = 0.317$, $n = 5$). These findings suggest that ethanol may alter the expression of the α-synuclein variants differently. MiRNAs may regulate the expression of these variants and could be responsible for many of the gene expression changes that occur in the brain of chronic alcoholics.

Disclosures: P. Janeczek: None. **J.M. Lewohl:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: Interdisciplinary Center for Clinical Research Erlangen, Project E13

Deutsche Forschungsgemeinschaft (DFG) KO 947/15-1, GU 335/32-1, and MU 2789/8-1

Title: A sphingolipid pathway into co-morbidity of depression and alcoholism

Authors: *C. P. MUELLER[1], T. STÖCKL[1], E. SPRENGER[1], J. TIESEL[1], S. E. HUBER[1], D. AMATO[1], M. WITT[2], J. FUCHSER[2], J. BECKMANN[2], E. GULBINS[3], M. REICHEL[4], J. KORNHUBER[4];
[1]Section of Addiction Med., Dept. of Psychiatry and Psychotherapy, Erlangen, Germany; [2]Bruker Daltonik GmbH, Bremen, Germany; [3]Dept. of Mol. Biol., Univ. of Duisburg-Essen, Essen, Germany; [4]Dept. of Psychiatry and Psychotherapy, Univ. of Erlangen-Nuremberg, Erlangen, Germany

Abstract: Alcohol addiction is a very common psychiatric disorder with severe health consequences for the individual and for society. A common aetiology is the depression/anxiety-induced alcohol addiction. Stress, or a particular susceptibility lead to a primary depression and anxiety. In an attempt to reduce this aversive state, controlled alcohol consumption can develop into alcohol addiction. The acid sphingomyelinase (ASM)-ceramide pathway has been shown to

control the balance between neurogenesis and apoptosis in the brain. ASM hydrolyses the lipids sphingomyelin to ceramide. Levels of both lipids are crucial for the formation of lipid rafts in synaptic membranes. Genetically induced overexpression of ASM (tgASM) results in a depressive/anxious phenotype in mice. We used tgASM and heterozygous ASM knock out (hetKO ASM) mice and tested alcohol preference and escalation of consumption after repeated withdrawls. We found a significantly enhanced alcohol preference in tgASM mice, which is persistent over time for 16 vol.% alcohol solution. After repeated withdrawals, tgASM mice showed massively enhanced alcohol consumption compared to baseline and wild type. HetKO ASM mice in turn showed a reduced alcohol preference at medium alcohol concentrations and attenuated consumption after repeated withdrawals. This was in line with a significantly enhanced and reduced alcohol-induced conditioned place preference and locomotor sensitization in tgASM and hetKO ASM mice. Mass spectrometric analysis of brain slices performed by FTMS SolariX XR 12 T revealed that alcohol reduces content of sphingomyelin species in the ncl. accumbens (Nac) and hippocampus in WT animals. ASM overexpression reduced sphingomyelin species in the Nac, but not hippocampus, and reversed the alcohol effect in the Nac. These findings suggest the ASM-sphingomyelin/ceramide pathway as a potential mediator of depression-induced alcohol preference, and possibly, addiction, by controlling lipid levels in the reward system.

Disclosures: **C.P. Mueller:** None. **T. Stöckl:** None. **E. Sprenger:** None. **J. Tiesel:** None. **S.E. Huber:** None. **D. Amato:** None. **M. Witt:** A. Employment/Salary (full or part-time):; Bruker Daltonik GmbH. **J. Fuchser:** A. Employment/Salary (full or part-time):; Bruker Daltonik GmbH. **J. Beckmann:** A. Employment/Salary (full or part-time):; Bruker Daltonik GmbH. **E. Gulbins:** None. **M. Reichel:** None. **J. Kornhuber:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH AA021013 (HNR)

NSF HRD 0450339 (WV)

NIH-NIGMS 5R25GM099649-03 (AS)

Title: Effect of voluntary binge drinking on microglial activation in the medial prefrontal cortex of male and female rats

Authors: *A. SILVA-GOTAY[1], W. VARGAS[1], M. K. HOLDER[2], H. N. RICHARDSON[1]; [1]Univ. of Massachusetts Amherst, Amherst, MA; [2]Georgia State Univ., Atlanta, GA

Abstract: Adolescence is a period of development when teenagers are engaging in risky behaviors including binge drinking. Heavy alcohol use may be particularly hazardous at this time because prefrontal circuits are undergoing maturational processes that are important for increased cognitive function and behavioral control in adulthood. Alcohol is been shown to induce inflammation in the brain. Microglia, the brain immune cells, not only mediate inflammation but also play a role in neural development. The goal of the present study was to examine if voluntary alcohol activates microglia in the medial prefrontal cortex (mPFC) early in adolescence, as this could alter the trajectory of neural development. Adolescent male and female rats (PD 28-42) were exposed to two weeks of operant binge alcohol self-administration and brains were collected one day after the last drinking session. Activated microglia were labeled using an ionized calcium-binding adapter molecule 1 (Iba1) antibody. Preliminary results using threshold analysis suggest alcohol may differentially affect microglia in males and females, with a dampening effect on microglia activation in females. Future directions include detailed morphological analysis of microglia as well as examination of specific neuroinflammatory mediators to better understand that nature of this sex difference.

Disclosures: A. Silva-Gotay: None. **W. Vargas:** None. **M.K. Holder:** None. **H.N. Richardson:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant RO1 EY019277

NIH Grant R21 AA020855

NIH Grant T32 ES007026

Title: Synaptic dynamics and microglial phenotype in adolescent mice with deficient activity-dependent plasticity after early postnatal high binge ethanol exposure

Authors: *E. L.-Y. WONG[1], G. O. SIPE[2], C. E. LAMANTIA[2], A. K. MAJEWSKA[2];
[1]Dept. of Envrn. Med., [2]Neurobio. and Anat., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Fetal alcohol spectrum disorder (FASD) is the leading cause of life-long, non-heritable mental disability, but the mechanisms by which developmental ethanol (EtOH) exposure causes long-term changes in brain function are still poorly understood. Microglia are the brain's resident immune cells and the first responders to environmental insult, infection, or injury. Outside of pathology in the healthy brain, microglial processes contact neurons at synapses, influencing synaptic architecture during plastic events. We hypothesize that developmental EtOH exposure perturbs both these pathological and physiological functions of microglia, thus contributing to long-lasting changes in synaptic connectivity and experience-dependent synaptic plasticity. We modeled EtOH exposure during the human third trimester, a period of intense synaptogenesis and initial formation of neural networks, termed the 'brain growth spurt.' Daily from P4-P9, the peak of the 'brain growth spurt' in mice, C57/BL6 pups were subcutaneously injected with two doses of 1.8g/kg EtOH, delivered two hours apart in 20% v/v EtOH in saline. Two control groups of: 1) littermates handled at dosing times but not injected and 2) saline-injected littermates, were included for all experiments. We found that mice developmentally exposed to EtOH exhibited reduced ocular dominance plasticity, which is normally induced in the primary visual cortex (V1) by monocular deprivation during the visual critical period (peak at ~P28 in mouse). Because this plasticity relies on the dynamic remodeling of dendritic spines, the postsynaptic sites of most excitatory synapses, we used *in vivo* two-photon microscopy to chronically image dendritic spines through a thinned skull. We found no change in basal turnover rate of dendritic spines, suggesting that brain growth spurt EtOH exposure does not affect dendritic spine stability in adolescence. We also found that early EtOH exposure did not induce changes in microglial morphology, density, or distribution in V1, either early in development (P10) or in adolescence (P28). While these results indicate that a robust and persistent inflammatory response to developmental EtOH exposure is unlikely, we are currently examining whether basal microglial process dynamics, microglial interactions with dendritic spines, or the response of microglia to laser ablation injury are altered. Even subtle effects of developmental EtOH exposure on these microglial behaviors could have important implications for normal cognitive functions, including experience-dependent plasticity, and the brain's ability to resolve pathological insult.

Disclosures: E.L. Wong: None. **G.O. Sipe:** None. **C.E. Lamantia:** None. **A.K. Majewska:** None.

Poster

141. Alcohol: Molecular Mechanisms

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Program#/Poster#: 141.27/H30

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R21DA035594

Title: Impact of alcohol on human neural stem cells

Authors: *L. DE FILIPPIS, A. HALIKERE, Z. PANG;
Child Hlth. Inst. of New Jersey, New Brunswick, NJ

Abstract: Alcohol abuse causes an enormous impact on health, society, and the economy. Currently, there are very limited effective therapies available, largely due to the poor understanding of mechanisms underlying alcohol use disorders (AUDs). Neurogenesis from Neural Stem Cells (NSCs) is important in brain development and neuronal functions such as behavior, learning, and memory. Both neurons and NSCs are known to be sensitive to alcohol exposure, but most of the reported studies exploring effect of alcohol on NSCs are incomplete and often controversial. To better understand the effects of ethanol on NSC basal properties such as self-renewal and multipotency, we generated NSCs from induced pluripotent stem (iPS) cells (iPS-NSC) through epigenetic induction to the neural lineage and studied the neurotoxic effects of both acute or chronic exposure on both iPS cells and NSCs. We showed that transient exposure to a sub-lethal dose of ethanol (70mM, resembling alcohol blood concentration in heavy drinkers) does exert a similar effect on iPS cells and NSCs. In particular, ethanol exposure for 24 hours or 7 days does not affect the proliferation or the multipotency of iPS cells and neural progenitors but primes an innate immune-like response by activating the inflammasome-mediated pathway. We observed a partial impairment of the mitochondrial and lysosomal patterns with a decrease of the number of iPS cell-derived neurons following ethanol exposure. However, the electrophysiological activity of alcohol challenged NSC-derived neurons is preserved, since they are able to fire action potentials similar to untreated cells. Our hypothesis is that ethanol exposure increases the vulnerability of NSCs to further damage (with specific reference to oxidative damage) that could originate either from genetic background (gene risk variants) or the external environment. Hence, unraveling the mechanisms underlying the neurogenic dysfunction induced by alcohol abuse will allow us to better predict how AUD patients will develop age related disorders. This will also elucidate how increased vulnerability of neural progenitors may contribute to the development of multifactorial disorders such as Alzheimer's Disease, Parkinson's Disease and lysosomal storage diseases.

Disclosures: L. De Filippis: None. **A. Halikere:** None. **Z. Pang:** None.

Poster

141. Alcohol: Molecular Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 141.28/H31

Topic: B.02. Ligand-Gated Ion Channels

Title: Physicochemical determinants of alcohol binding in a model ligand-gated ion channel

Authors: *R. J. HOWARD[1], T. B. VOIGT[1], S. HEUSSER[2], G. KLEMENT[2], I. POUYA[2], A. R. MOLA[1], T. M. D. RUEL[1], J. R. TRUDELL[3], E. LINDAHL[2];
[1]Chem., Skidmore Col., Saratoga Springs, NY; [2]Kungliga Tekniska Högskolan, Stockholm, Sweden; [3]Anesthesia, Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Pentameric ligand-gated ion channels are heavily implicated in neurological effects of alcohol, yet high-resolution structural data in this family of receptors are limited. The prokaryotic ligand-gated ion channel GLIC is a potentially valuable model system whose structure has been determined in multiple conformations and bound to various ligands. In particular, modification of the 14' position in GLIC rendered it potently sensitive to alcohol modulation, and enabled co-crystallization with ethanol as well as other anesthetics. In order to elucidate the physicochemical determinants of alcohol modulation via the 14'-proximal site in further detail, we substituted a wide range of amino acids of varying volume, polarity, and charge at GLIC position 14', expressed the mutated channels in frog oocytes, and measured their gating and modulation properties by two-electrode voltage-clamp electrophysiology. We correlated the resulting changes in agonist and modulator sensitivity with standard amino acid properties and molecular modeling of the predicted binding site. Our results clarify the relative contributions of volume and hydrophobicity in alcohol binding in the intersubunit transmembrane region of GLIC, with implications for the system's relevance as a model for human alcohol targets.

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Poster

141. Alcohol: Molecular Mechanisms

Location: Hall A

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Program#/Poster#: 141.29/H32

Topic: C.07. Epilepsy

Title: Differential diagnosis of seizures for a patient with Alcohol abuse

Authors: *Z. KONE[1,2];
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Abstract: Introduction: Alcohol abuse is associated with 40% of seizure that may be the primary cause or trigger. Aim: This observation is to attract the attention of doctors on differential diagnoses of an epileptic seizure in the chronic alcoholic abuse. Observation: Man, 47 years, alcohol abuse and smoking since 20 years, presented abruptly a generalized tonico-clonic seizure and repeatedly. The EEG was normal. The MRI found a large mass iso-intense T1 and T2 with strong enhancement after injection. It has a wide base of implantation of the clivus lateralized to the right coming into contact with the mammillary body and insinuating on the right temporal fossa coming into contact with the hippocampus (46x30mm). Discussion: The occurrence of seizures in alcohol abuse is so frequents that their etiology is systematically relatead. The discovery in our patient with a newly formed processes character strongly epileptogenic, shows the problem of exclusivity etiopathogenic seizures in the alcohol abuse. The issue has been studied in the literature in a paper entitled " Alcohol and epilepsy: A case report between alcohol withdrawal seizures and neuroborreliosis", etiology was infectious. Conclusion: Every epileptic seizure in an alcohol abuser is not necessarily related to alcohol and/or is not solely because of alcoholism.

Disclosures: Z. Kone: None.

Poster

142. Nicotine: Neural Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 142.01/H33

Topic: C.17. Drugs of Abuse and Addiction

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NIH Grant R01HL040959-25A1

Title: Chronic intermittent nicotine delivery with lung alveolar region-targeted aerosol technology to rats produces circadian blood pharmacokinetics resembling human smokers

Authors: *X. M. SHAO[1], S. LIU[2], H. PEI[2], J. LIANG[2], R. MUDGWAY[1], J. ZHANG[3], J. L. FELDMAN[1], S. LOUIE[2], X. S. XIE[3];
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Abstract: Cigarette smoke is an aerosol containing concentrated tiny particles that carry nicotine (Nic) into lung alveolar regions to be rapidly absorbed into the circulation. Episodic inhalation of Nic aerosol during smoking activates nicotinic receptors followed by a cycle of desensitization/resensitization. Smokers' exposure to Nic is a chronic intermittent process, with intake during wakefulness and abstinence during sleep (withdrawal) resulting in circadian fluctuation of blood Nic levels. Withdrawal plays an important role in Nic addiction which has been difficult to study properly due to lack of clinically relevant animal model that mimics episodic inhalation of Nic and produces circadian blood concentration pattern resembling that of human smokers. We previously developed a non-invasive method with alveolar region-targeted aerosol technology for delivering Nic to rodents that produced blood pharmacokinetics resembling smoking a cigarette in humans. We have now developed an integrated platform with computer control where freely moving rodents in a chamber are exposed to episodic Nic aerosol on scheduled intervals and number of times. We have optimized the parameters of aerosol generation and exposure. Nic aerosol was generated with a Collison nebulizer that contained a solution of 0.12% Nic in saline. Rats were exposed to Nic aerosol for 1 min every half hour, for a total of 24 times in each 12-hour (h) dark phase of 12/12 h dark/light circadian cycles for multiple days. Rats were returned to their home cage during the light phase when there was no aerosol delivery. We collected rat blood samples every 2 h at the end of the half hour intervals between aerosol deliveries during the 12-h dark phases and every 4 h during the light phases. Plasma concentrations of Nic and its metabolite cotinine were determined with a LC-MS/MS method. Nic concentrations gradually increased during the dark phase and reached a plateau of 30 to 40 ng/ml in 8 to 10 h from the start of the daily Nic aerosol deliveries. Nic levels gradually decreased to 5 to 10 ng/ml during the light phase when no aerosol was delivered. The circadian fluctuation patterns of blood Nic and cotinine levels match the circadian blood pharmacokinetics of human smokers (Benowitz et al., 1982). In summary, we have developed methods and devices that produce chronic intermittent Nic exposure animal models with the route of administration and circadian blood pharmacokinetics equivalent to those of human smokers. This methodology is a powerful tool for studies of behavior, pharmacology and toxicology of chronic Nic exposure, nicotine addiction, tobacco-related diseases, teratogenicity, and for discovery of therapeutics.

Disclosures: X.M. Shao: None. **S. Liu:** None. **H. Pei:** None. **J. Liang:** None. **R. Mudgway:** None. **J. Zhang:** None. **J.L. Feldman:** None. **S. Louie:** None. **X.S. Xie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Afasci Inc.

Poster

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Support: DFG grant NI 683/4-2

Title: Mechanisms of nicotinic modulation of glutamatergic neuroplasticity in humans

Authors: *M. A. NITSCHE[1,2], G. BATSIKADZE[2], M.-F. KUO[2], W. PAULUS[2], S. FRESNOZA[2], J. GRUNDEY[2], M. LUGON[3], E. NAKAMURA-PALACIOS[3];
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Abstract: Nicotine (NIC) alters cognitive functions by modulating neuroplasticity and cortical excitability in animals and humans. It was recently shown that NIC can not only stabilize, but also disrupt formation of glutamatergic plasticity in the human cerebral cortex. The impact of NIC on plasticity is thought to be primarily determined via calcium channel properties of nicotinic receptor subtypes, and glutamatergic plasticity is likewise calcium-dependent. Therefore glutamatergic plasticity is likely modulated by the impact of nicotinic receptor-dependent neuronal calcium influx. We tested this hypothesis for transcranial direct current stimulation (tDCS) -induced long-term potentiation-like plasticity, which is abolished by nicotine in healthy non-smokers, possibly due to calcium overflow. To reduce calcium influx under NIC, we blocked NMDA receptors, which have calcium channel properties. We hypothesized that NMDA receptor block alone will prevent tDCS-induced plasticity, but should re-establish it under nicotine due to reduction of calcium influx. We applied anodal tDCS in 13 healthy non-smokers combined with 15 mg nicotine patches and the NMDA receptor antagonist dextromethorphan (DMO) in three different doses (50, 100 and 150 mg) or placebo medication. Corticospinal excitability was monitored by single-pulse transcranial magnetic stimulation (TMS)-induced motor evoked potential (MEP) amplitudes for up to 36 h after plasticity induction. NIC abolished the anodal tDCS-induced motor cortex excitability enhancement, which was however restituted under the medium dosage of DMO. In contrast, low-dosage DMO did not affect the impact of nicotine on tDCS-induced plasticity and high-dosage DMO abolished plasticity. For DMO alone, the low dosage had no effect, but medium and high dosages abolished tDCS-induced plasticity. These results enhance our knowledge about the proposed calcium-dependent impact of nicotine on plasticity and might be relevant for the development of novel nicotinic treatments for cognitive dysfunction.

Disclosures: **M.A. Nitsche:** F. Consulting Fees (e.g., advisory boards); Neuroelectrics. **G. Batsikadze:** None. **M. Kuo:** None. **W. Paulus:** None. **S. Fresnoza:** None. **J. Grundey:** None. **M. Lugon:** None. **E. Nakamura-Palacios:** None.

Poster

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Title: Alpha6-containing nicotinic acetylcholine receptors in midbrain dopamine neurons are poised to govern dopamine-mediated behaviors and synaptic plasticity

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Abstract: Acetylcholine acts through nicotinic and muscarinic acetylcholine (ACh) receptors in ventral midbrain and striatal areas to influence dopamine (DA) transmission. This cholinergic control of DA transmission is important for processes such as attention and motivated behavior, and is manipulated by nicotine in tobacco products. Identifying and characterizing the key ACh receptors involved in cholinergic control of DA transmission could lead to small molecule therapeutics for treating disorders involving attention, addiction, Parkinson's disease, and schizophrenia. Here, we used genetic, pharmacological, behavioral, and biophysical approaches to study nicotinic ACh receptors (nAChRs) containing the α6 subunit. α6-containing nAChRs are highly and specifically expressed in midbrain DA neurons, making them an attractive drug target. For many experiments, we used mice expressing mutant α6 nAChRs ("α6L9S" mice) that increase the sensitivity of these receptors to agonists such as ACh and nicotine. Taking advantage of a simple behavioral phenotype exhibited by α6L9S mice, we compared the ability of full versus partial α6* nAChR agonists to activate α6* nAChRs *in vivo*. Systemic

administration of nicotine (0.02-0.05 mg/kg, i.p.) and the partial agonists varenicline (0.01-0.03 mg/kg, i.p.) and ABT-089 (1-3 mg/kg, i.p.) increased locomotor activity in α6L9S mice. Using local infusions of both agonists and antagonists into brain, we demonstrate that neurons and nAChRs in the midbrain are sufficient to account for this behavioral response. Intra-VTA infusion of nicotine (1.7 nmol) increased locomotor activity in α6L9S mice but not their wildtype counterparts, while co-infusion with the α6* antagonist αCtxMII (10 pmol) blocked the enhanced locomotor activating effects of nicotine in α6L9S mice. Ongoing studies are investigating the role of dopamine D1 receptors in the nucleus accumbens in the locomotor activation induced by nicotine in α6L9S mice. To complement these behavioral studies, we studied the ability of *in vivo* α6* nAChR activation to support plasticity changes in midbrain DA neurons that are relevant to behavioral sensitization and addiction. By coupling local infusions of drugs and brain slice patch clamp electrophysiology, we show that activating α6* nAChRs in midbrain DA areas is sufficient to enhance glutamatergic transmission onto VTA DA neurons. Together, these results from *in vivo* studies suggest that α6* nAChRs residing on VTA DA neurons are positioned to strongly influence both DA-mediated behaviors and the induction of synaptic plasticity by the addictive drug nicotine.

Disclosures: J.N. Berry: None. **S.E. Engle:** None. **J.M. McIntosh:** None. **R.M. Drenan:** None.

Poster

142. Nicotine: Neural Mechanisms

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Title: NRG3 modulates long-term synaptic plasticity in orbital frontal cortex

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Abstract: Cigarette smokers have difficulty quitting, which is thought to be related to deficient impulse control. Previous studies from our lab have shown that single-nucleotide polymorphisms

across the gene for neuregulin 3 (NRG3) are linked to failed smoking cessation. A recent preclinical study indicates that NRG3 in the frontal cortex regulates certain aspects of impulsivity. However, the mechanism by which NRG3 regulates neuronal signaling in this area, and hence impulsivity, is unknown. To begin to address this, we used electrophysiological field recording to assess whether NRG3 alters long-term potentiation (LTP) in orbital frontal cortex (OFC). We developed a protocol for LTP induction in OFC and found that NRG3 attenuated the expression of LTP. This NRG3-attenuated LTP was selectively rescued by Afatinib, an ErbB4 inhibitor. These data suggest that NRG3 may influence general impulsivity through modulation of long-term synaptic plasticity in the OFC. Current studies are evaluating the effect of chronic nicotine treatment on NRG3-mediated synaptic plasticity in the OFC. This study was supported by NIH/NIDA grants DA032681 (JRT) and DA031747 (PIO).

Disclosures: **L. Zhou:** None. **P. Ortinski:** None. **J. Turner:** None.

Poster

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Ministère de l'ESR, France

Title: Modulation of dopaminergic neurons by endogenous acetycholine and nicotine

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Abstract: Dopaminergic (Daergic) system is linked to a wide range of brain functions, including motivation, attention, decision making and reinforcement. The release of DA is modulated by a number of factors, and its deregulation has been implicated in multiple psychiatric disorders, such as addiction. In particular, nicotinic acetylcholine receptors (nAChRs) has been shown to be a key modulators of DA cells. However to date there are no electrophysiological recordings of the VTA DA cells in awake nicotinic mutant mice. To understand how the activity of DAergic

neurons in the ventral tegmental area (VTA) is modulated by endogenous ACh and nicotine, we recorded in awake wild type and mutant mice lacking the β2 subunit of nAChRs (β2 - /-), i) the spontaneous activity of DA neurons, ii) their response to nicotine injections, and iii) their activity in different behavioral context. Our results showed that in awake animals, the absence of β2-/- modify the bursting patterns but more importantly seems to change the synchrony among neurons. Changing the dynamics of the DA neurons activity could explain modification of DA release in the target structure and the loss of behavioral flexibility observed in the β2 - /- mice.

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Poster

142. Nicotine: Neural Mechanisms

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Title: Does nicotine speed up subjective time or induce impulsivity?

Authors: C. W. DANIELS[1], C. C. HERRERA[1], *F. SANABRIA[2];
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Abstract: Systemic nicotine induces premature responses when timing in the seconds-to-minutes range. It has been suggested that this effect reflects a nicotine-induced acceleration of the internal clock. An alternative explanation for this evidence posits that nicotine induces impulsive behavior by reducing the threshold for responses that result in more reinforcement. The clock-speed and response-threshold hypotheses were tested in rats in two experiments using a novel timing task. In this task, rats were trained to seek food at one location after 8 s since trial onset and at a different location after 16 s. In Experiment 1, rats either received the same reward at both times (group SAME) or received a larger reward at 16 s (group DIFF). In Experiment 2, rats either received a larger reward at 8 s (group SHORT) or received a larger reward at 16 s (group LONG). Steady baseline performance was followed by 3 days of subcutaneous nicotine administration (0.3 mg/kg), baseline recovery, and, in Experiment 1, an antagonist challenge (mecamylamine, 1.0 mg/kg). Empirical and modeling analysis revealed that nicotine induced an immediate reduction in latencies to switch (LTS) between locations in all groups, but this effect

was more prominent in groups DIFF and LONG than in groups SAME and SHORT. This effect was sustained throughout nicotine administration. Mecamylamine pretreatment and nicotine discontinuation rapidly recovered baseline performance. Additionally, the modeling analysis suggested that anomalous effects of nicotine on LTS dispersion may be due to a general loss of temporal control of behavior. Taken together, these results support the response-threshold hypothesis of nicotinic effects on timing, and suggest that the response threshold may be mediated by central nicotinic acetylcholine receptors.

Disclosures: C.W. Daniels: None. **C.C. Herrera:** None. **F. Sanabria:** None.

Poster

142. Nicotine: Neural Mechanisms

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NIH Grant DK083452

Title: Catecholamine neurons in the nucleus of the solitary tract become hypersensitive to nicotine during spontaneous nicotine withdrawal

Authors: *S. PAGE[1], M. ZHU[1], S. M. APPLEYARD[2];
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Abstract: Nicotine is a highly addictive drug that has broad effects throughout the brain. One site of action is the nucleus of the solitary tract (NTS), where nicotine initiates a stress response and modulates cardiovascular and gastric function through nicotinic acetylcholine receptors (nAChRs). Catecholamine neurons in the NTS influence both stress responses and cardiovascular reflexes, making them potential targets of nicotine; however the effect of chronic nicotine on these neurons is not known. Here we examined the effect of chronic nicotine and spontaneous nicotine withdrawal on NTS catecholamine neurons using patch-clamp electrophysiology in horizontal brain slices from transgenic mice that express enhanced green florescent protein driven by the tyrosine hydroxylase promoter (TH-EGFP). TH-EGFP mice were implanted with osmotic minipumps which contained either nicotine or saline. Pumps delivered nicotine at a rate of 24 mg/kg/day. Implanted mice were split into ‘chronic’ and ‘withdrawn’ treatment groups. Mice in the chronic group were sacrificed 14 days after pump

insertion and horizontal brain slices were prepared for patch-clamp recording. Mice in the withdrawn group were also treated for 14 days, but underwent a 24-hour abstinence from nicotine/saline prior to sacrifice. Neurons positive for EGFP were recorded in voltage-clamp mode. A puff of nicotine (200μM) applied to the soma of TH-EGFP neurons induced both a direct post-synaptic current mediated by α4β2-nicotinic acetylcholine receptors (nAChRs) and an increase in the frequency of spontaneous glutamatergic excitatory post-synaptic currents (sEPSCs), a pre-synaptic effect mediated by α7 nAChRs. The average amplitude of post-synaptic nicotine currents recorded in chronic nicotine-treated animals was not statistically different from control animals that received chronic saline. The pre-synaptic effect of nicotine to increase sEPSC frequency was also not significantly altered. However, both direct post-synaptic nicotine current, and pre-synaptic effect of nicotine to increase sEPSC frequency, were significantly increased in chronic nicotine-treated animals 24 hours after the pump was removed (nicotine-withdrawn animals) compared to chronic saline-treated animals whose pumps were removed. The baseline sEPSC frequency was not changed across all treatment groups. These results indicate that NTS catecholamine neurons undergo adaptation to chronic nicotine treatment that renders them hypersensitive, both pre- and post-synaptically, to acute nicotine during nicotine withdrawal, but not during continued chronic treatment.

Disclosures: S. Page: None. **M. Zhu:** None. **S.M. Appleyard:** None.

Poster

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CONICET (PIP Nº 11420090100303)

Title: The GABAB receptors modulates dysphoric manifestations induced by mecamylamine-precipitated nicotine withdrawal in mice

Authors: A. P. VARANI[1], V. T. PEDRÓN[1], A. AON[1], B. BETTLER[2], R. MALDONADO[3], *G. N. BALERIO[4,1];
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Neurofarmacologia, Facultat de Ciències de la Salut i de la Vida, Univ. Pompeu Fabra, Barcelona (C/Dr. Aiguader, 88, 08003), Spain; [4]Univ. Buenos Aires Sch. Pharm & Biochem, Buenos Aires, Argentina

Abstract: A growing body of evidence suggests that GABAB receptors modulate the addictive properties of nicotine, the main component of tobacco that produces dependence. In our study, complementary genetic and pharmacological approaches were used to test the hypothesis that the GABAB receptors modulate aversive motivational states associated with nicotine dependence in mice. For this purpose, the expression of the conditioned place aversion induced by mecamylamine-precipitated nicotine withdrawal was investigated in baclofen (GABAB agonist) and 2-OH-saclofen (GABAB antagonist) pretreated mice (pharmacological approach) and in GABAB1 knockout mice (genetic approach). Interestingly, baclofen prevented ($p<0.001$) the expression of conditioned place aversion induced by mecamylamine-precipitated nicotine withdrawal. Similarly, conditioned place aversion induced by nicotine withdrawal was not observed in GABAB1 knockout mice ($p<0.05$). In contrast, the antagonist 2-OH-saclofen increased ($p<0.01$) the conditioned place aversion induced by nicotine withdrawal. These findings would indicate that GABAB receptors could play a role in the conditioned place aversion of nicotine withdrawal. Taken together, these results support the hypothesis that GABAB receptor agonist may offer therapeutic advantages to treat tobacco dependence.

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Poster

142. Nicotine: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Title: Age-dependent effects of nicotine or fluoxetine on 5-HT1A receptor activity

Authors: *S. J. CROSS[1], M. YUAN[2], J. O. AFAGA[1], D. LARRY-SAR[2], M. T. PHAN[2], F. M. LESLIE[1,2];
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Abstract: Adolescence is a sensitive developmental period when the initiation of smoking and the onset of psychiatric disorders typically occur. Clinical data indicate that teen smokers are more likely to develop substance abuse and psychiatric disorders. Preclinical data support these

findings, demonstrating that adolescent exposure to nicotine, the main psychoactive constituent in tobacco, produces lasting changes in neuronal signaling, brain development, and motivated behavior. Our lab has shown that nicotine enhances behavioral sensitivity to cocaine reward in adolescents but not adults (Dao et al., 2011). This effect is mimicked by fluoxetine, a selective serotonin reuptake inhibitor commonly prescribed for teen depression. Additionally, both co-administration of the 5-HT_{1A} receptor antagonist WAY-100,635 during drug exposure and acute administration of WAY-100,635 after drug exposure blocks the enhancing effects of nicotine and fluoxetine on cocaine self-administration. Since this indicates that these drugs increase endogenous 5-HT_{1A}R signaling in adolescents but not adults, the current study examines whether nicotine or fluoxetine age-dependently alter 5-HT_{1A}R activity, particularly in brain regions important for drug reward and motivated behaviors. Adolescent or adult rats received saline, nicotine (60 µg/kg, i.v.), or fluoxetine (1 mg/kg, i.v.) for 4 consecutive days. The next day, brains were collected and processed for agonist stimulation of [^{35}S]GTPγS binding to determine 5-HT_{1A}R activity. Our data indicate that adolescent nicotine or fluoxetine exposure increases 5-HT_{1A}R activity in brain regions mediating executive function and addiction-related behaviors, while adult exposure produces different patterns of regional 5-HT_{1A} activity. These results present region-specific understanding of the effects of nicotine or fluoxetine on adolescent brain development, as well as clarifying the underlying mechanisms in which 5-HT_{1A}Rs mediate sensitization of cocaine reward during adolescence. Keywords: adolescence, nicotine, 5-HT_{1A} receptor, fluoxetine

Disclosures: S.J. Cross: None. **M. Yuan:** None. **J.O. Afaga:** None. **D. Larry-Sar:** None. **M.T. Phan:** None. **F.M. Leslie:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Title: Adolescent nicotine exposure enhances fear memory expression in ABA renewal

Authors: *R. C. BARNET, E. LUNER, A. HOGENMILLER;
Col. William & Mary, Williamsburg, VA

Abstract: Adolescent nicotine decreases the number of cells in the midbrain, hippocampus, and cortex (Trauth et al., 2000) and hippocampus-dependent context conditioning in rats is impaired in adulthood following adolescent nicotine exposure (Spaeth et al., 2010). Here we examined

whether adolescent nicotine would produce long-term impairments in a more complex form of context learning known as “renewal”. In renewal, an animal learns a task in one context (A) then receives extinction treatment in a different context (B). If returned to the original training context (A) the previously extinguished behavior recovers, or “renews”. Renewal implies that one form of context learning involves the ability of contextual cues to modulate memory retrieval (Bouton, 2002). If adolescent nicotine impairs complex aspects of context learning (renewal) in the same way as simpler forms of context learning (context conditioning) - presumably by disrupting hippocampus function - then adolescent nicotine should impair renewal. Adolescent rats received 2x/day intraperitoneal injections of nicotine (saline, 0.15 mg/kg, 0.40 mg/kg) from PD28-PD54 followed by a 20-day abstinence interval. Beginning on PD75 all animals were exposed to pairings of a light with footshock (Light-shock) then subsequent light extinction, presentation of the Light with no shock (L-). Extinction occurred either in the same or different context as original Light-shock training. Animals were tested for fear of the light in the original training context using fear-potentiated startle (FPS). Adolescent nicotine did not impair but actually facilitated context-dependent memory retrieval (renewal). Findings suggest adolescent nicotine may facilitate encoding or retrieval of traumatic fear memory long after the nicotine exposure period.

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Poster

142. Nicotine: Neural Mechanisms

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NIDA T32 DA024635

NIDA K23 DA027734

Title: Sex-differential effect on midbrain dopamine receptors of smoking

Authors: *M. JOHNSON[1], K. OKITA[2], N. PETERSEN[2], C. L. ROBERTSON[2], A. C. DEAN[2], M. A. MANDELKERN[3], E. D. LONDON[2];
[2]Semel Inst., [1]UCLA, Los Angeles, CA; [3]Physics, Univ. of California Irvine, Irvine, CA

Abstract: Evidence shows that female smokers have more difficulty in attaining long-term abstinence from cigarettes than male smokers. Although sex differences are important to consider when constructing a treatment plan, there still is a dearth of biological insight behind sex differences in smoking behavior. Previous studies show sex differences in dopaminergic transmission in the ventral striatum, the brain region through which nicotine produces rewarding effects. Given that, we hypothesize that cigarette smoking has a differential effect on D2-type receptors in the substantia nigra and ventral tegmental area (SN-VTA), which contains predominantly D2-short receptors having an inhibitory effect on dopamine release, between male and females. Twenty four smokers (14 men and 10 women) and 26 non-smokers (12 men and 14 women) participated. They were tested for dopamine D2-type receptor availability, indicated by binding potential (BPnd), in the SN-VTA using positron emission tomography (PET) with [^{18}F] fallypride, a radiotracer with high affinity for D2-type receptors. In an exploratory analyses, other brain regions were tested by voxel-wise analysis and volume of interest analysis, depending on voxel significance. The interaction effect between sex and group (smokers vs. non-smokers) was significant, whereas main effects were not. The data revealed a trend of higher SN-VTA BPnd in female smokers than in female non-smokers, as well as a higher SN-VTA BPnd in female smokers than in males. This highlights the sex difference in dopamine D2-type receptors in the SN-VTA, suggesting it may be have relation to dopamine release in the striatum of smokers. In addition, voxel-wise analyses uncovered an interaction effect between sex and group on D2-type receptor availability in regions other than SN-VTA, such as amygdala, insula, temporal lobe, and striatum. These results support findings in previous studies and provide a platform to extend research in biological sex-differential effect of tobacco smoking.

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Poster

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Title: Nicotine exposure causes parallel increases in prefrontal cortex gamma oscillations and visual attention

Authors: *L. S. BUENO-JUNIOR[1], N. W. SIMON[2], M. A. WEGENER[2], B. MOGHADDAM[2];
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Abstract: Nicotine exposure has been shown to improve attentional performance, although the neural mechanism of this phenomenon is not well understood. To address this, we recorded local field potential (LFP) oscillations and single unit activity in medial prefrontal cortex (mPFC) of awake, behaving rats during five consecutive sessions of experimenter-administered nicotine (.2 mg/kg freebase concentration). Then, in a separate group of subjects, we tested visual attention performance during a comparable five day nicotine schedule using a well-established behavioral paradigm (McGaughey and Sarter, 1995). In the electrophysiology experiment, substantial modulations of mPFC low gamma LFP power (40-60 Hz) manifested on day three of nicotine exposure, whereas a consistent reduction of theta and beta power (5-25 Hz) was evident across all days. Nicotine induced a net inhibition of mPFC single unit activity after the first injection, but this effect was not observed in subsequent sessions. In the second experiment, we observed that nicotine did not affect visual attention on day one, then improved attentional performance beginning on day three. This time scale was remarkably similar to the progression of effects of nicotine on low gamma oscillations, with effects on both attention and gamma beginning on day three and persisting across subsequent treatment sessions. These parallel findings suggest that nicotine may improve attention via modulation of low gamma oscillations in mPFC.

Disclosures: L.S. Bueno-Junior: None. **N.W. Simon:** None. **M.A. Wegener:** None. **B. Moghaddam:** None.

Poster

142. Nicotine: Neural Mechanisms

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

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National Science Foundation

Title: Subsecond modulation of dopamine release by the tobacco product flavorant menthol

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Abstract: Flavorants, such as menthol, are commonly added to tobacco cigarettes in order to increase appeal. Therefore, it is not surprising that menthol, and potentially other added flavorants, alters smoking behavior. Specifically, menthol smokers have their first cigarette of the day earlier compared to non-menthol, have a harder time quitting, and smoke fewer cigarettes per day. We postulate that these differences in smoking behavior arise from menthol's actions in the midbrain dopamine (DA) pathway, a critical circuit in the brain for mediating the reinforcing effects of natural rewards and drugs of abuse. Here, in male Sprague-Dawley rats, we demonstrate a novel approach for examining the interaction between flavorants and nicotine on nicotine taking and subsequent effects on phasic DA release. Combining intraoral delivery of flavorants (0.2mL/infusion) with fast scan cyclic voltammetry in freely-moving rats, we found that intraoral administration of sucrose (10%) and menthol (0.005%), but not water, elevates phasic DA release in the nucleus accumbens core in naïve rats. Each rat received water, menthol, and sucrose, in blocks of 25 infusions. The order of flavorant presentation was counterbalanced across rats. Moreover, there was no effect on flavorant presentation order nor within a block of infusions. In order to test the reinforcing value of each flavorant, we examined operant behavior for intraoral flavorant delivery using a FR-1 schedule of reinforcement. Rats readily self-administered intraoral sucrose but not menthol nor water. Ongoing experiments include combining intraoral flavorant and intravenous nicotine self-administration in order to examine the effects of flavorants on nicotine taking.

Disclosures: **R. Wickham:** None. **E. Nunes:** None. **J. Park:** None. **N. Addy:** None.

Poster

142. Nicotine: Neural Mechanisms

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Åhlén-stiftelsen

Wilhelm och Martina Lundgrens vetenskapsfond

Bror Gadelius minnesfond

Title: Age-dependent effects of nicotine on behavioral adaptations and striatal neurotransmission in Wistar rats

Authors: *A. LOTFI, J. MORUD LEKHOLM, B. SÖDERPALM, M. ERICSON, L. ADERMARK;
Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Nicotine addiction is one of the leading contributors to the global burden of disease, and users with an early onset display more severe addictive behaviour and have a lower chance of cessation than those starting at later stages in life. In addition, there appears to be an age-dependent component to the neurophysiological effects of nicotine, with adolescents being more prone to the reinforcing and rewarding effects by the drug. The striatal nucleus is a key structure in reward-guided behaviors, and integrative effects on striatal microcircuits appear to play major roles in the development of addiction. The aim of this study was to define age-dependent effects displayed by nicotine on behavior and neuronal function in striatal subregions. To this end, sensitization to the locomotor stimulatory properties of nicotine was studied in rats of three different ages (3-4 weeks, 8-10 weeks, >20 weeks). In addition, field potential recordings in brain slices determined the acute effects of nicotine on striatal neurotransmission in nicotine naïve or nicotine pretreated rats. Our data show that repeated nicotine administration produces a faster onset of behavioral sensitization in young animals (<10 weeks old). In addition, nicotine produced a depressant effect on rearing activity, to which tolerance developed faster in the younger age groups. Field potential recordings revealed that behavioral sensitization was accompanied by a decrease in input/output function in the dorsomedial striatum (DMS) of nicotine-treated rats. Acute administration of nicotine (1μM) to brain slices from nicotine naïve rats showed an age-dependent and partially subregion specific mechanism. Nicotine produced a robust decrease in evoked field excitatory post-synaptic potential (fEPSP) amplitude, which was significantly stronger in the dorsolateral striatum (DLS) as compared to the DMS. The decrease in fEPSP amplitude involved alpha4-containing nicotinic receptors, which was blocked by the GABA-A receptor antagonist, bicuculline (2-20μM), and the glycine receptor antagonist, strychnine (1μM), and was sensitive to modulation of dopaminergic neurotransmission. The acute depressant effect displayed by nicotine on evoked fEPSPs was reduced in brain slices from older rats, as was the acute effect by the dopamine D2 receptor agonist quinpirole. The data presented here suggests that nicotine modulates behavior and striatal neurotransmission in an age-dependent manner, which might be important in understanding the underpinnings of higher risks of developing nicotine addiction in adolescents.

Disclosures: **A. Lotfi:** None. **J. Morud Lekholm:** None. **B. Söderpalm:** None. **M. Ericson:** None. **L. Adermark:** None.

Poster

142. Nicotine: Neural Mechanisms

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Support: FAPESP (2014/08881-9)

FAPESP (2013/12769-7)

FAPESP (2013/10067-5)

Title: Environmental tobacco smoke (ETS) in the early postnatal period decreases the endocannabinoid related enzymes and receptors in the brainstem

Authors: ***T. MARCOURAKIS**, N. T. BALESTRIN, N. N. MONTEIRO, R. C. T. GARCIA, S. OLIVEIRA, T. ANDRIOLI, A. C. C. S. DURAO, L. H. L. TORRES;
Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Introduction: Environmental tobacco smoke (ETS) in the early postnatal period induces impairment in brain development, evidenced by disturbs synaptic proteins and spatial learning and memory from late infancy to early adulthood. Moreover, ETS disrupts myelination in brainstem, which controls the cardiorespiratory function (Torres et al. 2014). This study investigated the effects of the early postnatal ETS exposure in the endocannabinoid system that has been described as being involved in main stages of neural development and activity. Methods: C57/Bl mice were exposed to a mixture of mainstream and sidestream smoke generated from 3R4F reference research cigarettes since postnatal day (P) 3 to P14 during 2 hours/day. Animals (n=6) were euthanized in P15 (infancy), P35 (adolescence) and P65 (adult). Enzymes and receptors of the endocannabinoid system, such as CB1, CB2, NAPE-PLD (enzyme involved in anandamide synthesis) and FAAH (enzyme involved in anandamide metabolism) were evaluated by immunoblotting in the brainstem. Results: Mice exposed to ETS showed a significant decrease in CB1 ($p<0.05$), CB2 ($p<0.05$), NAPE-PLD ($p<0.05$) and FAAH ($p<0.05$) levels in infancy and a significant decrease in FAAH ($p<0.05$) levels in adulthood in the brainstem. Discussion: Decreases in elements of the endocannabinoid system levels in the brainstem are particularly relevant. The exposure to ETS is considered one of the major risk

factors for sudden infant death syndrome (SIDS). The mechanism of SIDS is still unknown, but requires immature cardiorespiratory control and impairment in sleep arousal, involving the brainstem. Torres LH, et al. Environmental tobacco smoke in the early postnatal period induces impairment in brain myelination. Arch Toxicol. 2014a [Epub ahead of print]. Supported by FAPESP

Disclosures: T. Marcourakis: None. **N.T. Balestrin:** None. **N.N. Monteiro:** None. **R.C.T. Garcia:** None. **S. Oliveira:** None. **T. Andrioli:** None. **A.C.C.S. Durao:** None. **L.H.L. Torres:** None.

Poster

142. Nicotine: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: MFDS Grant # 14182MFDS977

Title: Repeated nicotine exposure regulates BDNF expression in the dorsal striatum via stimulation of mGluRs in glia

Authors: J. KIM, I. RYU, S. SEO, K. SHIM, *E. CHOE;
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Abstract: BDNF regulates synaptic plasticity related to drug addiction. Our preliminary study demonstrated that fourteen daily subcutaneous injections of nicotine (1.0 mg/kg) significantly increased BDNF expression in the dorsal striatum, suggesting that BDNF in glia plays a crucial role in the regulation of glutamatergic transmission in response to repeated nicotine exposure. Based on this speculation, we will determine molecular mechanisms underlying the repeated nicotine-induced BDNF releases by investigating the following hypothesis: increases in the releases of glutamate after repeated nicotine administration regulate BDNF expression in the dorsal striatum through stimulation of signaling cascades coupled to group I mGluRs (mGluR1/5) in glia. Supported by Ministry of Food and Drug Safety, Korea (Grant # 14182MFDS977, E.S.C.).

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Poster

142. Nicotine: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: MFDS Grant 14182MFDS977

Title: Nicotine challenge-induced behavioral sensitization requires glutamate releases and MAP kinase phosphorylation in the rat dorsal striatum

Authors: *I. RYU, J. KIM, S. SEO, K. SHIM, E. CHOE;
Pusan Natl. Univ., Pusan, Korea, Republic of

Abstract: The dorsal striatum is a key structure of the basal ganglia circuitry integrating dopaminergic and glutamatergic transmission associated with drug addiction. Our preliminary studies demonstrated that nicotine challenge (0.4 mg/kg) after six days withdrawal following fourteen daily systemic nicotine injections (0.4 mg/kg/day, s.c.) significantly increased extracellular glutamate releases, ERK1/2 and p38 MAP kinase phosphorylation in the dorsal striatum, and locomotor activity as well as stereotypy movement. Based on these findings, we will also determine neuropsychopharmacological mechanisms underlying the nicotine challenge-induced behavioral sensitization via activation of calcium signaling cascades linked to glutamate receptors in the dorsal striatum of rats. *Supported by Ministry of Food and Drug Safety, Korea (Grant # 14182MFDS977, E.S.C.).*

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Poster

142. Nicotine: Neural Mechanisms

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Title: Functional upregulation of α4* nicotinic acetylcholine receptors in VTA GABAergic neurons increases sensitivity to nicotine reward

Authors: *J. NGOLAB, L. LIU, R. ZHAO-SHEA, G. GAO, P. D. GARDNER, A. R. TAPPER; BNRI - Univ. of Mass Med. Sch., Worcester, MA

Abstract: Chronic nicotine exposure increases sensitivity to nicotine reward during a withdrawal period which may facilitate relapse in abstinent smokers, yet the molecular neuroadaptation(s) that contribute to this phenomenon are unknown. Interestingly, chronic nicotine use induces functional upregulation of nicotinic acetylcholine receptors (nAChRs) in the mesocorticolimbic reward pathway potentially linking upregulation to increased drug sensitivity. In the ventral tegmental area (VTA) functional upregulation of nAChRs containing the α4 subunit (α4* nAChRs) is restricted to GABAergic neurons. To test the hypothesis that increased functional expression of α4* nAChRs in these neurons modulates nicotine reward behaviors, we engineered a Cre recombinase-dependent gene expression system to selectively express α4 nAChR subunits harboring a "gain-of-function" mutation (a leucine mutated to a serine residue at the 9′ position: Leu9′Ser) in VTA GABAergic neurons of adult mice. In mice expressing Leu9′Ser α4 nAChR subunits in VTA GABAergic neurons ($Gad2^{VTA}$:Leu9′Ser mice), sub-reward threshold doses of nicotine were sufficient to selectively activate VTA GABAergic neurons and elicit acute hypolocomotion which developed tolerance with subsequent nicotine exposures compared to control animals. In the conditioned place preference procedure, nicotine was sufficient to condition a significant place preference in $Gad2^{VTA}$: Leu9′Ser mice at low nicotine doses that failed to condition control animals. Together, these data indicate that functional upregulation of α4* nAChRs in VTA GABAergic neurons confers increased sensitivity to nicotine reward and points to nAChR subtypes specifically expressed in GABAergic VTA neurons as molecular targets for smoking cessation therapeutics.

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Poster

142. Nicotine: Neural Mechanisms

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Robert Wood Johnson Foundation

Title: Differential nicotine responses in human dopaminergic neurons derived from iPSC carrying CHRNA5 N398 variant

Authors: *E. ONI[1,2], A. HALIKERE[4,5], A. J. TORO-RAMOS[1], M. R. SWERDEL[1], J. C. MOORE[2,3], J. A. TISCHFIELD[2,3], Z. P. PANG[4,5], R. P. HART[1,2];
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Abstract: Many addictive drugs such as nicotine mediate reward and reinforcing mechanisms within the mesolimbic pathway involving midbrain dopaminergic (mDA) neurons via nicotinic acetylcholine receptors (nAChRs). Previously, genome-wide association analyses (GWAs) identified single nucleotide polymorphisms (SNPs) associated with increased risk of addictive phenotypes including a SNP encoding a D398N (aspartate to asparagine) variation in the CHRNA5 gene encoding the alpha 5 subunit of nAChR. Since nicotine mediates reward within mDA neurons of the mesolimbic pathway, we differentiated iPSC to mDA cultures to test functional properties and response to nicotine. Patient-derived induced pluripotent stem cell (iPSC) lines were prepared from age- and gender-matched D398 and N398 variants with clinically defined nicotine use. We generated mature, nAChR-expressing, DA-releasing neurons using published methods. Gene expression and immunohistochemistry studies confirm that the majority of cells expressed standard mDA markers in neuronal cultures derived from iPSCs carrying either N398 or D398 variants. A minor fraction of cells expressed glutamatergic or GABAergic markers. The N398 variant differentially expresses lower levels of genes associated with glutamate, serotonin, and dopamine receptor signaling, suggesting an indirect effect on signaling. While both groups exhibited physiological properties consistent with neuronal function, the N398 neuronal population responded more actively to application of nicotine in electrophysiological analyses, consistent with reports of an enhanced initial response to nicotine in N398 subjects. Our results are consistent with altered N398 signaling of DAergic neurons, possibly due to effects in presynaptic glutamatergic cells present in our cultures.

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Poster

142. Nicotine: Neural Mechanisms

Location: Hall A

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Topic: F.02. Animal Cognition and Behavior

Title: α5 nAChR modulation of the effects of nicotine on ventral-striatal DA release and cue-reward learning

Authors: *W. M. HOWE, P. TIERNEY, A. ROSSI, D. YOUNG, R. KOZAK;
Pfizer, INC, Cambridge, MA

Abstract: Previous work has shown that the α5 nicotinic acetylcholine receptors (nAChR) in the habenula-interpeduncular nucleus (IPN) pathway are potent modulators of nicotine consumption. Emerging data suggests that the role of α5 control of drug seeking extends beyond nicotine intake, but also to other drugs of abuse (e.g. alcohol, Giorgio et al., 2014), a result that might be via this receptor's actions in circuits beyond the habenula and IPN. A substantial body of evidence shows that reward and drug seeking is controlled by mesolimbic circuitry and the modulation of dopamine (DA) release. Importantly, α5 is found throughout the mesolimbic system, including presynaptically on DA terminals in the ventral striatum where it is poised to contribute to cholinergic modulation of DA release. In the present studies, we sought to characterize the role of the α5 nAChR in modulating ventral-striatal DA release and behaviors controlled by this circuitry. First, we developed and validated an RNAi strategy to manipulate a5 expression in-vivo. We then selectively knocked-down α5 expression in the nucleus accumbens, or in the DA cell bodies in the ventral tegemental area using this shRNA. Fast-scan cyclic voltammetry (FSCV) was employed to monitor stimulated DA release in the core of the nucleus accumbens; both at baseline and in response to a nicotine challenge. We next assessed the functional impact of α5 modulation of mesolimbic circuitry with a Pavlovian conditioned approach task, and further probed the behavioral response to nicotine administration. Our results indicate that α5 knockdown reduces the impact of nicotine administration on DA release in the nucleus accumbens (n=5 for knockdown and luciferase control conditions). In the Pavlovian conditioned approach paradigm (n=16 for knockdown and luciferase control groups), α5 knockdown diminished the capacity of nicotine to augment probability (~20% decrease in approach behavior) and speed of cue-evoked approach behavior. Our combined results suggest that the α5 nAChR, via its ability to modulate mesolimbic circuitry, could be a critical mediator of fundamental motivation and reward processes, and that this effect is independent of its ability to modulate activity at the level of the habenula and IPN.

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Poster

142. Nicotine: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: Grant in Aid for Young Scientists (B) 25780443

Grant in Aid for Scientific Research C 24530914

Title: Effects of adolescent nicotine exposure on intracranial self-stimulation behavior in rats

Authors: ***T. SUENAGA**[1], S. GAO[2], Y. ISHIDA[3], D. NAKAHARA[4];
[1]Tokyo Univ. and Grad. Sch. of Social Welfare, Tokyo, Japan; [2]The Lab. of Cell Imaging, Henan Univ. of Traditional Chinese Med., Henan province Zhengzhou City, China; [3]Dept. of Psychiatry, Univ. of Miyazaki, Miyazakishi, Japan; [4]Dept. of Psychology and Behavioral Neurosci., Hamamatsu Univ. Sch. of Med., Hamamatsu, Japan

Abstract: Tobacco is one of the most commonly used addictive drugs in the world. It is suggested that exposure to nicotine during adolescence affects vulnerability to nicotine abuse later in adulthood. In the present study, we investigated the effects of nicotine during adolescence on nicotine-induced self-stimulation reward threshold in adulthood. Male Sprague-Dawley rats were divided into three groups. They were intrapenetonally treated with saline (0 mg/kg), low-dose (0.1 mg/kg), or high-dose (0.4mg/kg) of nicotine for 10 days on postnatal day 34-43. Five weeks after drug administration, rats were implanted with a monopolar electrode into the medial forebrain bandle under anesthesia. After a one-week recovery period, they were trained in intracranial self-stimulation (ICSS) tests. During the preliminary session, the frequency was held constant at 65 pps and the current intensity was progressively increased until the subjects showed vigorous self-stimulation. The current-threshold of each rat was defined as the value of stimulus that evoked 50% of the maximal rate of self-stimulation. These intensity values were then held constant for the subsequent testing of the frequency-threshold. The rats were again tested using two alternating series of ascending and descending pulse frequencies. Drug tests began after the rate-frequency curve (once daily) was stable for at least three consecutive days. A base line rate-frequency curve was measured three hours before the drug

administration. Then 0, 0.4, 0.8, or 1.2 mg/kg nicotine was administered 15 min before ICSS test. The percent shift in threshold under drug conditions was analyzed using mixed-design analysis of variance. Saline-pretreated control group showed a decrease in ICSS reward threshold under 0.4 and 0.8 mg nicotine conditions and an increase under 1.2 mg nicotine condition. On the other hand, nicotine-pretreated groups during their adolescence showed decreased ICSS threshold under all nicotine conditions. Furthermore, rats pretreated with high-dose of nicotine showed a rightward-shift in thresholds compared to control rats. The number of reinforced response under nicotine did not differ between nicotine doses and groups. Thus ICSS thresholds were unaffected by nicotine-induced hyper-locomotion. The results reveal adolescent nicotine exposure induces a long-lasting alteration of the sensitivity of brain reward systems to the nicotine.

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Poster

142. Nicotine: Neural Mechanisms

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Topic: C.17. Drugs of Abuse and Addiction

Support: Conacyt 182208

CIC UMSNH 26.10

Title: Chronic toluene exposure and nicotine administration modify adrenergic responses in isolated rat heart

Authors: N. ALVARADO-GOMEZ[1], M. CARREON-GARCIDUEÑAS[1], L. ORTEGA-VARELA[2], D. GODINEZ-HERNANDEZ[1], *M. Y. GAUTHEREAU[3];
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Abstract: Toluene is a volatile solvent that can be inhaled to achieve intoxicating states. It is found in thinner, paints and adhesives. *In vitro* studies indicate that toluene inhibits nicotinic acetylcholine receptors. Nicotine is one of the alkaloids extracted from tobacco and is considered mainly responsible for cigarette addiction. There is evidence indicating that tobacco abuse is often combined with inhalation of solvents, such as toluene. Reports indicate that inhaling

solvents can induce cardiac arrhythmias and sudden sniffing death, by mechanisms not completely understood. However, there are some reports indicating that cardiac arrhythmias due to sensitization of the heart to epinephrine are probably the most common cause of death. In addition, tobacco smoking is a risk factor for the presentation of cardiac diseases and sudden death; nevertheless, the effects of the combined administration of toluene and nicotine have not been studied. The purpose of this study was to investigate the effect of chronic administration of toluene and nicotine on the reactivity of the heart to epinephrine. Male Wistar rats (250-300 g) were placed in a static exposure chamber and exposed to 6000 ppm toluene or air (control group) during 30 minutes twice a day during 30 days. Other groups of rats received nicotine (0.5 mg/kg, p.o.) and then were exposed to toluene or air as described above. On 31st day rats were anesthetized with sodium pentobarbital (50 mg/kg), and the hearts were isolated and perfused according to Langendorff method. Concentration-response curves to epinephrine were made and perfusion pressure, heart rate and strength of ventricular contraction were measured. Toluene-exposed hearts had a lower basal perfusion pressure compared to air-exposed hearts, and nicotine and toluene-nicotine treated hearts showed greater basal perfusion pressure values. In response to epinephrine, toluene produced a lower increase in perfusion pressure. Toluene exposure, nicotine and toluene-nicotine combination produced significant increases in basal heart rate and in heart rate values in response to epinephrine; this effect was more pronounced with toluene-nicotine combination. It was observed an increase in basal strength of ventricular contraction in all treated groups; in contrast, toluene exposed hearts and nicotine treated hearts had greater increases in strength of ventricular contraction in response to epinephrine and hearts that received toluene-nicotine combination showed a decrease in this parameter. Our results suggest that both toluene and nicotine alter cardiac responses and these effects are more pronounced if toluene and nicotine are administered together.

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Poster

142. Nicotine: Neural Mechanisms

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Title: Inhalation of aerosolized nicotine enhances cognitive function in aged mice using an automated aerosol delivery system

Authors: *X. S. XIE[1], C. A. LIEU[1], B. ZOU[1], C. PASCUAL[1], J. ZHANG[1], X. M. SHAO[2]; [1]Afasci Res. Laboratories, Afasci, Inc., Redwood City, CA; [2]Dept. of Neurobio., David Geffen Sch. of Med. at UCLA, Los Angeles, CA, CA

Abstract: Tobacco use is the leading cause of preventable disease and disability in the U.S. Nicotine is the key chemical causing addiction to tobacco smoking. Current smoke cessation drugs produce about 20% long-term abstinence of smoking. Therefore, understanding nicotine pharmacology, reward effects and toxicology is critical to the development of more effective pharmacotherapies. However, animal models that mimic human nicotine kinetics, such as episodic inhalation and chronic intermittent exposure are lacking. We have developed an aerosolized nicotine delivery device integrated with our SmartCage technology. The system allows researchers to deliver a controllable amount of nicotine rapidly into the animal's systemic circulation. To test the system, we examined if nicotine aerosol inhalation in the amount similar to smoking cigarettes in human enhances learning and memory in aged mice. We administered either 0.5% aerosolized nicotine or saline to free moving mice (C57BL/6, male, 16 - 20 months old) in the aerosolized chamber for 5 min daily for consecutive 5 days. There were 91 ng/ml nicotine and 101 ng/ml cotinine in mouse plasma 5 min after the last exposure. The levels of nicotine and cotinine 30 min after exposure were 57 ng/ml and 222 ng/ml in mouse plasma, respectively, which resemble levels seen in heavy smokers. Thirty minutes after nicotine inhalation, mice were subject to the Morris Water Maze test. Probe finding was trained daily for consecutive 4 days and memory retention was assessed in the probe test. Inhalation of aerosolized nicotine did not produce a significant decrease time in finding the probe; but significantly enhanced memory in aged mice manifested by an increased time (21 s) in test quadrant compared to the aerosolized saline-control group (14 s) in test quadrant during the probe test. In the Y-maze and step through passive avoidance test, nicotine-treated mice showed modest but an insignificant increase in memory compared to saline-control. Furthermore, the system enables rodents to perform self-administration of aerosolized nicotine by either nose-poking or lever pressing. Initially food pellet reward accelerated the training of nose-poking or lever-pressing behavior. Optimization and evaluation of nicotine self-administration are underway. In summary, we show that nicotine aerosol inhalation in the amount similar to smoking cigarettes in human enhance memory in aged mice. The aerosol generation system can deliver a controllable amount of nicotine, and potentially other drugs of abuse, which induce behavioral changes, pharmacological or toxicological effects in a preclinical setting similar to that occurs in human drug addicts.

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Poster

143. Nicotine Seeking, Reward, and Relapse

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Title: The role of the dynorphin-kappa-opioid system in reinstatement of nicotine-seeking in mouse self-administration

Authors: *A. M. GOMEZ[1], M. R. BRUCHAS[1,2];
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Abstract: Nicotine is the most widely used addictive substance, and its use is accompanied by a high propensity for relapse. However, the neurobiological mechanisms underlying nicotine relapse/reinstatement remain unclear. Prior studies have shown that in rodents, activation of the kappa-opioid receptor (KOR) system via stress-induced dynorphin release elicits negative affective states, and thereby triggering reinstatement of drug-seeking behaviors. Therefore, our goal is to establish the role of the dynorphin/KOR system in stress-induced reinstatement of nicotine-seeking. We first trained male C57BL/6 mice to self-administer nicotine intravenously (0.03 mg/kg/infusion, 60 min sessions) on a fixed ratio-5 schedule of reinforcement for a minimum of 14 days. After stable levels of nicotine intake were established, mice underwent extinction training until criterion was reached (≤ 20% of responding compared to last nicotine self-administration session). We then investigated whether activation of KORs were sufficient to induce reinstatement of nicotine-seeking. Indeed, mice showed a robust reinstatement response after administration of the KOR agonist, U50,488 (2.5-5.0 mg/kg, i.p., 30 mins prior to reinstatement test). This data suggests that activation of the KOR system is sufficient to cause nicotine-seeking in mice. Currently, we are exploring whether KORs are necessary for stress-induced reinstatement of nicotine-seeking via administration of systemic KOR selective antagonists. Future follow-up studies will focus on dissecting the specific neural circuitry underlying KOR-mediated reinstatement of nicotine-seeking.

Disclosures: A.M. Gomez: None. **M.R. Bruchas:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.02/I9

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/NIH and FDA Center for Tobacco Products U54DA031659

Title: Partial inhibition of monoamine oxidase (MAO) increases nicotine self-administration in rats

Authors: T. T. SMITH[1], S. N. CWALINA[1], L. E. RUPPRECHT[1], M. J. OMINUS[1], S. E. MURPHY[2], E. C. DONNY[1], *A. F. SVED[1];
[1]Univ. Pittsburgh, Pittsburgh, PA; [2]Univ. of Minnesota, Minneopolis, MN

Abstract: Monoamine oxidase (MAO) is inhibited in the brains of chronic cigarette smokers by approximately 40%. While some compounds in cigarette smoke that inhibit MAO have been identified, it is not clear to what extent these compounds are responsible for the MAO inhibition seen in smokers. Prior studies examining the significance of the MAO inhibition to smoking behavior have used known MAO inhibitors to examine their effect on nicotine self-administration in rats. These studies have shown that MAO inhibitors can enhance responding for intravenous (i.v.) infusions of nicotine, especially at low doses of nicotine. However, these studies have relied on large doses of MAO inhibitors that produce near complete inhibition of MAO, and in some studies the doses are large enough to produce substantial off-target effects. The goal of the present study was to examine the effects of doses of tranylcypromine (TCP) that produce partial inhibition of MAO on i.v. nicotine self-administration in adult rats. Rats responded for a low dose of nicotine (10 ug/kg/infusion) in daily 1-hr sessions and received an i.p. injection of 0, 0.1, 0.3, or 1.0 mg/kg of TCP 1-hr prior to each self-administration session. TCP produced a dose-dependent increase in the rate at which rats acquired stable nicotine self-administration, as well as the rate of nicotine self-administration during stable maintenance. The average number of infusions earned during the final three days of self-administration on a fixed-ratio 2 schedule of reinforcement was 7.75 in the saline group (SEM=1.59, n=21). Rats receiving 0.3 mg/kg TCP earned an average of 18.48 infusions (SEM=2.08, n=21), and MAO activity, measured in the dorsal striatum from a subset of brains collected at the end of the final session, was 20% of that in control animals. Across all doses of TCP, there was a significant correlation between nicotine self-administration and MAO inhibition. These data suggest that in the range of partial MAO inhibition seen in cigarette smokers, MAO inhibition increases responding for nicotine. Therefore MAO inhibition caused by cigarette smoking may interact with nicotine to

promote smoking behavior, and this may be particularly relevant at low doses of nicotine. These data may be important for the FDA as they consider a mandated reduction in the nicotine content of combustible products. These data suggest that cigarette constituents that inhibit MAO are likely to shift the reinforcing value of low-nicotine products.

Disclosures: **T.T. Smith:** None. **S.N. Cwalina:** None. **L.E. Rupprecht:** None. **M.J. Ominus:** None. **S.E. Murphy:** None. **E.C. Donny:** None. **A.F. Sved:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.03/I10

Topic: C.17. Drugs of Abuse and Addiction

Title: Individual differences in nicotine self-administration and approach to nicotine cues

Authors: *C. P. KING, C. L. VERSAGGI, L. JACKSON, P. J. MEYER;
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Abstract: Drug cues are important in the regulation of drug taking and relapse behaviors, and can produce craving and elicit cue-directed approach. Individual differences in the response to these drug cues may be related to the responses to food cues. To test this, we preformed two experiments examining the relationship between food- and nicotine cue responsivity. In the first experiment, the response to food predictive cues was measured in Sprague-Dawley rats during a Pavlovian conditioned approach procedure. Rats categorized as “sign-trackers” (n=6) approached a food-paired cue, and “goal-trackers” (n=5) approached the food delivery location. We then examined whether sign- and goal-tracker rats had different patterns of intravenous nicotine self-administration, extinction, and cue-induced reinstatement. Specifically, rats were trained to nose poke for a cue light and either saline or intravenous nicotine (.03mg/kg/infusion) on a FR5 schedule of reinforcement. We found that nicotine self-administering rats performed more responses and received more infusions than controls. Responding was then extinguished and rats were tested for cue-induced reinstatement of operant responding. During this test, sign-trackers responded more than goal-trackers when nicotine-paired cues were returned. In a second experiment, we examined whether a cue predictive of an intravenous nicotine infusion could produce a sign-tracking response in rats during Pavlovian conditioning. Rats (n = 13) received infusions of intravenous nicotine (0.03 mg/kg/infusion) on a variable interval schedule. Nicotine infusions were either paired with a lever cue immediately preceding the infusion, or presented in an unpaired fashion. We found that the paired group showed higher nicotine cue directed

approach compared to the unpaired group, showing that a nicotine cue can elicit approach behavior in rats. Taken together, these results suggest that sign-tracking and nicotine cue-induced reinstatement are subserved by common neurobehavioral processes. Further, we demonstrate that nicotine cues can also acquire incentive motivational properties, as measured by approach behavior. These cues may influence drug-taking during addiction but the effect of these cues may vary among individuals.

Disclosures: **C.P. King:** None. **C.L. Versaggi:** None. **L. Jackson:** None. **P.J. Meyer:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.04/I11

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA027840

Title: Histamine and Nicotine Self-administration

Authors: ***E. D. LEVIN**[1], K. YANG[2], S. JUNAID[2], K. MURGAS[2], C. WELLS[2], S. SLADE[2]; [1]Duke Univ. Med. Ctr., Durham, NC; [2]Duke Univ., Durham, NC

Abstract: Histamine is a neurotransmitter in the brain as well as a modulator in the periphery. As such, it does more than control secretions in the stomach and sinuses. Histaminergic neural circuits play important roles in a variety of behavioral functions. Histamine H1 receptors have been found in our earlier studies and others' to play critical roles in a variety of behavioral functions from sensorimotor modulation, to cognition to maintenance of reinforced behavior. Concerning reinforced behavior, we found that acute or chronic dosing of the histamine H1 antagonist pyrilamine significantly reduced nicotine self-administration in rats. H1 antagonist treatment may be useful as an aid for smoking cessation. To further the characterization of histaminergic involvement in nicotine self-administration, we investigated the effects of an H1 histamine receptor agonist, betahistine. Female young adult Sprague-Dawley rats were trained to self-administer nicotine (FR1, 0.03 mg/kg/infusion) in one-hour sessions. In the first study, betahistine (0, 2, 4, 8 and 16 mg/kg) was acutely administered to determine the dose-effect range for potentially increasing nicotine self-administration. Trends were found that 8 mg/kg of betahistine increased nicotine self-administration. This dose was not found to affect locomotor activity. In the second study a different set of rats were trained in the same way to self-administer nicotine. The rats were divided into two groups matched for baseline nicotine self-

administration. One group was given 8 mg/kg of betahistine before every subsequent nicotine self-administration session (N=17), while the other group was given control saline injections (N=19). Chronic betahistine significantly ($p<0.05$) escalated increasing nicotine self-administration. This further shows that histamine plays important roles in the level of nicotine self-administration, particularly further elevating higher levels of nicotine self-administration. Histaminergic system involvement in nicotine self-administration may be related to stress effects increasing tobacco addiction.

Disclosures: **E.D. Levin:** None. **K. Yang:** None. **S. Junaid:** None. **K. Murgas:** None. **C. Wells:** None. **S. Slade:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant(R01DA033396)

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DECODE (Inscopix)

Title: Imaging CA1-hippocampal neuronal ensembles during nicotine-dependent contextual associations

Authors: ***L. XIA**[1], S. K. NYGARD[2], B. ACLAND[4], N. J. HOURGUETTES[2], G. SOBCZAK[3], M. BRUCHAS[3];
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Abstract: Learned associations between environmental cues and the rewarding properties of addictive drugs are a major cause for relapse among drug addicts. The hippocampus (HIP) is therefore a likely key player in the development of addictive behaviors. The link between learning and memory systems, contextual cues, and reward circuitry is largely unknown. The conditioned place preference (CPP) paradigm attempts to model this aspect of drug reward-

associations and can be useful in examining the underlying neural circuitry involved in the formation of drug-associated memories. Here we combine *in vivo* calcium imaging of CaMKIIa CA1 HIP neurons with CPP to study the role of the HIP in nicotine-induced behaviors. We injected AAV5-CaMKIIa-GcAMP6f-eYFP into the HIP CA1 area and, implanted a 1 mm diameter and 4 mm length GRIN lens is 100 microns above the injected region. After another 1-2 weeks, we use a microscope camera from INSCOPIX to observe this the neuron activity in the CA1 area can be observed from the microscope. We then used a standard unbiased, counterbalanced CPP protocol to observe neuronal activity during the development of nicotine CPP. Mice were pre-tested in the CPP boxes on day 1, and days 2-3, they received saline in the AM and nicotine (0.5mg/kg, s.c.) in the PM for a 20 min session. On day 5, they were tested for nicotine place preference, as determined by the time they spent in the drug-paired chamber post-test minus pre-test. After collecting all data from each session, Mosaic™ is used to preprocess the data by reducing dataset, meaning filer, motion correction. By PCA/ICA, single neuron activity was separated and sorted manually. Each session retrieved one dataset for single neuron spatial filters and one dataset set for their time course. After comparing neuron activity between two CPP chamber within posttest section, we found distinct patterns of neuronal activity when a mouse enters the nicotine-paired chamber compared to the saline-paired chamber potentially indicating that cue-reward neuronal activity is potentiated during conditioning and formation of preference.. Follow-up studies were conducted in which AAV5-CaMKIIα-HA-hM4D(Gi)-IRES-mCitrine was injected into the CA1 HIP region to show that silencing CA1, CAMKII+ cell activity in this is sufficient to block the development of nicotine-induced CPP. Taken together, our data provide unique evidence for a key role of the CA1 HIP region in nicotine-contextual associations and begin to dissect the circuitry mediating the development of drug-reward cue associations.

Disclosures: **L. Xia:** None. **S.K. Nygard:** None. **B. Acland:** None. **N.J. Hourguettes:** None. **G. Sobczak:** None. **M. Bruchas:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.06/I13

Topic: C.17. Drugs of Abuse and Addiction

Support: U54DA031659

Title: Nicotine self-administration is enhanced in obesity-resistant rats

Authors: *L. RUPPRECHT, T. T. SMITH, E. C. DONNY, A. F. SVED;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Cigarette smoking and obesity represent the largest challenges to public health. Smokers with higher body mass index (BMI) smoke more cigarettes per day and may be more nicotine dependent than lean smokers. However, very little is understood about the relationship between obesity and nicotine reinforcement. Furthermore, the FDA is considering a policy of markedly reducing the allowable nicotine content in cigarettes; obese smokers may respond differently to nicotine reduction in cigarettes. To model obese smokers, adult male Sprague-Dawley rats (Charles River; Kingston facility) were maintained on high fat diet (HFD; 31.8 kcal from fat) for two weeks. Body weight gain distributed into distinct tertiles: diet-induced obese (DIO), DIO-resistant (DR), and a middle group, which was maintained on chow for the remainder of the experiment, as a diet control (Chow). Rats learned to self-administer 60 μg/kg/infusion nicotine on a fixed-ratio (FR) 2 for ten days before the schedule was escalated to FR5. To establish a nicotine dose response curve, and to model nicotine reduction policy, nicotine dose was halved every seven days to reach 3.75 μg/kg/infusion, a dose previously reported to be sub-threshold for nicotine reinforcement in rats eating a restricted standard chow diet. The Chow group took more nicotine than DR and DIO rats at 60 μg/kg/infusion, although there were no differences in the frequency or proportion to acquire self-administration behavior across groups. Together, these data indicate that at a population level, there are no differences in the probability that rats will acquire self-administration, but that Chow rats respond at higher rates, at least for high doses of nicotine. At all other doses, however, the dose response curve for the DR group was shifted upwards, such that the rats took more infusions than the DIO group ($p < 0.05$). The increase in infusions in the DR group was not explained by increased activity, as measured by inactive responding. The differences between groups are also not explained by differences in body weight, as there is no relationship between nicotine consumption or infusions earned and body weight. Together, nicotine self-administration, particularly at moderate and low doses, is enhanced in rats resistant to diet-induced obesity. These data indicate that current lean smokers eating a densely caloric diet may continue to smoke at high rates following the reduction of nicotine in cigarettes, prolonging the exposure to the harmful chemicals in cigarette smoke.

Disclosures: L. Rupprecht: None. **T.T. Smith:** None. **E.C. Donny:** None. **A.F. Sved:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01DA033396

T32DA007261-22

Title: Activation of the kappa-opioid receptor system is both necessary and sufficient for reinstatement of nicotine place preference

Authors: *S. K. NYGARD[1,2], N. J. HOURGUETTES[1,2], R. AL-HASANI[3,1], G. SOBCZAK[1], M. R. BRUCHAS[1,2,3];
[1]Anesthesiology/Psychiatry, [2]BP-Endure Neurosci. Pipeline, [3]Anat. and Neurobio., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: The Kappa-opioid receptor (KOR) system plays a conserved role in stress-induced behavioral responses including reinstatement of drug seeking behavior for nearly every major drug of abuse. Due to nicotine's high propensity for stress-induced relapse, we hypothesized that stress would also induce reinstatement of nicotine seeking behaviors in a KOR-dependent manner. We used a standard unbiased, counterbalanced conditioned place preference (CPP)/reinstatement protocol in mice to investigate the role of KORs in mediating stress-induced behavioral responses to nicotine. Mice were pre-tested in the CPP boxes on day 1, and on days 2-3 mice were conditioned, receiving saline in the AM and nicotine (0.5mg/kg, i.p.) in the PM for 20 min sessions. On day 5, mice were tested for nicotine CPP, determined by the time they spent in the drug-paired chamber post-test minus pre-test. Animals were extinguished for two days using saline pairings, prior to the reinstatement phase. We found that the widely used pharmacological stressor Yohimbine (Yoh) (2mg/kg, i.p.) 5 minutes prior to reinstatement post-testing causes reinstatement of nicotine CPP. This reinstatement of nicotine CPP is NorBNI sensitive, indicating that KOR activity is necessary for Yoh-induced nicotine CPP reinstatement. To determine if KOR activation alone is sufficient for reinstatement of nicotine CPP, we injected the KOR agonist U50,488 (5mg/kg) 30 minutes prior to reinstatement, and found that KOR activation was sufficient to reinstate nicotine place preference. Two hours following the reinstatement test, mice were perfused to examine the effects on Yoh on neuronal activation (c-fos) in the presence and absence of KOR signaling. We visualized robust c-fos expression in the Basolateral Amygdala (BLA) following Yoh treatment, which was significantly reduced in mice pre-treated with norBNI prior to Yoh-exposure. Follow-up studies were conducted to locally inactivate KOR or neuronal activity in the BLA, to assess the influence of KOR-expressing cells and neural circuits on nicotine CPP. NorBNI injected locally into the BLA blocked Yoh-induced nicotine-CPP reinstatement without affecting the acquisition of nicotine CPP. In a separate experiment, we found that activation of hM4D(Gi) DREADDs in the BLA by CNO is sufficient to reinstate nicotine CPP. These data suggest a role for BLA KORs in stress-induced nicotine

seeking. Future studies will attempt to further dissect this BLA circuitry to identify cell type-specificity of these KOR circuits involved with stress-induced reinstatement of nicotine CPP.

Disclosures: **S.K. Nygard:** None. **N.J. Hourguettes:** None. **R. Al-Hasani:** None. **G. Sobczak:** None. **M.R. Bruchas:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Support: K01 DA030445

R01 DA037897

Title: Trans-generational effects of paternal nicotine self-administration

Authors: ***J. MAURER**, D. VAN NEST, H. SCHMIDT;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Recent evidence indicates that paternal smoking is associated with nicotine dependence and increased incidence of childhood cancer in offspring. These findings indicate that voluntary nicotine taking influences behavioral phenotypes in future generations. The goal of this study is to establish a novel rodent model in which nicotine-experienced sires confer enhanced/increased vulnerability to nicotine reinforcement in their offspring and grand offspring. Male Sprague Dawley rats were allowed to self-administer nicotine (0.03 mg/kg/infusion) on a fixed-ratio 1 (FR1) schedule of reinforcement for 60 consecutive days. Each nicotine-experienced rat was paired with a yoked saline control rat that received the same number and temporal pattern of infusions. Following nicotine self-administration, nicotine-experienced and yoked saline control rats were allowed to mate with drug-naïve dams. When the offspring (F1) reached 60 days of age, acquisition and maintenance of nicotine self-administration were assessed. Both female and male nicotine-sired offspring self-administered significantly more nicotine than saline-sired offspring. Based on these results, drug-naïve, nicotine- and saline-sired male and female F1 littermates were bred with drug-naïve counterparts in order to produce an F2 generation (grand offspring of nicotine- and saline-experienced sires). Acquisition and maintenance of nicotine self-administration was then assessed in the male and female grand-offspring. Our preliminary results indicate that F2 males of nicotine-sired F1 females had a

propensity to self-administer more nicotine than controls. Taken together, these data are consistent with human epidemiological studies and indicate that voluntary paternal nicotine taking increases susceptibility to nicotine taking in subsequent generations. Identifying novel epigenetic mechanisms underlying the transmission of enhanced vulnerability to nicotine dependence will aid in the development of novel smoking cessation medication in generations at high risk for chronic smoking behavior.

Disclosures: **J. Maurer:** None. **D. Van Nest:** None. **H. Schmidt:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA 037844

Title: Azacytidine regulates socially-acquired nicotine intravenous self-administration (IVSA) in rats

Authors: ***W. HAN**, H. CHEN;
Pharmacol., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Social environment is a critical factor in cigarette smoking. Previous studies demonstrated that social learning reversed conditioned flavor aversion (CFA) induced by self-administered nicotine in rats. We first determined the effect of 5-Azacytidine (5-Azac), a DNA methyltransferase inhibitor, on socially-acquired nicotine IVSA. Carbon disulfide (CS2), a component of the rodent breath, was used as a surrogate for the demonstrator rat to mediate social learning as reported. Licking on the active drinking spout in operant chambers meeting a fixed-ratio 10 reinforcement schedule resulted in the concurrent delivery of nicotine (i.v.) and an olfactogustatory (OG) cue containing CS2 (0 or 500 ppm), 0.4% saccharin and 0.1% unsweetened grape KoolAid. CS2 did not enhance the acquisition of nicotine SA in the three daily training sessions. However, CS2 significantly increased the number of active licks on CFA tested on day four (OG + CS2 vs OG: p0.05). However, it significantly diminished the effect of CS2 (5-Azac vs aCSF, $p<0.01$). In saline rats, CS2 enhanced the operant response during both the acquisition and test sessions. This effect was inhibited by 5-Azac (i.c.v., training: $p<0.01$; testing: $p<0.05$). Similarly, bilateral infusions of 5-Azac (5 ng/ul, 0.2 ul/side) into the infralimbic cortex (IL) reduced the number of active licks on the CFA test (OG+CS2 vs OG, $p<0.01$). In

contrast, a low dose of 5-Azac (1 ng/ul, 0.2 ul/side) elevated the number of active licks on the first day of IVSA training (aCSF vs 5-Azac: $p<0.01$) and on CFA test (aCSF vs 5-Azac: $p<0.001$) in rats received OG+CS2. These data indicated that the effect of 5-Azac on social transmission of nicotine preference is region and dose specific. While i.c.v. administration and high dose IL administration reduced social learning, low dose application limited to the IL enhanced social learning. Thus, DNA methylation in IL is an important mechanism via which social learning promotes voluntary nicotine intake.

Disclosures: W. Han: None. **H. Chen:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: UTHSC Center for Integrative and Translational Genomics

Title: Gene expression and alternative splicing changes in infralimbic cortex (IL) underlies socially-acquired nicotine self-administration

Authors: *H. CHEN, T. WANG, W. HAN;
Dept Pharmacol, Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Social environment plays a critical role in the initiation of cigarette smoking among adolescents. We modeled this social effect in rats. We found adolescent rats developed conditioned aversion when an appetitive flavor cue was used for intravenous nicotine self-administration. However, this aversion was reversed by interacting with a conspecific consuming the same flavor cue (but not nicotine). We also have shown that infralimbic cortex (IL) is a critical brain region for the effect of social learning in reversing nicotine conditioned aversion. We used RNA-seq to further investigate the role of infralimbic cortex in socially acquired nicotine self-administration. Rats were trained to self-administer intravenous nicotine with contingent olfactogustatory cues in either an inducing or a neutral social environment for three days. Conditioned aversion were tested on day four. RNA extracted from IL were prepared for transcriptome sequencing. Approximately 15-20 million reads were mapped to the reference genome (rn5) per sample, which accounted for ~80-90% of the total reads. Average read length were approximately 150 bp. Gene expression levels were estimated based on Ensembl gene annotations. DESeq2 was used for data normalization and the identification of differential gene

expressions. Sixteen genes were detected with a false discovery rate of 0.1, including Egr1, Egr3, Nr4a3, etc. We also found ~ 60 genes significantly changed their splicing patterns. Many of these genes were involved in the glutamate receptor signaling pathway, such as Shisa9, Nfkb1, Dnm1, Fyn, etc. Further, GESA analysis showed that genes involved in transcription regulation, synaptic plasticity are unregulated by social learning. These data supported the hypothesis that social learning modulates nicotine intake via regulating gene expression in the infralimbic cortex.

Disclosures: H. Chen: None. **T. Wang:** None. **W. Han:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA 037844

Title: Genetic factors contribute to nicotine intravenous self-administration (IVSA) with menthol cue and sensation seeking

Authors: *T. WANG, H. CHEN;
Dept. of Pharmacol., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Menthol is the most widely used tobacco additive and is preferred by ~25% of US smokers. We previously reported a rat model of nicotine IVSA with orally delivered menthol as a sensory cue (Front Behav Neurosci 2014). We found that although taste and odor were associated with the aversive effect of nicotine, the cooling sensation of menthol was a conditioned cue for the reinforcing effect of nicotine. Genetic factors contributing to the preference for menthol-flavored tobacco products were difficult to ascertain from human studies, partly because of targeted advertisement in the minority populations. We studied the amount of nicotine intake with menthol cue in eight inbred strains of rats. These strains included the Brown Norway, Buffalo, Copenhagen, Dahl salt sensitive, Fisher 344, Lewis, Spontaneous hypertensive rat, and Wistar-Kyoto. All rats were implanted with jugular catheter on approx. postnatal day 38. Daily 2.5 h nicotine IVSA sessions were conducted in operant chambers equipped with two lickometers. Licking on the active spout completing a fixed-ratio 10 schedule trigged the concurrent delivery of 60 µl menthol solution (0.01%) to the spout and iv nicotine (30 µg/kg). No audio or visual cue was used. Rats were not food or water deprived. Nicotine intake was

highest in the Dahl salt sensitive strain (9.6 ± 1.1 infusions per session) and lowest in the Buffalo strain (0.55 ± 0.22 infusions per session) during the last 3 sessions. The estimated heritability (h2) was 0.68. Each rat was also trained in an operant sensation seeking (OSS) protocol. These 1 h sessions were conducted in operant chambers fitted with two nose poke holes. Activating the active hole (fixed-ratio 2) turned on one of the two randomly chosen cue lights at a random frequency (0.5, 1, 2, 4 Hz) for a random duration (2, 4, 6, 8 s). Heritability for the number of rewards earned during the last three OSS sessions was 0.87. There was a moderate correlation between nicotine intake with menthol cue and reward earned during OSS sessions (Pearson = 0.53, p = 0.18). These data suggested that both nicotine intake with menthol cue and sensation seeking are heritable, and that sensation seeking is likely contributing to voluntary nicotine intake with menthol cue.

Disclosures: T. Wang: None. **H. Chen:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

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Program#/Poster#: 143.12/I19

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA027840

Title: Hypothalamic involvement nicotine self-administration in rats

Authors: *B. J. HALL, C. WELLS, E. D. LEVIN;
Dept. of Psychiatry and Behavioral Sci., Duke Univ. Med. Ctr., Durham, NC

Abstract: The hypothalamus is a brain region that has typically been overlooked regarding its potential contributions to processes of drug addiction. Classic literature has shown hypothalamic involvement in consummatory behavior. Drug addiction shares with feeding regulation key behavioral aspects of the appetitive urge. In addition to its primary role as a regulator of metabolic and autonomic function, the hypothalamus is a limbic structure composed of several distinct nuclei that both project to and receive input from several regions of the brain, including areas involved in memory, attention, emotion, and reinforcement learning. The functional output of these projections is responsive to, and regulated by, dopaminergic, serotonergic, noradrenergic, and cholinergic activity. We are investigating the contributions of hypothalamic nuclei to nicotine addiction in a rat model of nicotine self-administration. The first series of experiments targeted D1 dopamine receptors located in the supramammillary nucleus (SuM) of

the hypothalamus; a region that has been implicated in the process of positive reinforcement. Young adult female Sprague-Dawley rats were fitted with jugular catheters and given access to self-administer nicotine (0.03 mg/kg) on an FR1 schedule of reinforcement. Each self-administration session lasted 45 min. Bilateral infusion cannulae were implanted in the SuM to allow local infusion of the D1 receptor antagonist SCH23390. Infusions of SCH23390 occurred 5 min prior to the start of each session. Doses of SCH23390 (1, 2, and 4 μg/side) were infused in a repeated measures, counterbalanced design two times for each rat. Bilateral infusions of SCH23390 into the SuM caused significant reductions in the number of nicotine infusions per session at the 2 ($p<0.05$) and 4 ($p<0.01$) μg/side doses when compared to infusions of the aCSF vehicle. These results demonstrate that hypothalamic D1 dopaminergic innervation plays an important role in the process of nicotine self-administration. Hypothalamic mechanisms may be key components of the neural circuitry underlying addiction. Future experiments will examine the contributions of other transmitters innervating hypothalamic nuclei to nicotine addiction including the involvement of serotonergic and cholinergic receptor mechanisms to this process.

Disclosures: B.J. Hall: None. **C. Wells:** None. **E.D. Levin:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.13/I20

Topic: C.17. Drugs of Abuse and Addiction

Support: NHMRC Grant 1047899

Title: Effect of the dual orexin receptor antagonist tcs1102 on intravenous nicotine self-administration and reinstatement in the rat

Authors: *S. Y. KHOO, G. P. MCNALLY, K. J. CLEMENS;
Sch. of Psychology, Univ. of New South Wales, Sydney, Australia

Abstract: Background: Smoking is the leading preventable cause of morbidity and mortality worldwide. Animal models have shown orexin antagonists reduce alcohol, cocaine and opioid seeking behaviour. Recent evidence also shows that nicotine promotes orexin release. We therefore examined impact of a dual orexin antagonist on nicotine self-administration and reinstatement. Method: Male Sprague-Dawley rats (n = 16) were implanted with chronically indwelling jugular vein catheters and intracranial guide cannulae targeting the lateral ventricle. After recovery, they received 10 days of self-administration training where each active nosepoke

(FR1) was reinforced with a 30 µg/kg/100µL infusion of nicotine. On separate test days, rats received i.c.v. infusion of 1, 3 or 10µg TCS1102 or vehicle 10min prior to self-administration. After self-administration testing and a total of 29 days of nicotine self-administration, they underwent extinction (at least 6 days until 2 consecutive days of ≤30% active nosepoke responding), during which time active nosepokes had no consequences. Relapse-like behaviour was precipitated by presentation of drug-associated cues, a priming injection of nicotine (0.3mg/kg, s.c.) or both the cue and prime, with extinction sessions in between. Rats received i.c.v. infusion of 10µg TCS1102 or its vehicle prior to each reinstatement session. Results: Central administration of 10 µg TCS1102 prior to self-administration had no significant effect on responding for nicotine. Reinstatement was precipitated by drug-associated cues, a priming injection of nicotine or a cue+prime compound. Compound reinstatement was significantly lower in TCS1102 treated animals relative to vehicle controls but there was no significant difference between the vehicle and TCS1102 treatment groups for cue-induced or nicotine-primed reinstatement sessions. Importantly, there was no effect on locomotor activity. Conclusion: The dual orexin antagonist, TCS1102, selectively reduced compound reinstatement over other nicotine-seeking and self-administration behaviours. This suggests that dual orexin antagonists, like the recently approved suvorexant, may have limited clinical utility for nicotine addiction.

Disclosures: S.Y. Khoo: None. **G.P. McNally:** None. **K.J. Clemens:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

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Program#/Poster#: 143.14/I21

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant 1R01DA037277

NIH Grant 1R01DA017288

Title: Effects of menthol on nicotine-taking and -seeking behavior in rat models of nicotine dependence: implications for tobacco product control policy making

Authors: *X. LIU, L. BISWAS, E. HARRISON, J. LEE;
Psychiatry and Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Tobacco smoking is a leading preventable cause of premature death in the US. Menthol is a significant additive in tobacco products. Clinical evidence suggests that menthol

may promote tobacco smoking and nicotine dependence. However, it is not clear whether menthol enhances the reinforcing actions of nicotine and thus facilitates nicotine consumption. Investigating how menthol influences nicotine-addictive behavior may provide important information for making policies to regulate tobacco products. This study employed rat models of nicotine use to examine effects of menthol on nicotine-taking and -seeking behavior. Male Sprague-Dawley rats were trained in daily 1-h sessions to press a lever for intravenous nicotine self-administration under a fixed-ratio 5 schedule. A nicotine-conditioned cue was established via association of a neutral sensory stimulus with each nicotine infusion. In the subsequent extinction sessions, responding was extinguished by withholding nicotine delivery. On the following day after extinction, reinstatement tests were performed. Menthol administration (5 mg/kg, intraperitoneal or intraoral) was given 5 min prior to sessions. The preliminary results showed that menthol increased self-administration of nicotine at 0.015 mg/kg/infusion, a dose on the ascending limb of the inverted "U" shaped nicotine dose-response curve. Continued administration of menthol sustained active lever responses in the extinction sessions. Moreover, re-administration of menthol after extinction effectively reinstated active lever responses. These data demonstrate a facilitative effect of menthol on nicotine-taking and -seeking behavior, suggesting that menthol in tobacco products may promote consumption of nicotine and contribute to the perseverance of tobacco-seeking behavior.

Disclosures: X. Liu: None. **L. Biswas:** None. **E. Harrison:** None. **J. Lee:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.15/I22

Topic: C.17. Drugs of Abuse and Addiction

Support: CIHR

Title: Nicotine enhances responding for a conditioned reinforcer via α4β2 nicotinic acetylcholine receptors in the ventral tegmental area

Authors: *R. I. TABBARA[1,3], P. J. FLETCHER[1,3,2];
[1]Psychology, [2]Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [3]Biopsychology, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Environmental stimuli associated repeatedly with natural or drug reward can acquire incentive value and elicit reward-seeking behaviors. Nicotine, the main psychoactive component

in tobacco smoke, enhances the motivational properties of conditioned stimuli (CSs) previously paired with reward, such that rodents respond more for presentations of these CSs following exposure to nicotine. This enhanced responding suggests that these CSs function as conditioned reinforcers (CRfs) and that nicotine potentiates responding for CRfs. This work examined whether the response-enhancing effect of nicotine is mediated within the ventral tegmental area (VTA), as this dopamine cell body region has been heavily implicated in nicotine reinforcement. Male Long-Evans rats were implanted with a bilateral guide cannula aimed at the VTA. Next, thirsty rats received a nicotine injection (0.4 mg/kg; s.c.) prior to 12 sessions of Pavlovian conditioning. Each session consisted of 30 trials in which a 5-sec tone-light CS was paired with 0.05 ml of water. Tests for conditioned reinforcement were then conducted during which presses on a 'CR' lever produced the CS without the US, whereas presses on an 'NCR' lever had no consequences. To determine whether the enhancing effect of systemic nicotine on responding for a CRf is acting via the VTA, experiment 1 involved infusing the α4β2 nicotinic antagonist Dihydro-β-Erythroidine (DHβE; 10 nmol) into the VTA prior to a nicotine injection (0.2 mg/kg; s.c.) at test. Results indicated that nicotine systemically enhanced responding for a CRf and DHβE infused into the VTA selectively blocked the response-enhancing effect of nicotine. Next, to determine whether stimulation of nicotinic acetylcholine receptors (nAChRs) in the VTA is sufficient to enhance responding for conditioned reinforcement, experiment 2 involved infusing nicotine into the VTA at doses of 8, 16 or 32 nmol at test. Results indicated that nicotine infused into the VTA dose-dependently enhanced responding for a CRf, with the greatest enhancement produced at the 32 nmol dose. Together, these findings suggest that nicotine enhances the motivating properties of reward-paired CSs via α4β2 nAChRs in the VTA.

Disclosures: **R.I. Tabbara:** None. **P.J. Fletcher:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA012262

NIH Grant AA020396

Title: Cotinine: a critical player in nicotine addiction?

Authors: *Z. DING, C. M. INGRAHAM, A. M. SENTIR, E. A. ENGLEMAN, R. A. CHAMBERS, W. J. MCBRIDE;
Indiana Univ. Sch. of Med., IPR, Neurosci. Res. Building, Indianapolis, IN

Abstract: Nicotine (NIC) addiction exerts an enormous heath and economic burden to both individuals and society. Limited effectiveness of current NIC-cessation treatments warrants development of new targets and therapeutics. Cotinine (COT) is the major metabolite of NIC and is mainly used as a bio-marker for NIC usage due to its long half-life. Limited research suggests that COT is biologically active. However, it remains unknown whether COT contributes to NIC effects. The objectives of this study were to test the hypotheses that COT itself can produce rewarding effects and COT interacts synergistically with NIC. Four experiments were conducted in the rat. The 1st experiment examined the rewarding effects of COT with an intra-venous (IV) self-administration procedure. The 2nd experiment tested the local rewarding effects of COT in the posterior ventral tegmental area (pVTA) with an intra-cranial self-administration (ICSA) procedure. The 3rd experiment used a microdialysis procedure to examine the effects of COT on the mesolimbic dopamine projections from the pVTA to the nucleus accumbens shell (NACsh). The 4th experiment used the ICSA procedure to examine the synergistic interaction of COT with NIC in the pVTA. The results demonstrate that: 1) rats readily acquired IV self-administration of COT (0.03 mg/kg/infusion, n = 5), responded significantly more on the active than inactive lever ($p < 0.05$). COT self-infusions gradually increased and reached approximately 0.9 mg/kg/session (~ 30 infusions / session) toward the end of the experiment. Blood COT levels immediately after the last session were 435 ± 110 ng / ml, which are within levels observed in human heavy smokers. 2) COT (0, 0.44, 0.88, 1.76 ng/100 nl/infusion, n = 3-5/group) was dose-dependently self-infused into the pVTA (10 ± 1, 11 ± 2, 26 ± 4 and 35 ± 8 infusions/session, respectively). In addition, lever discrimination was observed with the 2 higher COT concentrations. 3) Intra-pVTA microinjection of COT (1.76 ng/100 nl/infusion) significantly increased dopamine release in the NACsh by ~ 60-70% above baseline (n = 5). 4) A mixture of sub-threshold concentrations of COT (0.44 ng/100 nl/infusion) and NIC (0.16 ng/100 nl/infusion) was readily self-infused into the pVTA (~ 35 infusions/session), whereas each compound alone was not self-infused (9 - 11 infusions/session). Taken together, these results indicate that 1) COT produces its own rewarding effects; 2) activation of the mesolimbic dopamine system may mediate, at least in part, these effects; 3) COT and NIC interact synergistically to produce rewarding effects in the pVTA. Thus, COT may play a critical role in NIC reinforcement, abuse and addiction.

Disclosures: Z. Ding: None. **C.M. Ingraham:** None. **A.M. Sentir:** None. **E.A. Engleman:** None. **R.A. Chambers:** None. **W.J. McBride:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant 2-R01 DA020686 (PI - Kenny)

Title: Role for Tcf7l2 in regulating nicotinic acetylcholine receptor function and nicotine intake

Authors: *A. D. DUNCAN[1], M. P. HEYER[1], A. GEURTS[2], H. O'NEILL[3], P. J. KENNY[1]; [1]Mt. Sinai Sch. of Med., New York, NY; [2]Med. Col. of Wisconsin, Milwaukee, WI; [3]Univ. of Colorado Boulder, Boulder, CO

Abstract: Tobacco smoking is a major cause of premature death and disease, resulting in a significant economic burden on the United States healthcare system. Nicotine is the major psychoactive component of tobacco responsible for sustaining the tobacco smoking habit. Nicotine acts in the brain by stimulating neuronal nicotinic acetylcholine receptors (nAChRs). The positive reinforcing effects of nicotine are related to activation of high-affinity α4β2 nAChRs in the midbrain dopamine system. Conversely, aversive effects of nicotine are regulated by nAChRs containing α5, α3 and/or β4 subunits in medial habenula (MHb) neurons that project to interpeduncular nucleus (IPN), with these subunits highly enriched in the MHb-IPN circuit. The molecular mechanisms that restrict α5, α3 and/or β4 subunit expression to the MHb-IPN system, and the thereby control nicotine intake, are unknown. Here, we report that components of the Wnt signaling cascade, including Wnt glycoproteins, Fzd receptors and Tcf7l2, show remarkable enrichment in the MHb-IPN pathway in adult brain. We find that Wnt signaling is constitutively active in MHb neurons. Genetic disruption of Wnt signaling in the brains of rats, accomplished by genetic deletion of the Wnt transcription factor Tcf7l2, decreased α5 subunit gene expression in the MHb. Moreover, we found that nAChR-mediated transmission in the MHb and IPN were disrupted in the Tcf7l2 knockout rats, measured using 86Rubidium [86Rb+] efflux from synaptoneurosomes. As nAChR transmission in MHb and IPN is known to dependent largely on nAChRs that contain α5 subunits, these data further support a role for Wnt signaling in regulating nAChR function in the MHb-IPN system. Finally, we found that Tcf7l2 knockout rats consumed significantly more nicotine than their wildtype counterparts. Together, these data identify a key role for Wnt signaling, mediated through the transcription factor TCF7L2, in regulating the function of α5 subunit-containing nAChRs in the MHb-IPN system. Moreover, Wnt signaling in this system appears to play a key role in controlling the motivational properties of nicotine. *The first two authors contributed equally to this work

Disclosures: **A.D. Duncan:** None. **M.P. Heyer:** None. **A. Geurts:** None. **H. O'Neill:** None. **P.J. Kenny:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.18/I25

Topic: C.17. Drugs of Abuse and Addiction

Title: Nicotine-induced behavioral sensitization is not affected by early-life methylphenidate exposure in adolescent male and female rats

Authors: S. SENG, A. SU, A. MCELROY, *A. R. ZAVALA;
Psychology, California State Univ., Long Beach, CA

Abstract: The long-term consequences of early use of methylphenidate (MPH) to treat ADHD in preschool age children are not fully understood. Preclinical studies suggest that MPH exposure during postnatal days (PD) 11-20, a period of rat development analogous to preschool age children, enhances the rewarding effects of morphine and the reinforcing effects of cocaine in adult rats. Surprisingly, the functional consequences of early MPH on other drugs of abuse commonly used during adolescence (e.g., nicotine) have not been examined. Thus, the present study examined whether MPH exposure modulates nicotine-induced locomotor sensitization in adolescent rats. Male and female rats were pretreated twice-daily with MPH (0 or 4 mg/kg, intraperitoneally) from PD 11-20. Beginning on PD 32, rats underwent daily nicotine injections (0, 0.2, or 0.6 mg/kg, subcutaneously) for 7 consecutive days, during which locomotor activity was recorded. On PD 40 all rats were then challenged with nicotine (0.2 mg/kg, intraperitoneally) and locomotor behavior was assessed. Nicotine increased locomotor activity and induced sensitization in all rats, but MPH pretreatment during PD 11-20 did not modulate the development of sensitization. The inability of MPH to enhance nicotine's sensitization effects may be due to modulation of different neurocircuitry associated with drug reward- from that of drug-induced locomotor behavior.

Disclosures: S. Seng: None. **A. Su:** None. **A. McElroy:** None. **A.R. Zavala:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

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Topic: C.17. Drugs of Abuse and Addiction

Support: CENIC Pilot Fund

Title: Chronic nicotine exposure alters cue-evoked reward strategy in rats

Authors: *N. W. SIMON[1], C. C. PATTERSON[2], B. MOGHADDAM[2];
[1]Neurosci., [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Nicotine exposure enhances the salience of reward associated cues, which increases the prevalence of cue induced nicotine relapse. Therefore, it is important to understand how nicotine affects the behavioral patterns and neural processing induced by cue presentation. To begin to address this, we exposed rats to 16 days of repeated experimenter-administered nicotine at either a high dose (.3 mg/kg freebase concentration), low dose (.1 mg/kg), a reduced nicotine schedule (8 days of .3, 8 days of .1), or saline vehicle. Following this chronic nicotine exposure, rats were administered nicotine, then run in a Pavlovian conditioning task for 7 consecutive days. This task utilized a 10 second compound cue consisting of a light and extension of a lever directly beneath the light, followed by pellet delivery. We observed that the high-dose nicotine group preferred a "sign-tracking" strategy during the cue, reflected by increased contact with the lever. This strategy is typically indicative of enhanced cue incentive salience, and is correlated with increased sensitivity to drugs of abuse. Conversely, the saline vehicle group demonstrated a "goal-tracking" strategy during the cue, reflected by increased time in the food trough anticipating reward delivery. Surprisingly, both the low and reduced dose groups consistently failed to develop a strong preference for either behavioral strategy throughout training. To elucidate how nicotine influences the neural processing of reward cues, we are currently recording neuronal activity in orbitofrontal cortex during sign- and goal-tracking behavior following nicotine exposure. Collectively, these data will provide novel insight on both the role of nicotine in cue-evoked reward strategy and the neural correlates of sign- and goal-tracking behavior.

Disclosures: N.W. Simon: None. **C.C. Patterson:** None. **B. Moghaddam:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

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Support: NIH Grant T34GM008074

NIH Grant SC2GM109811

Title: Time-dependent nicotine-induced condition place preference in female and male adolescent rats

Authors: *K. HERNANDEZ[1], B. J. SALINAS[1], *N. SOLLENBERGER[1], S. E. FOX[1], S. D. IÑIGUEZ[2], N. S. PENTKOWSKI[3], A. R. ZAVALA[1];
[1]Psychology, California State University, Long Beach, Long Beach, CA; [2]Psychology, The Univ. of Texas at El Paso, El Paso, TX; [3]Psychology, Univ. of New Mexico, Albuquerque, NM

Abstract: Research investigating the rewarding properties of nicotine, using the condition place preference (CPP) paradigm, has produced varying results with the degree to which place conditioning is observed. One factor that may mediate these differences is the duration of the conditioning session post nicotine exposure, as studies employ 10-30 min conditioning sessions. Thus, the present study examined whether conditioning session length (15 vs. 30 min) results in varying expression of nicotine-induced CPP in female and male adolescent Sprague-Dawley rats. Specifically, rats were assessed for nicotine-induced CPP beginning on postnatal day (PD) 27 using a 14-day CPP procedure. On day 1, rats were habituated to the CPP apparatus by confining them to one side of the two chamber CPP apparatus for 7.5 min followed by immediate confinement to the other side for equal duration, with order of exposure counterbalanced. On days 2 and 14, rats were tested for their preconditioning and postconditioning place preferences, respectively, during 15-min sessions. On days 5-12, rats were conditioned for 15- or 30-minutes per day with either nicotine (0, 0.022, 0.067, 0.2 or 0.6 mg/kg, subcutaneously) or saline on alternating days. Days 3, 4, and 13 were rest days. Preliminary results reveal sex differences in the rewarding effects of nicotine in rats that were conditioned for 15 or 30 min, as well as differences in the strength of CPP between the two conditioning durations. Overall, these results suggest that the length of the conditioning sessions may be important in interpreting the rewarding effects of nicotine.

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Poster

143. Nicotine Seeking, Reward, and Relapse

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Support: NHSN Pilot Grant

Department of Psychology

MARC*U*STAR program

Sally Casanova Scholarship

Title: One-trial nicotine-induced locomotor sensitization in male and female adult rats

Authors: *B. P. SCHUESSLER, A. SU, R. MERCER, A. R. ZAVALA;
Psychology, 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. Adult rats readily exhibit one-trial locomotor sensitization to cocaine, but only within the context, or the environment where the initial drug administration took place. However, one-trial locomotor sensitization to nicotine has not been well characterized and little is known about potential sex differences. Thus, the present study examined sex differences in one-trial nicotine-induced locomotor sensitization. For the context-dependent condition, separate groups of male and female rats were injected with nicotine (0.6 mg/kg) immediately before being placed in a novel test chamber for 45 min on PD 75. Following pretreatment, rats were brought back to their home cage and after 45 min received a saline injection. For the context-independent condition, separate groups of male and female rats were injected with saline immediately before being placed in the novel test chamber, followed by an injection of nicotine (0.6 mg/kg) 45 min after being returned to their home cage. Control rats were injected with saline immediately before being place in the novel test chamber, followed by another injection of saline 45 min after being returned to their home cage. After 24 hours, rats from each pretreatment group were randomly assigned to receive a challenge injection of nicotine (0, 0.15 or 0.3 mg/kg, SC) immediately before being placed in the test chamber for 45 min. Results show that female rats exhibit nicotine-induced context-dependent locomotor sensitization, given that female rats pretreated with nicotine in the novel test chamber and challenged with either 0.15 or 0.3 mg/kg nicotine exhibited more robust locomotor responding compared to control rats and rats receiving nicotine for the first time during the challenge test day. Conversely, male rats failed to exhibit any nicotine-induced locomotor sensitization. The results demonstrate sex differences in nicotine-induced locomotor sensitization. These results are not in agreement with a recent study that demonstrated nicotine locomotor sensitization in male mice pretreated with nicotine and tested 7 days later with a challenge injection of nicotine. The number of days between the two nicotine injections may be an important factor mediating one-trial nicotine-induced sensitization in males.

Disclosures: **B.P. Schuessler:** None. **A. Su:** None. **R. Mercer:** None. **A.R. Zavala:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.22/I29

Topic: C.17. Drugs of Abuse and Addiction

Title: Effects of chronic stress on nicotine-seeking behavior and reinstatement

Authors: *J. J. CORTRIGHT, H. KLIMEK, M. ERB, A. MILLER, A. JANKE, T. HARMAN; Psychology, Univ. of Wisconsin River Falls, River Falls, WI

Abstract: Drug addiction is a major public health and serious economic concern in the United States costing taxpayers billions of dollars annually. Experimental evidence shows that exposure to stress is not only a factor in the development of addiction; but also a trigger for drug relapse, or reinstatement. As tobacco use has been linked to a number of cancers and represents the leading cause of preventable death in the United States, elucidation of the effects of stress on nicotine-seeking behavior and relapse is critical. A critical role of chronic stress in the compulsion to seek tobacco and other nicotine delivering products has long been suspected. Although many studies have provided compelling evidence for a role of chronic stress in the enhanced sensitivity to cocaine-seeking behavior and relapse, few have assessed the contribution of chronic stress on nicotine-seeking behavior. In fact, stress induced cross-sensitization to nicotine remains controversial. Additionally, there have been no studies investigating the effects of chronic stress on nicotine-seeking relapse, or reinstatement. Thus, these experiments assess the ability of repeated exposure to variable stress to augment nicotine-seeking behavior and relapse in an animal model of drug addiction. Male Long-Evans rats were exposed to variable stress that consisted of the exposure to different stressors twice a day in random order for 14 days. During this period the control group was left undisturbed except for cage cleaning. Rats were allowed to self-administer nicotine (0.03 mg/kg/infusion) under fixed ratio schedules of reinforcement across 15 consecutive daily sessions. Responding under a progressive ratio schedule of reinforcement was assessed over the following six daily sessions. This schedule allows for break points to be analyzed, a measure that reflects the motivation to self-administer nicotine. Following up to 20 days of extinction training, rats were tested for nicotine-seeking behavior reinstatement by a non-contingent injection of nicotine (0.4 mg/kg, IP). Rats exposed to chronic stress acquired nicotine self-administration at a faster rate relative to controls and exhibited enhanced motivation to obtain the drug. Further, we hypothesize that exposure to chronic variable stress will lead to resistance to nicotine self-administration extinction and

enhancements in nicotine-primed reinstatement, or relapse. Collectively, these findings indicate that chronic stress can enhance the motivational effects of nicotine.

Disclosures: **J.J. Cortright:** None. **H. Klimek:** None. **M. Erb:** None. **A. Miller:** None. **A. Janke:** None. **T. Harman:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.23/I30

Topic: C.17. Drugs of Abuse and Addiction

Title: Predatory behavior predicts high motivation to self-administer nicotine in rats

Authors: *A. BROWN, E. WILLIAMS, C. A. BRADLEY, C. N. SWYMER, M. I. PALMATIER;
East Tennessee State Univ., Johnson City, TN

Abstract: The etiology of tobacco dependence derives from predispositional (i.e., genetic) and ontological (i.e., environmental) influences. Tobacco related death and illness may be prevented or reduced by determining predispositional factors that predict nicotine reinforcement and dependence. One method for investigating predispositional influences is by using phenotypic markers that correlate to drug reward - one of these is a tendency to engage in predatory behaviors in an artificial prey paradigm. In rats, predatory behavior is mediated by the same central nervous system circuits that become disregulated in substance dependence. We hypothesized that a 'high predation' endophenotype would be associated with increased nicotine self-administration. Predatory behavior was measured in 30 male Sprague Dawley rats in an 'artificial prey' chamber. The artificial prey item (marble) was allowed to roll through a trough in the chamber floor. The marble traversed the chamber in approximately 5 s and a sucrose reward (20% w/v) 5 s after the start of each trial. Predatory behavior was operationally defined as marble contacts and chasing and was measured during five sessions (12 trials per session). Third splits were used to separate the high and low predation groups (HPr and LPr, respectively) prior to nicotine self-administration. Both groups were then shaped to lever press in an operant chamber and subsequently instrumented for intravenous nicotine self-administration. There was no difference between HPr and LPr groups in acquisition of self-administration under a fixed ratio (FR) 1, 2, and 5 schedule of reinforcement. However, subsequent tests under a progressive ratio (PR) schedule, a measure motivation to obtain nicotine, the HPr's earned more infusions relative to the LPr's, indicating that predatory aggression is a predictor of the motivation to self-

administer nicotine. This expands the scope of endophenotypes associated with nicotine dependence to ethologically relevant behaviors that rely on midbrain dopaminergic function.

Disclosures: A. Brown: None. **E. Williams:** None. **C.A. Bradley:** None. **C.N. Swymer:** None. **M.I. Palmatier:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA P50 DA027840

Gilead Sciences, Inc

Title: Inhibition of aldehyde dehydrogenase-2 (ALDH-2) suppresses nicotine self-administration in rats

Authors: *A. H. REZVANI[1], M. P. AROLFO[2], M. GRAUPE[2], E. D. LEVIN[1], I. DIAMOND[2]; [1]Dept. of Psychiatry, Duke Univ., Durham, NC; [2]Gilead Sci., Palo Alto, CA

Abstract: ALDH-2 inhibitors have been shown to reduce cocaine and alcohol self-administration in rats by reducing drug-induced dopamine (DA) production in the VTA and DA release in the nucleus accumbens (Arolfo et al, 2009; Yao et al., 2010). The main goal of this study was to explore the potential of a selective ALDH-2 inhibitor for reducing nicotine self-administration. Rats were trained to self-administer nicotine intravenously (iv) via their jugular vein. After acquiring a stable baseline for nicotine self-administration, rats were given an oral administration of one of the three doses (5, 10 or 30 mg eq/kg, calculated based on parent drug) of the pro-drug GS-6637 or vehicle 1 hr before nicotine self-administration session. Results showed that the acute administration of GS-6637 at 10 and 30 mg eq/kg significantly reduced nicotine-self-administration when compared with vehicle treatment (46% and 67%, respectively). Similarly, chronic oral administration of GS-6637 for 7 consecutive days showed a significant effect by reducing nicotine self-administration at 10 and 30 mg eq/kg g (39% and 61 % inhibition, respectively) without the development of tolerance. In order to make a direct comparison with varenicline (Chantix®), one of the few therapies approved as an aid to smoking cessation , a separate group of animals was administered single doses of varenicline at 1.6, 3.2 and 6.4 mg/kg. Consistent with previous reports (Rollema et al., 2007), significant inhibitions of

nicotine self-administration was observed at the 3.2 and 6.4 mg/kg doses (52% and 49%, respectively). In conclusion, GS-6637 administered orally either acutely or chronically, can reduce nicotine self-administration without development of tolerance suggesting its promise as an aid for smoking cessation (Supported in part by NIDA P50 DA027840 and Gilead Sciences Inc.).

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Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

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Topic: C.17. Drugs of Abuse and Addiction

Support: PJ Smith Freemasons travelling fellowing 2014

NHMRC 1050766

Title: Investigating risk and reward based decision making in nicotine dependent subjects

Authors: *L. E. CURLEY[1,2], R. R. KYDD[3,2], I. J. KIRK[4,2], B. R. RUSSELL[1], R. HESTER[5]; [1]Sch. of Pharm., [2]Ctr. for Brain Res., [3]Dept. of Psychological Med., [4]Sch. of Psychology, The Univ. of Auckland, Auckland, New Zealand; [5]Sch. of Psychological Sci., Univ. of Melbourne, Melbourne, Australia

Abstract: To date, studies investigating regional brain activation associated with risk-based decision making in drug users have not typically dissociated probability of risk from magnitude of reward, i.e. the increase in reward has been associated with an increase in the risk-associated decision. In addition, research investigating whether the outcome of a previous decision effects future decision-making has been limited. This study aimed to determine functional changes in response to risk in nicotine dependent participants using functional magnetic resonance imaging (fMRI). Two novel tasks were developed to investigate aspects of risk and reward-based decision making. The passive task attempts to dissociate probability of risk from magnitude of reward, by modulating the risk based on the difficulty of the task. The active (“choice”) task allowed

subjects to choose their own level of risk for each trial based on the monetary gamble that they select. Subjects could win or lose money which allowed the evaluation of whether outcome (i.e. whether subjects won or lost) affected future selection. Nicotine-dependent subjects and healthy control subjects, aged 18-40 years, underwent fMRI whilst completing these two tasks. Echo-planar images were collected using a Siemens 3.0 Trio scanner and were analysed using SPM8. Behavioural results (reaction time (RT) and accuracy) were compared between group using a oneway ANOVA in SPSS. The influence of previous outcomes on future decision making was also assessed. Regional brain activation was identified during the tasks when high risk trials were compared to low-risk trials. Data analysis showed a significant change in activation for the nicotine-dependent subjects in comparison to the controls in the medio-frontal regions. There were no significant differences in either RT or accuracy between groups. The effect of a positive or negative outcome on future decision-making was also different between nicotine-dependent subjects and controls. This study shows clear differences in functional activation in medio-frontal regions during the evaluation of risk between nicotine-dependent and healthy control subjects. The medio-frontal regions alongside the nucleus accumbens are affected by drugs of abuse and are involved in the development of drug-dependence. These findings in combination with the differences in reward selection after positive/negative feedback reflect changes in reward processing and decision-making in those with nicotine dependence.

Disclosures: **L.E. Curley:** None. **R.R. Kydd:** None. **I.J. Kirk:** None. **B.R. Russell:** None. **R. Hester:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.26/I33

Topic: C.17. Drugs of Abuse and Addiction

Support: Intramural Research Program, National Institute on Drug Abuse, NIH

Title: Nicotine reinforcement and relapse are prevented by pharmacological enhancement of kynurenic acid in rats and squirrel monkeys

Authors: ***M. SECCI**[1], A. AUBER[1], L. V. PANLILIO[1], G. H. REDHI[1], E. B. THORNDIKE[1], C. W. SCHINDLER[1], R. SCHWARCZ[2], S. R. GOLDBERG[1], Z. JUSTINOVA[1];
[1]Preclinical Pharmacol., NIDA, IRP, NIH, DHHS, Baltimore, MD; [2]Psychiatry, MPRC Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Tobacco smoking remains one of the leading causes of illness and death in the United States. Current anti-smoking medications, such as bupropion and varenicline, have limited effectiveness and are associated with high rates of relapse. Therefore, there is a pressing need for newer, more effective treatment strategies. Recently, we demonstrated that enhancing brain levels of kynurenic acid (KYNA)_which is an endogenous neuroinhibitory product of tryptophan metabolism and an allosteric modulator of alpha-7 nicotinic receptors_selectively counteracts the abuse-related behavioral and neurochemical effects of cannabinoids. However, there have been no studies examining whether increasing endogenous levels of KYNA can decrease nicotine reinforcement and relapse to nicotine seeking after a period of abstinence. In the present study, we enhanced KYNA levels by administering the kynurenine 3-monooxygenase (KMO) inhibitor Ro 61-8048. We investigated the effects of this treatment on: (1) nicotine self-administration in squirrel monkeys and rats; (2) drug-induced and cue-induced relapse to nicotine-seeking behavior in abstinent rats and monkeys; and (3) nicotine-induced elevation of dopamine levels in the nucleus accumbens shell (NAcS) of freely-moving rats. In these experiments, enhancing endogenous KYNA levels blocked nicotine self-administration and attenuated nicotine-induced dopamine release in the NAcS. Moreover, it prevented relapse-like effects induced by re-exposure to either nicotine or cues that had previously been associated with nicotine. These findings suggest that KMO inhibition should be further investigated as a promising new approach for the treatment of nicotine addiction.

Disclosures: M. Secci: None. **A. Auber:** None. **L.V. Panlilio:** None. **G.H. Redhi:** None. **E.B. Thorndike:** None. **C.W. Schindler:** None. **R. Schwarcz:** None. **S.R. Goldberg:** None. **Z. Justinova:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.27/I34

Topic: C.17. Drugs of Abuse and Addiction

Title: Effects of single and repeated N-acetylcysteine on cue-induced nicotine-seeking behavior in rats

Authors: *F. MORO[1], G. GIANNOTTI[2], C. MARZO[1], A. DI CLEMENTE[1], F. FUMAGALLI[2], L. CERVO[1];
[1]Irccs-Mario Negri Inst. For Pharmacol. Re, Milan, Italy; [2]Dept. of Pharmacol. and Biomolecular Sci., Univ. of Milan, Milan, Italy

Abstract: The inability of smokers to control relapse to drug-seeking behavior is a key feature of nicotine addiction. A large body of evidence indicates that the cysteine pro-drug N-acetylcysteine (N-AC) may have beneficial therapeutic effects in the treatment of drug addiction. In humans, pilot studies have shown that N-AC decreases drug cues-induced craving for cocaine, number of cigarettes smoked, and marijuana use and craving. Pre-clinically N-AC reduced conditioned cues-induced cocaine- and heroin-seeking by restoring cystine-glutamate exchange system Xc- and the glial glutamate transporter GLT-1, normalizing extracellular glutamate in the nucleus accumbens (Nacc), thus blunting the activation of glutamatergic neurons associated with drug cues-induced reinstatement. Although nicotine-associated cues reinstate drug-seeking, it is still not clear whether N-AC can inhibit cue-induced reinstatement in abstinent rats after nicotine self-administration. It is also not clear whether restoring Glu homeostasis by chronic N-AC treatment can enhance the outcome of cue-exposure therapy (CET) for smoking cessation. To gain this information we used rats trained to associate discriminative stimuli (SDs) with intravenous nicotine or oral saccharin self-administration vs. no-reward in two-lever operant cages. Reinforced response was followed by cue signaling 20-second time-out (CSs). Re-exposure to nicotine or saccharin SD+/CS+, but not no-reward SD-/CS-, revived responding at the previously reinforced lever. A single dose of N-AC (100 mg/kg) induced a short-term reduction of cues-induced nicotine-seeking that was completely prevented by pre-treatment with the selective mGluR2/3 antagonist LY341495 (1 mg/kg i.p.). Chronic treatment with N-AC (100 mg/kg) during 14 days of CET, but not forced abstinence, induced lasting anti-relapse activity that was still present 50 days after the end of treatment. To provide a molecular mechanism underlying behavioral results, separate groups of rats that underwent nicotine self-administration and treated either with N-AC or vehicle in combination with CET were killed at different time points for molecular analysis. Interestingly, western blot analysis of brain punches containing Nacc-core and Nac-shell revealed that chronic N-AC reverted changes in the levels of the catalytic subunit of system Xc- and GLT-1 found in vehicle-treated rats. These results, provided they could be extrapolated to smokers, suggest the potential therapeutic use of N-AC for acute cue-controlled nicotine-seeking and to promote extinction of nicotine-cue conditioned responding.

Disclosures: **F. Moro:** None. **G. Giannotti:** None. **C. Marzo:** None. **A. Di Clemente:** None. **F. Fumagalli:** None. **L. Cervo:** None.

Poster

143. Nicotine Seeking, Reward, and Relapse

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 143.28/I35

Topic: C.17. Drugs of Abuse and Addiction

Support: NCI U19-CA157345

Minneapolis Medical Research Foundation (MMRF) Translational Addiction Research Program

Title: Effects of nicotine and minor tobacco alkaloids on intracranial-self-stimulation in rats

Authors: *A. C. HARRIS[1,2], L. TALLY[1], P. MUELKEN[1,2], A. BANAL[1,2], C. SCHMIDT[1,2], Q. CAO[2], M. LE SAGE[1,2];
[1]Med., Minneapolis Med. Res. Fndn., Minneapolis, MN; [2]Univ. of Minnesota, Minneapolis, MN

Abstract: While nicotine is the primary addictive compound in tobacco, other tobacco constituents including minor alkaloids (e.g., nornicotine, anabasine) may also contribute to tobacco addiction by mimicking or enhancing the effects of nicotine. Further evaluating the behavioral effects of minor alkaloids is essential for understanding their impact on tobacco addiction and informing development of tobacco product standards by the FDA. This study compared the addiction-related effects of nicotine and the minor alkaloids nornicotine, anabasine, myosmine, anatabine, and cotinine on intracranial self-stimulation (ICSS) thresholds in rats. Acute injection of nicotine produced reinforcement-enhancing (ICSS threshold-decreasing) effects at low to moderate doses, and reinforcement-attenuating/aversive (ICSS threshold-increasing) effects at high doses. Nornicotine and anabasine produced similar biphasic effects on ICSS thresholds, although with lower potency compared to nicotine. Myosmine only elevated ICSS thresholds at relatively high doses, while anatabine and cotinine did not influence ICSS thresholds at any dose. None of the alkaloids significantly influenced ICSS response latencies, indicating a lack of nonspecific motoric effects. These findings indicate that some minor tobacco alkaloids can either fully (nornicotine, anabasine) or partially (myosmine) mimic nicotine's addiction-related effects on ICSS, albeit at reduced potency. These findings emphasize the need for further study of the abuse potential of minor alkaloids, including evaluation of their effects when combined with nicotine and other tobacco constituents to better simulate tobacco exposure in humans. Such work is essential for informing FDA regulation of tobacco products and could also lead to the development of novel pharmacotherapies for tobacco addiction.

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Poster

143. Nicotine Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant 1R01DA023281-01S1

State of Florida Executive Office of the Governor's Department of Economic Opportunity

Title: Effect of α4β2 and α3β4 nAChR-directed compounds on nicotine and alcohol co-addiction

Authors: A. CIPPITELLI[1], G. BRUNORI[1], N. T. ZAVERI[2], J. WU[1], M. GIULIANOTTI[1], C. ARMISHAW[1], *L. TOLL[1];
[1]Torrey Pines Inst. For Mol. Studies, Port Saint Lucie, FL; [2]Astraea Therapeut., Mountain View, CA

Abstract: Alcohol and nicotine addiction are highly co-morbid, indicating overlapping neural substrates. Recently, considerable interest has been attributed to the habenular/interpeduncular (MHb/IPN) cholinergic pathway, thought to play a role in nicotine addiction as well as addiction to other drugs of abuse. The MHb and IPN highly express nicotinic acetylcholine receptors (nAChR), particularly the α3β4 nAChR subtype, which is thought to be implicated in both nicotine and alcohol taking behaviors. Also, it has been proposed that the smoking cessation medication varenicline, commonly considered a α4β2 nAChR partial agonist, blocks alcohol self-administration by stimulating α3β4 nAChR. We examined the effect of α3β4 and α4β2 nAChR-directed compounds in an operant co-administration procedure in which rats concurrently self-administer nicotine (30 μg/kg through a jugular catheter) and alcohol (10% orally). The tested compounds were the α3β4 partial agonist AT-1001, the α4β2 partial agonist varenicline, TPI-202, a selective α4β2 antagonist; and TPI-2212-59, a selective α3β4 antagonist. Results demonstrated that the two α3β4 nAChR compounds, AT-1001 and TPI-2212-59, both attenuated nicotine self-administration at doses that did not alter alcohol self-administration whereas the α4β2 ligands, varenicline and TPI-202, both attenuated the self-administration of both reinforcers. When alcohol was used as the only reinforcer, AT-1001 was found to affect self-administration only at doses that also reduced food-maintained responding. However, a dose of AT-1001, which had no effect on alcohol or food self-administration (1.5 mg/kg), essentially eliminated reinstatement of alcohol seeking induced by yohimbine (0.625 mg/kg) whereas, reinstatement induced by alcohol-associated cues was not altered, nor did AT-1001 induce reinstatement of extinguished self-administration on its own. These data suggest that partial activation/functional inhibition at α3β4 receptors would primarily act on decreasing nicotine-reinforced behavior, while targeting α4β2 appears to modulate both alcohol and nicotine taking. However, α3β4 nAChR plays a role in mediating stress-related alcohol disorders.

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Poster

143. Nicotine Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant 1R01 DA036487-01

Title: Behavioral and neural effects of cigarette craving regulation using a proximal/distal reappraisal strategy in young-adult smokers

Authors: C. M. COX, *D. G. GHAHREMANI, P. FAULKNER, E. D. LONDON;
Dept. of Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA

Abstract: Tobacco smoking is a leading preventable contributor to death and disease with an estimate of over 500,000 smoking-related, premature deaths in the U.S. alone. Craving for nicotine-containing products, such as cigarettes, is a major factor in the maintenance of use. Behavioral strategies to help with regulation of craving may be helpful for reducing cigarette smoking, yet remain underexplored. The current fMRI study uses behavioral and brain measures to examine craving regulation in young smokers. Fifteen, young-adult cigarette smokers (11 males, 18-25 years old), who abstained overnight from smoking, underwent fMRI scanning before and after smoking a cigarette. We adapted a regulation strategy, often used in emotion regulation studies and based on proximal/distal self-positioning, to the context of cigarette craving. Prior to viewing video clips of people either smoking cigarettes (smoke) or not smoking (non-smoke), participants were instructed to either imagine themselves immersed in the scene, 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 video. Behavioral results indicated main effects of smoking, proximity (close/far), and video type (smoke/nonsmoke videos) on cue-elicited craving – lower craving ratings were given after smoking, following the "far" versus "close" instructions, and viewing the non-smoke versus smoke videos. For the far-versus- close contrast (index of regulation), fMRI activation was greater after smoking a cigarette in brain regions associated with cognitive control, including right inferior frontal gyrus, bilateral anterior insula, anterior cingulate, bilateral putamen, and bilateral posterior parietal cortex. These preliminary results suggest that reappraisal strategies have an impact on cue-elicited self-reported craving, and that smoking a cigarette after a period of abstinence may increase capacity for neural processes related to cognitive control that are important for craving regulation.

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Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 144.01/I38

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01 DA034684 (RTL)

Title: D1 and D2 receptors in the infralimbic and medial orbitofrontal cortices differentially mediate the reinstatement of cocaine seeking in rats

Authors: ***C. V. COSME**, A. L. GUTMAN, A. PEPPLES, R. T. LALUMIERE;
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Abstract: Prior work indicates that the medial prefrontal cortex (mPFC) plays a crucial role in mediating drug-seeking behaviors. Specifically, the infralimbic cortex (IL) within the mPFC has been shown to suppress cocaine-seeking behaviors. Despite the established dopaminergic innervation of this structure, the specific role that infralimbic dopamine plays in mediating the reinstatement of cocaine seeking is unknown. Moreover, whether the medial orbitofrontal cortex (mOFC), a separate, more anterior region of the mPFC, regulates cocaine-seeking behaviors is not clear. Thus, the current set of experiments examined whether D1 or D2 receptor activation within the IL and the mOFC is involved in cocaine seeking during a variety of reinstatement tests. Male Sprague-Dawley rats underwent surgery for implantation of bilateral cannulas aimed at either the mOFC or the IL and insertion of an intravenous jugular catheter. After recovery, all animals underwent cocaine self-administration training for at least 12 days (2 h daily) followed by extinction training for a minimum of 7 days. After rats met extinction criteria, reinstatement testing began, which consisted of cued, cocaine-prime, and cue + cocaine-prime reinstatement tests. Immediately prior to reinstatement testing, rats received microinjections of the D1 antagonist SCH 23390, the D2 antagonist sulpiride, or their respective vehicles. Results indicated that D1 receptor blockade in the IL reduced cued reinstatement but had no effect on cocaine-prime and cue + cocaine-prime reinstatement. In contrast, D1 receptor blockade in the mOFC resulted in a blockade of all 3 reinstatement types. Additionally, blocking D2 receptors in the mOFC had no effect on any reinstatement type. Ongoing experiments have found that D2 receptor blockade in the IL reduces cocaine-prime reinstatement. These findings suggest that D1 receptor activation in the mOFC is required for all types of reinstatement examined whereas, in

the IL, such activation is involved in cued, but not cocaine-prime, reinstatement. Additionally, although D2 receptor blockade in the mOFC had no effect on cocaine-seeking behaviors, blocking these receptors in the IL appears to alter cocaine-prime reinstatement. Moreover, in contrast to previous work suggesting that IL activity is involved in suppressing cocaine seeking, our findings suggest that D1 and D2 receptor activation in the IL promotes cocaine seeking. Ongoing studies will further elucidate the role of D2 receptors in the IL to determine whether D1 and D2 receptors within the IL play discrete roles in mediating cocaine-seeking behaviors.

Disclosures: C.V. Cosme: None. **A.L. Gutman:** None. **A. Pepples:** None. **R.T. LaLumiere:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01 DA034684 (RTL)

Title: Optogenetic inhibition of the infralimbic cortex following unreinforced lever presses increases cocaine seeking in rats

Authors: *A. L. GUTMAN[1], C. V. COSME[1], R. VASQUEZ[2], R. T. LALUMIERE[1]; [1]Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA; [2]Psychological and Brain Sci., rachel-vasquez@uiowa.edu, Iowa City, IA

Abstract: The infralimbic cortex (IL), a region of the medial prefrontal cortex, is a component of the neural circuitry that mediates extinction learning and the active suppression of cocaine-seeking behavior. IL inactivation and activation immediately after extinction training impairs and enhances, respectively, the retention of extinction learning for cocaine seeking. However, the precise temporal relationship between IL activity, lever pressing, and extinction learning is unclear. Therefore, we examined whether selective IL inhibition immediately following each unreinforced lever press during extinction affected ongoing and subsequent cocaine-seeking behavior. The light-sensitive outward proton pump eArchT3.0 was selectively expressed in glutamatergic pyramidal neurons by injecting the adeno-associated virus encoding for eArchT3.0 under the CaMKIIα promoter bilaterally into the IL of male Sprague-Dawley rats. Rats underwent a minimum of 12 days of cocaine self-administration, during which each active (right) lever press resulted in an infusion of cocaine and the presentation of a light and tone cue. After

each right lever press, the lever was retracted for 20 s. Rats then underwent 5 days of shortened (30 min) extinction sessions, during which active lever presses did not produce cocaine infusions or cues. During these shortened extinction sessions, the IL was optically inhibited for 20 s following each unreinforced active lever press. This was followed by 7 days of full-length (2 hr) extinction sessions that served as retention tests for the extinction learning. Optical inhibition increased active lever pressing during the 5 sessions in which the inhibition occurred but had no effect on lever pressing during the 7 full-length extinction sessions. In a control experiment, similar 20-s periods of optical inhibition were provided, but in a manner not contingent upon lever pressing. In this case, IL inhibition did not increase lever pressing during the session itself or on the subsequent extinction sessions. Following extinction, rats underwent cue-induced reinstatement tests, in which active lever presses resulted in the delivery of a light and tone cue but no optogenetic manipulations were given. Rats that had received IL inhibition during extinction showed potentiated cue-induced cocaine seeking, whereas rats that had received non-contingent IL inhibition did not show any change in cue-induced reinstatement. These results suggest that IL activity immediately following an unreinforced lever press contributes to the suppression of ongoing cocaine-seeking behavior and is important for the suppression of subsequent cue-induced reinstatement.

Disclosures: **A.L. Gutman:** None. **C.V. Cosme:** None. **R. Vasquez:** None. **R.T. LaLumiere:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA037897

NIH Grant DA030445

Title: Glucagon-like peptide-1 receptor activation in the ventral tegmental area or the nucleus accumbens attenuates cocaine seeking in rats

Authors: *N. S. HERNANDEZ, E. G. MIETLICKI-BAASE, J. J. MAURER, D. S. VAN NEST, M. R. HAYES, H. D. SCHMIDT;
Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Glucagon-like peptide-1 (GLP-1) receptor signaling in the CNS is pharmacologically and physiologically relevant for energy balance control. The GLP-1 receptor agonist exendin-4 decreases intake of palatable food when administered into the ventral tegmental area (VTA) and nucleus accumbens (NAc) core. Since the VTA and the NAc mediate the reinforcing effects of food and drugs of abuse, we hypothesized that GLP-1 receptor activation in these two nuclei would attenuate cocaine reinstatement, an animal model of relapse in human addicts. Initially, rats were allowed to self-administer cocaine (0.25 mg/infusion i.v.) for 21 days on a fixed-ratio 5 (FR5) schedule of reinforcement. Cocaine self-administration was then extinguished by replacing cocaine with saline. Once cocaine taking was extinguished, rats received an acute priming injection of cocaine (10 mg/kg, i.p.) to reinstate cocaine-seeking behavior. During subsequent reinstatement test sessions, rats were pretreated with intra-cranial infusions of the GLP-1 receptor agonist exendin-4 (0, 0.005 and 0.05 μg) prior to a priming injection of cocaine. Here, we show that administration of exendin-4 directly into the VTA, NAc core or NAc shell dose-dependently attenuated cocaine priming-induced reinstatement of drug-seeking behavior. To determine if the suppressive effects of exendin-4 in the VTA and NAc on cocaine seeking were due to drug-induced motor impairments, we also examined the effects of intra-cranial exendin-4 infusions on the reinstatement of sucrose seeking. Administration of exendin-4 directly into the VTA, NAc core or NAc shell had no effect on sucrose reinstatement. Taken together, these results indicate that increased activation of VTA and NAc GLP-1 receptors is sufficient to reduce cocaine seeking and that these effects are not due to general motor suppressant effects of drug treatment. Thus, these findings support re-purposing GLP-1 receptor agonists, which are FDA-approved for treating diabetes type II and obesity, for treating cocaine addiction.

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Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS grant R01-DA006214

PHS grant F31-DA035561

Title: Hippocampal inputs to lateral septum drive context-induced, but not cue-induced cocaine seeking

Authors: *E. M. MCGLINCHEY[1,2], G. ASTON-JONES[2];
[1]Neurosciences, Med. Univ. of South Carolina, Charleston, SC; [2]Brain Hlth. Inst., Rutgers University/Rutgers Biomed. and Hlth. Sci., Piscataway, NJ

Abstract: Stimuli associated with drug experiences can trigger relapse in drug addicts. Drug-associated contexts and discrete drug cues initiate relapse by activating distinct brain regions. However, neural circuits distinctly involved in these different relapse modalities have not been fully characterized. Using a modified self-administration model, all rats self-administered cocaine with light/tone cues in one context, extinguished this behavior in an alternative context (without light/tone cues), and reinstated in either the training context without light/tone cues (context reinstatement, ABA) or in the extinction context with light/tone cues (cued reinstatement, ABB) to dissociate context vs. cued reinstatement of cocaine seeking. We then used Fos expression as a marker of neural activation in brain regions, a retrograde tracer combined with Fos to determine activated circuits, and pharmacologic and chemogenetic approaches to examine the role of hippocampal inputs to lateral septum (LS) in these reinstatement modalities. Based on the involvement of the hippocampus in contextual processing, its dense projections to LS, and previous results from our lab showing a functional CA3-LS-VTA circuit, we hypothesized that this circuit is important specifically for context-induced cocaine seeking, but not seeking driven by discrete cues. Results revealed that both dorsal hippocampus (CA1, CA3, and dentate gyrus) and LS (caudal and rostral LS) expressed a greater number of Fos cells during context compared to cued reinstatement. Furthermore, a greater percentage of CA3 neurons that project to LS express Fos during context compared to cued reinstatement or extinction, indicating this circuit is specifically activated during re-exposure to drug-associated contexts. Interestingly, pharmacological inhibition of LS attenuated both context and cued reinstatement. We then used DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to specifically inhibit hippocampal (CA3) terminals in LS. Inhibitory (hM4Di) DREADDs virally transduced into CA3 were transported to terminals in LS and activated by local microinjections of the ligand clozapine-N-oxide (CNO). Inhibition of hM4Di-expressing CA3 terminals in LS attenuated context, but not cued reinstatement. Together these findings highlight the importance of LS in cocaine-seeking behavior, and that hippocampal inputs to LS drive context-induced reinstatement, whereas other inputs to LS likely drive cue-induced reinstatement. Elucidating the circuitry involved in different relapse modalities will identify therapeutic targets for specific relapse triggers in recovering drug addicts.

Disclosures: E.M. McGlinchey: None. **G. Aston-Jones:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Program#/Poster#: 144.05/I42

Topic: C.17. Drugs of Abuse and Addiction

Support: PHS grant P50-DA016511.

Title: Drug seeking during initial abstinence is driven by hippocampal β-adrenergic and serotonergic receptors in a sex-dependent manner

Authors: *A. S. KOHTZ[1], A. CASON[2], G. ASTON-JONES[1];
[1]Neurosci., Brain Hlth. Inst., Piscataway, NJ; [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: Studies indicate that female rats exhibit greater drug seeking than male rats during initial drug abstinence. Moreover, females are more sensitive to the effect of stress to drive drug seeking than males. Locus coeruleus norepinephrine (LC-NE), dorsal raphe serotonin (DR-5HT) and corticotropin releasing factor (CRF) neurons are involved in stress responses, including the ability of stress to drive drug relapse. Notably, LC-NE neurons are more sensitive to CRF in females compared to males, and conversely, DR-5HT neurons are more sensitive to CRF in males compared to females. Dorsal hippocampus (DH) is a prominent focal point in the stress response that receives strong inputs from both LC-NE and DR-5HT neurons. DH has a number of structural and biochemical sex differences that modulate stress responsivity, including substantial differences in CRF receptor binding affinity, de novo serotonin synthesis, cholinergic enzyme activity as well as adrenergic, corticosterone, and GABA receptor expression. Notably, DH is required for context-dependent reinstatement of drug seeking, and drug relapse often occurs when addicts are re-exposed to drug-associated contexts. Thus, we hypothesized that β-adrenergic and serotonergic neurotransmission in DH is involved differentially in male and female rats in drug seeking during extinction day 1 (ED1), i.e., the initiation of abstinence when animals are re-exposed to the drug-associated context. Drug-seeking during this initial abstinence test was decreased by S-propranolol (β-adrenergic and 5-HT1A/1B receptor antagonist), R-propranolol (5-HT1A/1B receptor antagonist), and racemic (R/S mixture) propranolol in both male and female rats (10mg/kg, IP). We observed that hippocampal, locus coeruleus, and dorsal raphe Fos expression was increased on ED1 in both male and female rats, and that hippocampal Fos was decreased by systemic S-propranolol. Utilizing intrahippocampal infusions of S-propranolol, a Betaxolol/ICI-118,551 cocktail (selective β-adrenoceptor antagonists, β-AR), or a WAY-100635/GR-127935 cocktail (5-HT1a & 1b receptor antagonists), we investigated the role of hippocampal 5-HT and β-adrenergic neurotransmission in ED1 drug-seeking behavior. ED1 responding was reduced by 5-HT but not β-AR antagonists in males, and reduced by both 5-HT and β-AR antagonists in females. Thus, drug seeking during initial abstinence requires hippocampal 5-HT and β-AR neurotransmission in females, but only 5-HT neurotransmission in

males. Additional studies are underway to test if manipulations of hippocampal serotonergic or adrenergic systems during ED1 influences later relapse to cocaine seeking.

Disclosures: **A.S. Kohtz:** None. **A. Cason:** None. **G. Aston-Jones:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 144.06/I43

Topic: C.17. Drugs of Abuse and Addiction

Support: PHS grant R01-DA006214

PHS grant F32-DA036995

Title: Attenuation of orexin/hypocretin signaling decreases cocaine seeking and increases cocaine taking in rats with a history of self-administering cocaine and ethanol

Authors: *B. A. ZIMMER, G. ASTON-JONES;
Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ

Abstract: Abuse of psychostimulants such as cocaine has a high comorbidity with heavy use of alcohol with 85-88% of cocaine-dependent individuals engaging in co-use of alcohol. Such co-use is problematic given findings that the alcohol-cocaine combination is the most common substance use pattern reported in emergency rooms. The prevalent co-abuse of alcohol and cocaine leads us to hypothesize that alcohol abuse aggravates cocaine abuse, and facilitates the transition to cocaine addiction. Male, Sprague-Dawley rats were initially trained to drink ethanol in their home-cage using the intermittent access (IA) paradigm in which liquid ethanol (20%) was available every other day. Following two weeks of IA, subjects were trained to self-administer cocaine on a within-session threshold behavioral economics (BE) procedure. A median split was performed on voluntary drinking behavior to sort animals into high drinkers (HD) and low drinkers (LD). Results showed that HD rats had significantly higher baseline demand elasticity for cocaine, indicating lower motivation to self-administer cocaine relative to the LD rats. Over time this difference between groups decreased, such that initial LD rats maintained the same demand elasticity for cocaine, whereas HD rats decreased their cocaine demand elasticity (increased motivation) over the course of the experiment. These results imply that HD rats are initially resistant to cocaine, but with repeated exposure to both cocaine and ethanol this demand for cocaine increases. We hypothesize that this may reflect trait differences

in anxiety that are mitigated by alcohol consumption over time. To assess the roles of orexin signaling in free consumption and motivation for cocaine in cocaine-alcohol exposed subjects, rats from the above experiment were given an i.p. injection of the orexin 1 receptor antagonist SB334867 (30 mg/kg) 30 min prior to a cocaine BE self-administration test session. Results showed a significant increase in cocaine demand elasticity indicating a significant decrease in motivation for cocaine. Interestingly, a significant increase was also found in cocaine free consumption, indicating an increase in the desired level of cocaine in brain, or hedonic set point. These results indicate that attenuation of orexin signaling decreases motivation for cocaine (as observed previously in cocaine-only subjects) but increases cocaine free (low effort) consumption. Thus, alcohol plus cocaine exposure elicits adaptations in the orexin system not seen in subjects exposed to cocaine alone. Possible clinical implications of these adaptations deserve further study. Supported by PHS grants R01-DA006214 and F32-DA036995

Disclosures: B.A. Zimmer: None. **G. Aston-Jones:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS Grant R01-DA006214

Title: Long access self-administration increases cocaine demand: Dependence on orexin 1 receptor signaling

Authors: *C. M. STOPPER, G. ASTON-JONES;
Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ

Abstract: Orexin/hypocretin plays a key role in stimulus-driven drug-seeking. Blockade of the orexin 1 receptor (Ox1R) impairs cue-, context-, and stress-induced reinstatement. Using a novel behavioral economics procedure, our lab recently demonstrated that the selective Ox1R antagonist SB-334,867 decreases demand for cocaine, but only in high-demand animals trained with drug-paired cues (Bentzley & Aston-Jones, 2015). These results highlight the contribution of orexin to trait-based motivation for cocaine. Yet, cocaine dependence often results from state factors, rather than innate individual variations. The current experiment uses this rationale to test if Ox1R antagonism can also attenuate motivation for cocaine in rats exhibiting escalated intake following long access self-administration. Male Sprague-Dawley rats were implanted with

jugular catheters for i.v. cocaine self-administration. After initial FR1 self-administration training, subjects were trained until stable on the behavioral economics demand procedure (Bentzley et al., 2013). Rats were then trained for 14 days on long access (LgA; 6 Hrs) or short access (ShA; 1 Hr) FR1 self-administration. Following LgA or ShA self-administration, rats were given counterbalanced systemic injections of vehicle or two doses of the Ox1R antagonist SB-334,867 (SB; 10 and 30 mg/kg). Animals then went through extinction training followed by reinstatement tests with SB. As has been previously observed, LgA self-administration caused escalation of cocaine intake, particularly in the first hour. LgA also decreased demand elasticity and increased free consumption of cocaine (alpha and Q0 parameters), reflecting increased motivation and desired brain level for cocaine, respectively. SB altered these cocaine demand parameters in a dose-dependent fashion, towards pre-LgA values. These results demonstrate that the OxR1 is necessary for elevated cocaine demand following long access escalation. Whereas previous data from our lab show that OxR1 antagonism attenuates cocaine demand in animals with innate "trait-based" demand, the findings of the current experiments indicate that OxR1 blockade also decreases "state-based" demand following prolonged cocaine exposure. These findings have translational implications for cocaine addiction, wherein extended use triggered by environmental factors leads to a state of dependence that is difficult to treat. As LgA cocaine self-administration is anxiogenic and OxR1 blockade is known to be anxiolytic, we hypothesize that OxR1 blockade decreased cocaine demand by decreasing the anxiety involved in escalation of intake.

Disclosures: C.M. Stopper: None. **G. Aston-Jones:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

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NIH Grant P50DA016511

Title: Effects of central and peripheral oxytocin on reinstated cocaine seeking in male and female rats

Authors: *L. R. FREEMAN[1], K.-C. LEONG[2], S. M. GHEE[2], C. R. BERINI[2], T. A. STUBBS-STROUD[3], B. M. BROWN[3], M. C. AMEY[3], C. M. REICHEL[2];

[1]Biol. and Neurosciences, Furman Univ., Greenville, SC; [2]Med. Univ. of South Carolina, Charleston, SC; [3]Col. of Charleston, Charleston, SC

Abstract: Background: Oxytocin has gained increasing attention as a possible treatment for multiple neuropsychiatric disorders, including addiction. Oxytocin impacts natural and drug reward due to extensive innervation of central reward pathways. Sex differences clearly exist in psychostimulant addiction patterns. However, the underlying neurobiology and potential addiction therapies have typically only been studied in males. The oxytocin system is sexually dimorphic with a greater number of oxytocin receptors expressed throughout the addiction circuit in males relative to females. Here, we determined that oxytocin decreased reinstated cocaine seeking in males and females following systemic oxytocin treatment. We are currently investigating effects of intracerebroventricular (icv) administration of oxytocin with this paradigm. Methods: Male and female rats underwent 2 weeks of cocaine self-administration followed by extinction and reinstatement tests after systemic (1 mg/kg, i.p.) oxytocin or vehicle treatment in the presence of conditioned cues. Following testing, rats were perfused and the brains were processed for c-Fos staining and c-Fos/oxytocin double-labeling in the paraventricular nucleus of the hypothalamus (PVN). A separate group of rats received oxytocin treatment during extinction and were tested with vehicle or oxytocin in response to cocaine-conditioned cues. Results: An acute oxytocin injection (i.p.) reduced reinstated cocaine seeking in both males and females. Likewise, repeated oxytocin during extinction also reduced responding on a cue test in males and females. However, oxytocin during extinction had no lasting impact on a cued reinstatement test. Currently, we are double labeling c-Fos and oxytocin cell bodies in the PVN to determine whether oxytocin reduced reinstated cocaine seeking via similar mechanisms. To date, we found males have a higher number of oxytocin and Fos-positive neurons in the PVN relative to females. Additionally, we are quantifying c-Fos expression in terminal areas. Discussion: Oxytocin impacted reinstated cocaine seeking similarly in both sexes. This similarity is in contrast to the well-known sex differences in the role of oxytocin on peripheral organ sites of actions and the sexually dimorphic distribution of central oxytocin receptor cites in females, relative to males. This study will determine if similar behavioral outputs may be mediated by a different underlying neurobiology.

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Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Title: Treatment with the M1-selective muscarinic antagonist trihexyphenidyl attenuates cocaine-reinforced behavior

Authors: *K. W. GRASING, F.-C. YANG;
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Abstract: Medications that modify cholinergic tone can have pronounced effects on behaviors reinforced by natural and drug reinforcers. Systemic treatment with M1-preferential muscarinic agonists decreases self-administration across a broad range of cocaine doses. In contrast, M1-selective antagonists can enhance the discriminative-stimulus effects of cocaine. When administered with cocaine, M1 antagonists can potentiate increases in dopamine in the nucleus accumbens shell but not the core or prefrontal cortex. The present study examined effects of the M1-preferential muscarinic antagonist trihexyphenidyl (TXP) on cocaine- and food- reinforced behavior. METHODS: Rats were trained to respond for either cocaine or liquid food under a fixed-ratio-5 (FR-5) schedule during two-hour multiple-component sessions. Across components, either cocaine dose (0.1, 0.2, and 0.4 mg/kg per injection) or amount of 20% liquid food (30, 60, or 120 µl) was varied. Pretreatment with TXP was administered intraperitoneally at low, intermediate, or high doses (1.0, 3.2, or 10 mg/kg-injection). RESULTS: TXP decreased cocaine-reinforced responding by 10 to 30% relative to baseline, with similar actions at different doses of TXP, as well as different cocaine doses. Responding for 30 µl of liquid food was attenuated by high-dose TXP, but was otherwise unaffected. TXP increased spontaneous sniffing, rearing, and digging; without modifying inactive lever responding under any of the conditions tested. CONCLUSION: TXP can decrease drug self-administration across a broad, 10-fold range of cocaine doses. Effects on spontaneous behavior are less pronounced than for cholinergic agonists, and include increases in some normally observed scored behaviors. These behavioral effects may be mediated by increases in dopamine in the nucleus accumbens shell.

Disclosures: K.W. Grasing: None. **F. Yang:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Support: CURE Addiction Center of Excellence

NIDA U54 Cocaine Cooperative Medication Development Center

Title: Pathological persistence of the brain response to "unseen" 33 msec cocaine cues as a marker of relapse vulnerability

Authors: *A. R. CHILDRESS[1], K. JAGANNATHAN[1], Z. MONGE[1], J. SUH[1,2], K. YOUNG[1], P. REGIER[1], T. FRANKLIN[1], D. LANGLEBEN[1,2], Z. WANG[1], Z. LI[1], K. KAMPMAN[1,2], R. WETHERILL[1], R. EHRMAN[1,2], M. GAWRYSIAK[1], R. SZUCS-REED[1], C. P. O'BRIEN[1]; [1]Dept Psychiat, Univ. PENN Perelman Sch. Med., Philadelphia, PA; [2]Mirecc, Philadelphia Dept. of Veteran's Affairs Med. Ctr., Philadelphia, PA

Abstract: Aims: Our laboratory has shown that even "unseen" (subliminal) 33 msec drug cues can trigger the brain's subcortical motivational circuitry. Does this early brain response to drug cues constitute a relapse vulnerability? In a new cohort of treatment-seeking cocaine patients, we are examining whether the "pathological persistence" of the brain response to cocaine cues is linked to poor clinical outcome.**Methods:** In a "fast" event-related BOLD fMRI paradigm, cocaine inpatients were exposed to cocaine-related and to comparison (sexual, aversive and neutral) cues of 33 msec duration. Each cue (48 presentations of each cue category) was "backward-masked" by a 467 msec neutral stimulus to prevent conscious recognition. Pre-planned contrasts to characterize "persistence" (comparing the brain response during the first half vs. the second half of the task, for each cue category) were calculated within SPM 8 for two outcome subgroups "GOOD" ($\leq$30% cocaine-positive urines, n=9) vs. "POOR" (>90% cocaine-positive urines, n=15).**Results:** As hypothesized, cocaine patients with "POOR" clinical outcome evidenced a greater (e.g., drug2 > drug1; $p<0.05$) response to 33 msec cues in the **second** half of the task, for 3 (cocaine, aversive and neutral) of the 4 cue categories – among *a priori* limbic regions, this "persistence" was reflected in amygdala/v. pallidum, and in temporal pole. "GOOD" outcome patients lacked this pattern. **Conclusions:** The current data highlight the relapse relevance of the early brain response to "unseen" cues": patients with POOR outcome had cue-triggered brain responses that tended to persist -- despite the multiple, unreinforced presentation of the cues. "Pathological persistence" may be a sensitive predictor of relapse, complementing conventional "magnitude" measures in BOLD fMRI. These findings underscore the potential utility of the "unseen" cue paradigm, both as a tool for screening anti-relapse medications and for identifying the "cue-vulnerable" patients who need these medications most.

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Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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NIH/NIDA DA15758 (JRM)

Title: The group 1 metabotropic glutamate receptors regulation of cocaine seeking is receptor-selective and intake-dependent: Role of anatomical substrates

Authors: *M. GHASEMZADEH, P. HEASLIP, R. DIDOMINICIS, M. VUONCINO, C. SZEWCZYK, J. MANTSCH;
Dept Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: A major obstacle in the treatment of addiction has been the propensity to relapse, often mediated by drug-associated cues, even after prolonged period of abstinence from drug use. Repeated exposure to cocaine leads to enduring alterations in glutamatergic signaling in the brain reward circuitry that play an important role in long-lasting molecular, cellular and behavioral neuroadaptations. Therefore, glutamate signaling has been investigated as a target for the development of treatment for addiction. Recent studies have suggested that the group I metabotropic glutamate receptors (mGluR1/5) play important roles in drug reinforcement and seeking and, therefore, have been pursued as promising targets for drug development. Here, we examined the role of mGluR1/5 receptors in abstinence drug seeking using animal models of cocaine self-administration. Sprague-Dawley rats were trained to self-administer cocaine (FR1; 1.0 mg/kg/200 µl/inf) during either 2-hr (ShA) or 6-hr sessions (LgA) for 14 days. Subsequently, animals were left undisturbed in home cage for 3, 10, or 60 days. Following abstinence period, rats were tested under extinction condition for cocaine seeking after either saline or an mGluR1/5 receptor antagonist administration (MTEP or JNJ16259685). Following a short abstinence period (3 or 10 days), the blockade of mGluR5 receptor reduced drug seeking only in ShA subjects without affecting the LgA animals, while mGluR1 receptor blockade were equally effective in reducing drug seeking in both groups. However, after a long abstinence period (60 days), the blockade of either of receptors significantly reduced drug seeking in ShA and LgA rats.

Furthermore, mGluR5 blockade was effective in reducing drug taking (cocaine self-administration) in a dose dependent manner by ShA subjects but not by LgA animals. The results suggest that exposure to cocaine produced a transient intake dependent plasticity in mGluR5 signaling in the brain. The observed plasticity is specific to mGluR5 signaling since blockade of mGluR1 receptors reduced drug seeking similarly in both ShA and LgA animals. In order to identify the anatomical substrates contributing to the selective modulation of mGluR5 signaling, site-specific blockade of mGluR5 receptors in the nuclei of motive circuit will be performed. The selective, intake dependent, and transient plasticity in brain mGluR5 signaling mediated by exposure to cocaine suggest an important role for mGluR5 in cocaine mediated neuroadaptations and addiction behaviors. Understanding the mechanism of cocaine mediated effects may reveal new molecular targets for therapeutic development for the treatment of cocaine addiction.

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Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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NIH/NIDA Grant DA033344 (RMF)

Title: Orexin-A/hypocretin-1 in the paraventricular nucleus of the thalamus fails to reinstate cocaine-seeking behavior in animals with a history of cocaine dependence following a prolonged period of abstinence

Authors: *A. MATZEU, F. WEISS, R. MARTIN-FARDON;
The Scripps Res. Inst., La Jolla, CA

Abstract: Growing evidence implicates a role for orexin/hypocretin (Orx/Hcrt) neurons originating in the lateral hypothalamus (LH) and projecting to the paraventricular nucleus of the thalamus (PVT) in drug addiction. We previously reported that intra-PVT administration of orexin-A/hypocretin-1 (Orx-A/Hcrt-1) after 2 weeks of abstinence from cocaine or sweetened condensed milk (SCM) self-administration reinstated (primed) cocaine-seeking behavior in animals with short cocaine access (ShA, 2 h/day) or long cocaine access (LgA, 6 h/day, an

animal model of cocaine dependence), as well as SCM seeking-behavior, but with different dose-response profiles. Specifically, in LgA rats a left-upward shift of the dose-response function compared with SCM group and an upward shift compared with the ShA group were observed. This suggests that a history of cocaine dependence leads to neuroadaptive changes at the level of the PVT, resulting in the "sensitization" of PVT-Orx/Hcrt transmission, reflected by increased sensitivity (i.e., a leftward shift) and exacerbated behavioral responses (i.e., an upward shift) to the to the effects of Orx-A/Hcrt-1. The present study's aim was to investigate whether the intra-PVT priming effect of Orx-A/Hcrt-1 is preserved following 4 weeks of abstinence in animals that had a cocaine self-administration history. Male Wistar rats were trained to self-administer ShA cocaine, LgA cocaine, or SCM for a total of 21 days. After completion of the training procedure, the animals were maintained in their home cage for 2 weeks and then subjected to extinction training for 2 weeks (2 h/day). The following day, the rats received intra-PVT microinjections of 0.5 μg Orx-A/Hcrt-1 (a dose that produced equivalent reinstatement at 2 weeks of abstinence in all groups), or the respective vehicle, and then placed into operant chambers under extinction conditions for 2 h. At 4 weeks of abstinence, intra-PVT Orx-A/Hcrt-1 produced reinstatement of both SCM and ShA that was identical to what was observed at 2 weeks of abstinence. Surprisingly, Orx-A/Hcrt-1 did not trigger cocaine-seeking behavior in LgA rats. The data suggest that following cocaine dependence (i.e., LgA), functional changes in PVT Orx/Hcrt transmission occurred, reflected by a change in the pharmacological profile of Orx-A/Hcrt-1 at 2 weeks ("sensitization" to the effects of Orx-A/Hcrt-1) and 4 weeks (lack of Orx-A/Hcrt-1's priming effects) of cocaine abstinence. One tentative explanation is that the expression and/or functionality of Orx/Hcrt receptors fluctuates during cocaine withdrawal following dependence, as reflected by decreased sensitivity to Orx-A/Hcrt-1's priming effects.

Disclosures: **A. Matzeu:** None. **F. Weiss:** None. **R. Martin-Fardon:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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NIH/NIDA DA033344 (RMF)

Title: Orexin-A/hypocretin-1 in the paraventricular nucleus of the thalamus induces Fos expression in the medial prefrontal cortex in animals with a history of cocaine dependence

Authors: *R. MARTIN-FARDON, F. WEISS, A. MATZEU;
MCND, The Scripps Res. Inst., La Jolla, CA

Abstract: Orexin/hypocretin (Orx/Hcrt) projections from the lateral hypothalamus (LH) to the paraventricular nucleus of the thalamus (PVT) are implicated in drug addiction. We previously reported that administration of orexin-A/hypocretin-1 (Orx-A/Hcrt-1) in the PVT reinstates extinguished cocaine-seeking behavior in animals with short access (ShA, 2 h/day) or long access (LgA, 6 h/day, a model of cocaine dependence) to cocaine and sweetened condensed milk (SCM) seeking, but with different dose-response profiles. Specifically, in the LgA group a left-upward shift of the dose-response function compared with the SCM group and an upward shift compared with the ShA group were observed. This suggests that a history of cocaine dependence leads to neuroadaptive changes at the level of the PVT, resulting in "sensitization" of PVT-Orx/Hcrt transmission, reflected by increased sensitivity (i.e., a leftward shift) and exacerbated behavioral responses (i.e., an upward shift) to the effects of Orx-A/Hcrt-1. The present study sought to investigate whether the neural activation pattern following intra-PVT Orx-A/Hcrt-1 administration in animals that self-administered cocaine (ShA, LgA) or SCM is different and could partially explain the different Orx/Hcrt dose-response functions. Male Wistar rats were trained to self-administer short-access cocaine (ShA), long-access cocaine (LgA), or SCM for a total of 21 days. After completion of the training procedure, the animals underwent extinction training for 2 weeks in 2 h daily extinction sessions. Rats received intra-PVT microinjections of Orx-A/Hcrt-1 (0.5 μg) or the respective vehicle (saline) and then placed into operant chambers under extinction conditions for 2 h. At the end of the behavioral tests, the brains were prepared for Fos immunohistochemistry and analyzed for Fos expression in the medial prefrontal cortex (mPFC), nucleus accumbens (NAC) core, and shell (i.e., brain regions receiving inputs from the PVT and well known to regulate cocaine-seeking behavior). Intra-PVT administration of Orx-A/Hcrt-1 induced strong activation of the mPFC (i.e., increased Fos-expressing neurons) only in animals that had a history of cocaine dependence. In contrast, no significant activation was found in the NAC shell and core, with no differences between groups. These data suggest that the LH→PVT→mPFC pathway, through Orx/Hcrt transmission at the PVT interface, is a neuronal circuits that drives cocaine-seeking behavior in cocaine-dependent animals.

Disclosures: **R. Martin-Fardon:** None. **F. Weiss:** None. **A. Matzeu:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

Support: 5T32DA00788-22

Title: Experience-dependent changes in calcium-permeable AMPA receptors in the mPFC following cocaine conditioned place preference versus cocaine self-administration

Authors: *J. I. PEÑA BRAVO, C. M. REICHEL, A. LAVIN;
Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Relapse to cocaine seeking involves corticostriatal neurotransmission. Plasticity of glutamatergic synapses is a fundamental mechanism through which experience changes neural function to impact behavior. Here, we tested whether experience-dependent changes in levels of cortical Ca2+-permeable AMPA receptors (Cp-ARs) depend on re-exposure to a cocaine-associated context. AMPA receptor rectification index (RI) was calculated as [RI = (eEPSC amplitude at -70mV)/(eEPSC amplitude at +40mV)] from either prelimbic (PL-mPFC) or infralimbic (IL-mPFC) medial prefrontal cortex deep layer pyramidal neurons. We found that the RI was significantly decreased in PL-mPFC neurons and trended towards an increase in IL-mPFC neurons of rats that underwent cocaine self-administration, extinction and cue-induced reinstatement. However, this methodology does not allow for a distinction between discrete cues versus contextual mediated effects. To address this distinction we used a cocaine conditioned place preference (CPP) model to establish a contextual associative memory and address whether Cp-ARs are modulated by re-exposure to a cocaine-associated environment. In the first experiment, rats underwent conditioned place preference. On day 1, rats received a 10 minutes pre-conditioning test were they had free access to the entire apparatus. During the 8 days of conditioning, rats received daily single injections of either cocaine (20mg/kg i.p.) paired with a distinct compartment or saline paired with another compartment for 25 minutes. On the test day, animals were given free access to both compartments in a drug-free state and their preference was assessed for 10 minutes. After the first CPP test, animals were split into two groups: 8 days or 30 days of abstinence. These groups are further subdivided into rats that are either tested or not tested and killed 15 minutes later for whole-cell voltage clamp experiment. Our results show a increase in RI after 8 days of abstinence, 8 days of abstinence followed by a CPP test and 30 days of abstinence compared to saline controls. Interestingly, testing the rats for CPP after 30 days of abstinence lead to a decrease in RI. In the second experiment, rats underwent 10 days of cocaine self-administration (2mg/50µL). Following 2 weeks of abstinence, rats were killed without testing and we found no difference between saline controls RI values in PL and IL-mPFC neurons. This was not surprising given that the rats did not re-experience the drug associated context or discrete cues. Future work will parse apart the importance of the cue vs context association as well as the influence of abstinence vs extinction.

Disclosures: **J.I. Peña Bravo:** None. **C.M. Reichel:** None. **A. Lavin:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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APA Dissertation Research Award

Title: Involvement of the dorsal hippocampus and HDAC3 in cocaine drug-seeking

Authors: *L. N. HITCHCOCK[1], J. D. RAYBUCK[1], R. G. WILLIAMS, Jr[1], M. A. WOOD[2], K. M. LATTAL[1];
[1]Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Substance use disorder is a chronic, often relapsing disease that leads to a loss of behavioral inhibition and compulsive drug-seeking. Cues that are paired with acquisition of drug-seeking are thought to influence subsequent extinction (animal model of exposure-based therapy) and relapse-like behavior, both in humans and animals. Our previous work has implicated the dorsal hippocampus for developing and retrieving memories in a drug-seeking context. By inactivating the dorsal hippocampus, we found that extinction was impaired in a contextual learning paradigm (cocaine-induced conditioned place preference). In addition, the epigenetic enzyme histone deacetylase 3 (HDAC3), has been shown to be a negative regulator of cocaine-associated learning and memory. Here, we further investigate this hippocampal-based extinction model and determine whether inhibition of HDAC3 can enhance extinction after cocaine self-administration. Despite the fact that extended extinction does not eliminate contextual renewal or cue-induced reinstatement, we find that a systemic injection of a synthetic HDAC3 inhibitor creates persistent extinction and weakens renewal and cue-induced reinstatement. In addition, we test whether inhibition of HDAC3 in the dorsal hippocampus alone is sufficient to impair extinction. In contrast to our systemic manipulation, it appears that dorsal hippocampus-specific HDAC3 inhibition does not alter drug-seeking behavior. Results suggest that regions outside of the dorsal hippocampus likely contribute to HDAC3-mediated enhancements in extinction after cocaine self-administration.

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Poster

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Title: Inhibitory influence of basolateral amygdala cannabinoid CB1 receptors in instrumental cocaine memory reconsolidation

Authors: *J. A. HIGGINBOTHAM, N. MAHAN, K. HARMON, A. ARGUELLO, R. FUCHS;
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Abstract: Exposure to a cocaine-associated context can reinstate extinguished drug-seeking behavior and trigger the reactivation of contextual cocaine memories. Reactivated cocaine memories are labile and sensitive to manipulation until they are re-stabilized into long term memory stores through the process of memory reconsolidation. Our laboratory has shown that cannabinoid 1 receptors (CB1R) play a role in instrumental cocaine memory reconsolidation. In the present study, we evaluated the specific contribution of CB1R populations in the basolateral amygdala (BLA) to this phenomenon. We trained rats to lever press for cocaine infusions in a distinct environmental context followed by extinction training in a different context. In order to reactivate cocaine memories, the rats were re-exposed to the previously cocaine-paired context for 15 min. The selective CB1R antagonist, AM251 (300 ng/hemisphere), or vehicle was microinfused bilaterally into the BLA either immediately or 6 h following memory reactivation (i.e., outside of the time window of memory reconsolidation). Seventy-two h later, cocaine-seeking behavior (i.e., non-reinforced active lever presses) was assessed in the previously cocaine-paired context. Remarkably, intra-BLA AM251 administration immediately, but not 6 h, following cocaine memory reactivation *facilitated* subsequent cocaine-seeking behavior. This suggests that the stimulation of CB1Rs in the BLA *inhibits* instrumental cocaine memory reconsolidation. Thus, BLA CB1Rs may be novel therapeutic targets for disrupting the salience or intrusiveness of maladaptive drug memories and for preventing relapse.

Disclosures: **J.A. Higginbotham:** None. **N. Mahan:** None. **K. Harmon:** None. **A. Arguello:** None. **R. Fuchs:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 144.17/J6

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA R01 DA025646

NIDA K99 DA037271

NINDS T32 NS007431

Title: Time-dependent, opposite effects of glucocorticoid receptor antagonism in the basolateral amygdala on drug context-induced cocaine-seeking behavior

Authors: *R. A. FUCHS[1], S. J. STRINGFIELD[2], A. A. ARGUELLO[1], K. M. HARMON[1], J. A. HIGGINBOTHAM[1];
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Abstract: Drug context-induced relapse to cocaine-seeking is dependent on the integrity of context-cocaine associative memories and the recruitment of these memories and other processes that trigger goal-directed behavior. Here, we investigated the role of basolateral amygdala (BLA) glucocorticoid receptors (GR) in A) contextual cocaine memory reconsolidation, a process responsible for long-term memory maintenance, and B) the drug context-induced reinstatement of cocaine-seeking behavior. Rats were trained to lever press for cocaine infusions in a distinct context followed by extinction training in a different context. In Exp. 1, rats received bilateral intra-BLA microinfusions of the GR antagonist, RU038486 (3, 10 ng/hemisphere), or vehicle following exposure to the previously cocaine-paired context, a procedure that elicits cocaine memory reactivation and reconsolidation. Controls were exposed to an unpaired context (no reactivation control). Non-reinforced lever presses were assessed 72 h later in the cocaine-paired context. In Exp. 2, rats received the same pharmacological manipulations immediately prior to testing. Intra-BLA RU038486 administration dose-dependently increased cocaine-seeking behavior 72 h later. This effect did not depend on memory reactivation; therefore, it did not indicate enhancement in memory reconsolidation. This RU038486-induced increase in cocaine-seeking behavior was associated with a paradoxical decrease in BLA glutamate GluN2a and GluN2b subunit activation, which in turn positively correlated with GR levels and ERK1/2 activation, respectively. Intra-BLA RU038486 administration at test dose-dependently attenuated drug context-induced cocaine-seeking behavior. Furthermore, intra-BLA RU038486 failed to

alter locomotor activity immediately or 72 h after administration. Together these findings suggest that BLA GR stimulation is necessary for drug context-induced motivated behavior. However, compensatory changes precipitated by BLA GR antagonism can result in a protracted increase in cue reactivity.

Disclosures: R.A. Fuchs: None. **S.J. Stringfield:** None. **A.A. Arguello:** None. **K.M. Harmon:** None. **J.A. Higginbotham:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA025646

K99 DA037271

Title: Optogenetic inhibition of the dorsal hippocampus: effect on reconsolidation of cocaine-associated contextual memories and subsequent cocaine-seeking behavior

Authors: *A. A. ARGUELLO, J. A. HIGGINBOTHAM, P. N. BUSHANA, J. L. BARNES, R. WANG, R. A. FUCHS;
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Abstract: Cocaine addiction is a chronic relapsing disorder. Relapse can be triggered by drug-associated environmental stimuli, suggesting that maladaptive cocaine-associated memories contribute to the addiction cycle. The maintenance of these memories over time depends on their reconsolidation into long-term memory stores following reactivation-induced de-stabilization. We have shown that tetrodotoxin (TTX)-induced inactivation of, but not protein synthesis inhibition in, the dorsal hippocampus (DH) impairs cocaine memory reconsolidation and subsequent cocaine-seeking behavior. Accordingly, the DH may maintain labile memories prior to their re-stabilization at another site, such as the amygdala. Therefore, the DH may be more critical during the initial stages of the 4-hour memory reconsolidation window. We have begun to examine the temporal dynamics of DH recruitment for cocaine memory reconsolidation and hypothesized that optogenetic inhibition of the DH during the first hour following memory reactivation would impair memory reconsolidation. Sprague-Dawley rats received bilateral infusions of AAV5-hSyn-eArch3.0-YFP plus optic fibers into the DH. Rats were trained to lever

press for un-signaled cocaine infusions in a distinct context and underwent extinction training in a different context. Following extinction training, rats were re-exposed to the previously cocaine-paired context for 15 minutes in order to de-stabilize cocaine-associated memories and trigger memory reconsolidation. Rats were then placed in a third context, where they received bilateral laser stimulation (532 nm, 1 second on/off) or no stimulation for 1 hour. After 2 additional extinction training sessions, cocaine-seeking behavior (i.e., non-reinforced lever presses) was assessed in the cocaine-paired context. Optogenetic inhibition of the DH during the first hour following re-exposure to the cocaine-paired context was sufficient to impair subsequent cocaine-seeking behavior. This suggests that the DH plays a critical role in the early stages of cocaine memory reconsolidation.

Disclosures: **A.A. Arguello:** None. **J.A. Higginbotham:** None. **P.N. Bushana:** None. **J.L. Barnes:** None. **R. Wang:** None. **R.A. Fuchs:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH N01DA-13-8908

Title: Retrograde contextual learning induced by cocaine in a conditioned place preference paradigm

Authors: R. A. SHETTY, M. A. RUTLEDGE, *M. J. FORSTER;
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Abstract: The purpose of these studies was to evaluate the magnitude of appetitive trace conditioning of context that could be induced by cocaine when presented at different interstimulus intervals (ISI) following a contextual S+. Swiss-Webster mice were tested using a standard conditioned place preference (CPP) procedure in which simultaneous pairing of 15 mg/kg cocaine with a distinctive, non-preferred floor type (S+) (composed of stainless steel rods or perforated holes) yielded a CPP. Mice were assigned to either a cocaine control condition (receiving simultaneous pairing with the S+ floor), or to trace pairing, with cocaine administered in the home cage after an ISI of 0.25, 4, or 8 h following exposure to the S+ floor. Additional mice were assigned to a saline control (null) condition in which no cocaine was administered. On two consecutive days, all mice were injected with saline before a morning session on the S-

floor. During an afternoon session on the S+ floor, the mice received either saline (saline control and trace pairing groups) or 15 mg/kg cocaine (cocaine control group). Following each afternoon session, mice were returned to their home cages in the vivarium where they received a third injection of either saline (saline control, cocaine control) or cocaine (0.25-h ISI, 4-h ISI, 8-h ISI). A preference test was conducted on the next day in which all mice were injected with saline and placed in the apparatus with split floors consisting of rods and holes, and the time spent on each floor was recorded. A positive shift in preference for the S+ floor was considered to reflect appetitive conditioning to the context. Three of the groups of mice exposed to cocaine (cocaine control, 0.25-h ISI, 4-h ISI) expressed a strong preference for the initially non-preferred floor. However, the shift in preference for S+ was absent in the 8-h ISI group. These studies suggest that retrograde appetitive conditioning of cocaine may occur to a salient context that was present up to 4 hours prior to cocaine exposure. The unusually long time-frame of this retrograde interaction is similar to that observed in studies of conditioned taste aversion, and this property may account for the important role of context in psychostimulant addiction and relapse.

Disclosures: **R.A. Shetty:** None. **M.A. Rutledge:** None. **M.J. Forster:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Fondation NRJ

LABEX BRAIN (ANR-10-LABX-43)

Title: Extinction of cocaine craving is associated with a shutdown of neuronal activity in the insula-prefrontal cortex circuit

Authors: *P. GIRARDEAU[1,2], S. NAVAILLES[3,2], A. DURAND[3,2], K. GUILLEM[3,2], S. H. AHMED[3,2];
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Abstract: Relapse is often preceded by a strong desire for the drug of choice or craving. Preventing the expression of craving represents thus a major goal in the research and development of new addiction therapies. We recently developed and validated in rats a behavioral procedure that durably abolishes cocaine-primed reinstatement of cocaine seeking - a robust model of drug craving. This procedure consists of exposing rats with a long history of cocaine self-administration to repeated, passive drug priming in the absence of cocaine reinforcement. The efficacy of this procedure is thought to result from the extinction of the interoceptive stimuli of cocaine that have been conditioned to cocaine reinforcement during cocaine self-administration. Here we report using this procedure that the extinction of cocaine-primed craving is associated with a complete loss of neuronal responses to cocaine in the insular and prelimbic cortex. To obtain a more dynamic picture, we also recorded neuronal responses in the prelimbic (PL) cortex to repeated drug primings using *in vivo* electrophysiology. We found that most PL neurons fired in responses to the first cocaine priming but that this initial response eventually disappeared with repeated cocaine priming in parallel with the extinction of cocaine-primed craving. Overall, these findings show that extinction of cocaine craving is associated with a progressive extinction of neuronal responses to cocaine in the insula-prefrontal cortex circuit.

Disclosures: P. Girardeau: None. **S. Navailles:** None. **A. Durand:** None. **K. Guillem:** None. **S.H. Ahmed:** None.

Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

Support: FRM DPA20140629789

CNRS

Aix Marseille Université

Title: Subthalamic nucleus markers of compulsive cocaine seeking

Authors: *C. BAUNEZ, A. TIRAN-CAPPELLO, Y. PELLOUX;
INT CNRS & Aix-Marseille Univ., Marseille, France

Abstract: Loss of control over drug intake and compulsive drug seeking are key features of drug addiction. Uncovering neurobiological markers of these disorders may help refine our understanding of the disease and reveal new potential therapeutic strategies. Most theories regarding neurobiological substrate of addiction have focused on dopaminergic alterations. In patients suffering from dopaminergic neurodegeneration inherent to Parkinson's disease, the subthalamic nucleus (STN) exhibits pathological oscillations in the low frequency range (beta band between 13 to 30Hz) that disappear concomitantly to motor symptoms after L-DOPA or STN high frequency stimulation. In order to assess the presence of STN oscillations in a dopamine related disorder affecting more motivation processes, we measured local field potentials (LFPs) in animal models of addiction exhibiting aberrant responding for the drug. For this aim, rats were first trained under the seeking taking schedule of reinforcement for cocaine. Then, some animals were given the opportunity to escalate their cocaine intake across 15 sessions of long access to the drug (6-hour sessions). STN LFPs were recorded 15 minutes before (that is during acute withdrawal) and 15 minutes just after (that is under the effect of cocaine) long access sessions across the 15 days. Then, all animals were returned to the seeking taking task after which intermittent punishment upon seeking responding was introduced. As previously shown, a subpopulation of animals after escalation showed persistent responding despite intermittent punishment. Only these compulsive animals exhibited a progressive increase in low frequency oscillations (6-12 Hz) during acute withdrawal, which disappeared under the effect of cocaine. These results evidence a participation of STN low frequency oscillations in the development of compulsive cocaine seeking and suggest potential benefit of manipulation aiming at reducing low frequency stimulation such as L-DOPA or STN high frequency stimulation.

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Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

Support: ANII Grant FCE100466

PEDECIBA

Title: Cocaine motivational value is enhanced when co-administered with caffeine: relevance of adulterants in reinforcement

Authors: *J. P. PRIETO[1], C. SCORZA[2], G. SERRA[4], V. PERRA[4], G. PIRAS[4], M. GALVALISI[2], A. ABÍN-CARRIQUIRY[3], G. DI CHIARA[4], V. VALENTINI[4];
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Abstract: It is widely known that illicit drugs of abuse, such as cocaine hydrochloride, are usually sold adulterated. Adulteration implies the intentional addition of a pharmacologically active substance. Forensic data provide information about the common adulterants found in drugs of abuse. It has been reported that phenacetine, caffeine, lidocaine and levamisol are the most commonly substances detected in street cocaine samples. However, it is unusual that the influence of adulterants in the psychoactive effect of cocaine is considered. Animal studies have demonstrated that particularly caffeine is able to potentiate cocaine actions, although the enhancement of the cocaine reinforcing property by this adulterant is less reported, and the results depend on the paradigms and experimental protocols used. In the present study we examined the ability of caffeine to enhance the motivational and rewarding properties of cocaine using the intravenous self-administration paradigm in rats. Additionally, the role of caffeine as a primer cue during extinction was evaluated. To this end, we assessed in naïve Sprague-Dawley rats: 1) the ability of the combination of cocaine (0,125 mg/kg/infusion) and caffeine (0,0625 mg/kg/infusion) to maintain self-administration in fixed ratio 1 (FR 1) and progressive ratio 3-4 (PR 3-4) schedules of reinforcement compared with cocaine and caffeine alone; 2) the effect of caffeine in the maintenance of responding in the animals exposed to the combination of the drugs during cocaine extinction. Cocaine and the combination of cocaine and caffeine were self-administered on a FR 1 and PR 3-4 schedules of reinforcement, being the breaking point of the combination of the drugs higher than cocaine alone. Caffeine was not reliably self-administered, but was able to maintain a drug-seeking behavior in rats previously exposed to cocaine plus caffeine. These findings suggest that the presence of caffeine enhances the reinforcing effects of cocaine and the motivational value of the drug. Our results highlight the role of active adulterants commonly used in illicit street drugs. Finally, the permanent identification of substances used as adulterants can really contribute to identify the acute or chronic toxicity and dependence induced by drugs of abuse.

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Poster

144. Cocaine: Neural Mechanisms of Reinforcement and Relapse II

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Topic: C.17. Drugs of Abuse and Addiction

Support: FAPESP 2015/01877-9

Title: Neuronal activation in ventral hippocampus during context-induced reinstatement of cocaine self-administration in rats

Authors: *P. E. OLIVEIRA[1], P. C. BIANCHI[1], R. M. LEÃO[1], C. S. PLANETA[1], B. T. HOPE[2], F. C. CRUZ[3];
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Abstract: In human addicts, environmental contexts associated with previous drug use can provoke relapse to drug use after prolonged abstinence. Previous studies indicate that specific patterns of sparsely distributed neurons, called neuronal ensembles, mediate and likely encode learned associations between drug-associated contexts and drug-taking. In the present study, we investigated whether context-induced reinstatement of cocaine seeking is mediated by activation of neuronal ensembles in the ventral hippocampus. Rats were trained to self-administer cocaine (0.5-1.0 mg/infusion) 3 h/day for 12 days; drug infusions were paired with a discrete tone-light cue. Subsequently, lever responding was extinguished over 12 days in the presence of the discrete cue in a non-drug context with different sensory and circadian features than the drug self-administration context. Rats were then re-exposed to the cocaine-associated context (or the non-drug extinction context as the control condition) and lever-pressing was assessed under the same extinction conditions for 90 min as a measure of cocaine seeking. Neuronal ensembles in ventral hippocampus that were activated during context-induced reinstatement were identified using Fos immunohistochemistry. Re-exposure to the cocaine-associated context, but not the non-drug context, increased lever pressing as well as Fos-immunoreactivity in ventral hippocampus (lever pressing: 78±16 and 12±3, respectively; $p<0.05$), (Fos positive nuclei: 552±102 and 102±25, respectively; $p<0.05$). These data suggest that activation of ventral hippocampus neuronal ensembles might mediate context-induced reinstatement of cocaine self-administration

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Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: R21 NS078731 (VG)

Title: Altered capacity for cellular remodeling and neurotransmission in the hippocampus underlie memory deficit in a mouse model of perinatal hyperoxia

Authors: *J. ABBAH[1], L.-J. CHEW[1], C.-M. VACHER[1,2], V. GALLO[1];
[1]Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC; [2]Mol. Neuroendocrinology of Food Intake Lab., NeuroPSI - Univ. Paris Sud 11 - CNRS, Orsay, France

Abstract: Survivors of preterm birth are susceptible to brain injury due to perinatal hypoxia-ischemia, infection and hyperoxia (HO). These injuries result in neurological sequeleae such as cognitive impairment and learning disability, which frequently occur in this population. The cellular and physiological mechanisms underlying the deficit in memory and learning behavior in this population are still largely unknown, in particular hippocampal abnormalities that may potentially predispose to development of a disease phenotype. Our lab developed a mouse model of perinatal brain injury using short-term (48 h) exposure to hyperoxia (80-85% oxygen) from postnatal age 6 to 8 (P6 - P8), in which impaired white matter development was previously demonstrated. Here we evaluated the impact of perinatal hyperoxia (HO) on the proliferative capacity of dentate gyrus (DG) of the hippocampus using BrdU pulse-chase in proopiomelanocortin-EGFP (POMC-EGFP) transgenic mice. Perinatal HO leads to biphasic impairment in cell proliferation within the hippocampus. Specifically, HO causes an initial decline (P8) in the number of BrdU+ cells followed by recovery at P12 - P14. In parallel with reduction in all newly generated cells, we also found that the number of Sox2+ cells that represent the pool of stem/progenitor cells, and those of neuronal neuroblasts that are POMC+ were also reduced after HO treatment. However, the apparent recovery of cell proliferation in the DG following HO is not sustained, as the numbers of BrdU+, POMC+ and doublecortin positive cells are significantly reduced again at P60. HO also caused a significant reduction in the number of parvalbumin positive (PV+) interneurons within the CA1, and of the expression levels of PV mRNA. Protein levels of CaMKII and the α1 and α3 subunits of GABAA receptors were also reduced. Patch-clamp recordings revealed regional-specific reduction in inhibitory postsynaptic currents (IPSCs) in the CA1 at both P8 and P20, and an increase in excitatory postsynaptic currents (EPSCs) at P20. Mice exposed to HO also displayed impaired memory and learning ability in the water T-Maze and novel object recognition tests. These data indicate that HO selectively impairs the proliferation of neuronal-lineage cells in the SGZ and alters the balance of excitatory and inhibitory neurotransmission in the CA1. Impaired hippocampal remodeling

coupled with dysregulated GABAergic neurotransmission caused by HO contributes to deficits in learning and cognitive ability.

Disclosures: **J. Abbah:** None. **L. Chew:** None. **C. Vacher:** None. **V. Gallo:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: 2013/2014 Cord Blood Research Grant, Inner Wheel Australia

NIH Grant

Title: Early administration of cord blood cells reduces preterm brain injury following hypoxia-ischemia

Authors: ***J. LI**, T. YAWNO, A. SUTHERLAND, J. LOOSE, I. NITSOS, F. Y. WONG, G. JENKIN, S. L. MILLER;
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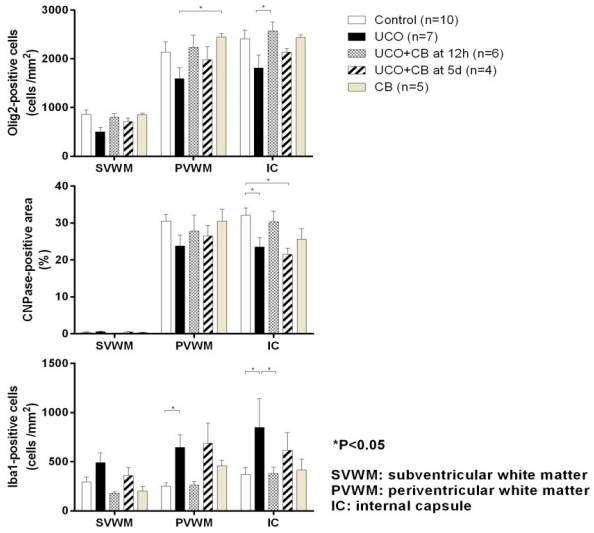
Abstract: Background: Preterm infants, particularly infants born <32 weeks gestational age, are at high risk for cerebral palsy and other neurological deficits. There is increasing evidence that umbilical cord blood (UCB) may have therapeutic potential in reducing brain injury in cases of term neonatal asphyxia. However, there is limited information on the potential benefits of UCB treatment for the preterm brain. Aim: This study examined the effectiveness and mechanism of action of allogeneic UCB mononuclear cell administration to preterm fetal sheep following umbilical cord occlusion (UCO). Methods: UCO or sham was performed for 25 minutes to fetal sheep at 100d gestational age (~0.7 gestation). 50 million CFSE-labelled UCB cells, derived from term lambs, or saline were administered intravenously to the fetus at 12 h or 5 d after UCO. Regular fetal plasma samples were collected, and brains obtained at 10 d after UCO, for analysis. Results: Brain histology revealed that CFSE-labelled cells when given i.v were detected in the fetal brain. UCO reduced the number of oligodendrocytes (olig2+) and myelinated axon density (CNPase+) in white matter, while UCB cells administered at 12 h, restored white matter development (Figure). UCO animals showed a significant increase in TUNEL- and Ki67-positive cells in the white matter, co-localised with olig2, while UCB cells, administered at 12 h, prevented these effects. The number of activated microglia (Iba1+) was also increased in the

white matter in UCO fetuses, whereas UCB cell administration at 12 h provided an anti-inflammatory effect. However, UCB cell administration at 5 d failed to show these, potentially, therapeutic effects (Figure). Additionally, UCB cell administration at 12 h reduced systemic oxidative stress that occurred in response to UCO. Conclusion: Early UCB mononuclear cell administration at 12 h after hypoxia-ischemia reduces white matter injury through its anti-inflammatory, anti-apoptotic, and antioxidant effects.



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Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: NIH Grant NS081936

Title: Perinatal tetrahydrobiopterin biosynthesis is changed in fetal brain in a chorioamnionitis animal model

Authors: *Z. SHI[1], K. LUO[1], A. DROBYSHEVSKY[1], J. VASQUEZ-VIVAR[2], S. TAN[1]; [1]Pediatrics, Northshore Univ. Hosp., Evanston, IL; [2]Biophysics, Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Aim: Tetrahydrobiopterin (BH_4) is an essential cofactor for nitric oxide (NO) formation in many organs. BH_4 levels in fetal rabbit brains are at very low levels at premature gestation and decreases with hypoxia-ischemia. We have developed a new model of chorioamnionitis in maternal rabbits using lipopolysaccharide (LPS) injection into the endocervix. **Methods:** LPS (0.5 ml, 200 μg/kg/ml, Escherichia coli O22:B55, Sigma) or sterile saline (0.5 ml, control) was injected into the endocervix of E28 (89% term) rabbit dams (New Zealand White) under hysteroscopy guidance. Brain tissues were collected 24h afterwards, and snap-frozen. Rabbit genes, which were previously unknown, were cloned using RACE-based techniques by lab-designed gene-specific primers, and confirmed by alignment with the predicted sequences at GenBank. Primers were designed by Primer-Blast programs. Quantitative RT-PCR was used for gene expression of enzymes in the tetrahydrobiopterin pathways. **Results:** We compared two key biosynthetic enzymes involved in the tetrahydrobiopterin pathway, GTP cyclohydrolase I (GTPCH) and sepiapterin reductase (SPR) between the LPS and saline groups. We found no change in neuronal nitric oxide synthase (nNOS) expression. However, a significant increase in GTPCH was observed in all brain regions from fetuses irrespective of the position of the uterus, with a trend to increase more in the fetuses at the uterine poles compared to the uterine base. There was a decrease in SPR expression in cortex of the pole samples. **Conclusions:** For the first time we can study the gene expression of biosynthetic enzymes of tetrahydrobiopterin in the rabbit. Different biosynthetic enzymes of the tetrahydrobiopterin pathway respond differently to an inflammatory insult with opposing results. Further studies are underway to characterize the other biosynthetic enzymes. **Key words** Lipopolysaccharide, Chorioamnionitis, Tetrahydrobiopterin, Neuronal nitric oxide synthase, GTP cyclohydrolase I

Disclosures: Z. Shi: None. **K. Luo:** None. **A. Drobyshevsky:** None. **J. Vasquez-Vivar:** None. **S. Tan:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

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Support: AIHS

CIHR

Title: Transcranial motor-evoked responses in children with perinatal stroke involved in an intensive leg training program

Authors: *A. SMITH[1], E. ZWEDIE[1], D. LIVINGSTONE[1], K. BRUNTON[1], C. HURD[1], A. MOIR[1], A. KIRTON[2], J. YANG[1], M. GORASSINI[1];
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Abstract: The sensorimotor regions of the brain are common sites of damage early in development. Individuals with cerebral palsy (CP) experience a reorganization of sensorimotor pathways in the developing brain resulting in limited limb activity and motor impairment. Over 70% of children with CP experience difficulties with walking. While current therapy interventions aimed at improving walking are still very broad, there is a growing shift from passive movements initiated by a therapist to an emphasis on more activity-based learning initiated by the child. These intensive practice programs in early infancy have shown promising functional results; however, the degree to which they influence underlying motor systems is unknown. Here we investigated the effects of intensive training to the affected leg in children with perinatal stroke to mainly one side of the brain. The strength of the corticospinal pathways involved and the latency of leg muscle responses at different ages were investigated before and after the training period. We examined the amplitude and onset latency of motor-evoked potentials (MEPs) evoked by transcranial magnetic stimulation (TMS) over the motor cortex supplying the lower limb muscles (vastus lateralis, tibialis anterior, hamstrings, and gastroc-soleus). Data will be presented from an on-going, 2-centre, randomized trial with a delayed-treatment group acting as controls. Intensive leg training was provided by physical therapists to improve walking function in children between 8 months and 3 years old. In 19 children, we examined if MEPs could be evoked in leg muscles when applying TMS over the less affected cortex. In this condition, ipsilateral MEPs could be evoked in 14 children in at least one of the four muscles tested, whereas contralateral MEPs were less prevalent, occurring in 13 children. In contrast, MEPs could only be evoked from the more affected cortex in 9 of the children, all of which showed contralateral responses and only 7 of which showed ipsilateral responses in at least one of the four muscles tested. The onset latency of both ipsilateral and contralateral MEPs evoked from the less affected cortex progressively decreased with age across the sample size, typically with latencies of 60 ms at 8 months of age to 30 ms at 54 months of age. Additionally, a strengthening of descending pathways was observed in 10 children where there was an increase in consistency and amplitude of MEPs in at least one of the muscles following the training period. These preliminary results suggest a reorganization of underlying motor systems in response to an early intensive training program.

Disclosures: **A. Smith:** None. **E. Zwedie:** None. **D. Livingstone:** None. **K. Brunton:** None. **C. Hurd:** None. **A. Moir:** None. **A. Kirton:** None. **J. Yang:** None. **M. Gorassini:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 145.05/J17

Topic: C.20.Perinatal Brain Injury

Support: NIH Grant NS081936

Title: Perinatal brain injury in a chorioamnionitis model in rabbits

Authors: *K. LUO[1], Z. SHI[2], A. DROBYSHEVSKY[2], S. TAN[2];
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Abstract: Introduction: Chorioamnionitis, an ascending infection from the vagina into the amniotic cavity, is believed to be a risk factor for perinatal brain injury. Previous animal models have used bacterial injection into the endocervix or lipopolysaccharide injection into the myometrium in rabbits. Rodent models that use postnatal intraperitoneal injection of lipopolysaccharide may not exactly mimic the human condition. **Objective:** Our objective was to develop a clinically relevant animal model by intra-cervical administration of the endotoxin, lipopolysaccharide (LPS), in pregnant rabbits to induce intrauterine inflammation. The study determined the postnatal neurobehavioral deficits and cytokine response in fetuses. **Methods:** Pregnant rabbits at 28 days gestation were anesthetized. A flexible cannula was inserted under hysteroscopy guidance 1-2 cm into the cervix. Saline or 0.5 ml of LPS (Escherichia coli O22:B55) in a dose 200 µg/kg/ml was injected in each cervical opening. One cohort of animals was allowed to deliver and a neurobehavioral battery of tests conducted at postnatal days 1 and 11 (P1, P11). Caesarean section was done on another cohort at 24 hours after LPS injection. Fetal cortex (CO), basal ganglia (BG), thalamus (TH), and cerebellum (CE) various regions of the uterus were assayed with RT-PCR for cytokines. Statistical significance was defined as $P < 0.05$ (Student t test). **Results:** LPS exposure caused a significant locomotion deficit at P1. The degree of hypertonia was mild. By P11 most animals had recovered and looked almost normal. The neurobehavioral deficits were transient compared to hypoxia-ischemia. Since some of the newborn kits looked normal we determined if the cytokine response was any different in the fetuses between the base and pole position in the uterus after 24 hours. TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-10 and TGF-β expressions were not different between the base and pole (n=3 in each group). Most cytokines showed increase in the LPS treated group except TNF-α, which showed a decrease. **Conclusions:** This is the first study to show neurobehavioral changes in kits and cytokine changes in fetal brain tissues in a LPS induced ascending chorioamnionitis model in rabbit. LPS inflammation affects fetuses to varying degrees, probably not depending on the

position of the fetus in the uterus. **Key words**: LPS; intra-cervical injection; RT-PCR; cytokine; IL-1β; IL-2; IL-6; TGF-β; TNF-α, IL-8; IL-10

Disclosures: K. Luo: None. **Z. Shi:** None. **A. Drobyshevsky:** None. **S. Tan:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

Location: Hall A

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Program#/Poster#: 145.06/J18

Topic: C.20.Perinatal Brain Injury

Support: NS060896

Title: Development of an LPS-sensitized mouse model of very preterm brain injury

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Abstract: Evidence supports a role for both infection/inflammation and hypoxic-ischemic (HI) injury in the pathogenesis of brain injury in premature human newborns. The Rice-Vannucci model (unilateral carotid ligation followed by hypoxia) has been combined with lipopolysaccharide (LPS) administration at P7 to either sensitize or precondition against brain injury depending upon timing of administration. However, this model has not been performed before P5. We have used the Rice-Vannucci procedure at P2 in rats to model brain injury in the very preterm human newborn (26-30 weeks) with injury to lower cortical layers including subplate and developing white matter. However, we have not been able to reproduce similar cortical injury in mice. We aim to develop a mouse model of very preterm brain injury (< P5) combining LPS sensitization with Rice-Vannucci procedure for cerebral hypoxia-ischemia. C57Bl/6 mice at P3 or P4 received subcutaneous injections of LPS 4 hours before unilateral right carotid artery ligation and subsequent exposure to 8% O2 for 30-40 min. Procedural and post-procedural mortality was recorded. At 10 days post-HI, injury was assessed by histology and immunohistochemistry. Infarct volume was measured using Cavalieri method and calculated as (hypoxia hemisphere area - HI hemisphere area)/(hypoxia hemisphere area). LPS (doses 0.1-1mg/kg) or HI alone did not cause detectable cortical injury. Surgical mortality for HI procedure was 14% (N=8/56). Mice pretreated with LPS doses of 0.3 and 1 mg/kg prior to HI all died 1-3 days after HI (N= 8). At a dose of 0.1 mg/kg, no mice survived a hypoxia duration of 40 minutes (N=5) performed at P4. When LPS-HI was performed at P3 with 30 minutes of 8% oxygen, post-

procedural mortality was 19% and at P4 mortality was 40%. At both ages, a range of injury was observed from mild (no histologically detectable injury) to severe (cortical injury with lateral cyst formation). Mild/moderate injury was found in 76% at P3 (N=16/21) and 91% at P4 (N=10/11). For P3 animals, infarct volume increased with categorical injury score: mild- (1.8 +/- 0.5%, N=3), moderate- (8.5+/-5.5%, N=2), severe- (25.6+/-5%, N=3). Immunofluorescence with layer specific markers is being analyzed for difference in subplate and lower cortical layer cell number in injured animals. Cortical injury with acceptable mortality can be produced by combining LPS pretreatment with HI at early ages in mouse. A mouse preterm brain injury model will facilitate utilization of genetically modified mouse strains for imaging, cell fate tracking and gain/loss of function experiments.

Disclosures: A. Mikhailova: None. **S. Ranasinghe:** None. **P.S. McQuillen:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: ERC-2013-AdG 341116-PressBirth

Jane and Aatos Erkko Foundation

Title: Graded restoration of normocapnia blocks pathophysiological suppression of sharp waves in a rat model of birth asphyxia

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Abstract: Spontaneous periodic network events are a characteristic feature of developing neuronal networks, and they are thought to play a crucial role in the maturation of neuronal circuits. In the rodent hippocampus *in vivo*, sharp waves (SPW) are the first and only population event during early postnatal development. Birth asphyxia is associated with pathophysiological changes in the EEG, including either seizures or suppression of EEG activity during and immediately following asphyxia, which are often predictive of a poor neurodevelopmental outcome. The mechanisms underlying brain trauma and developmental disorders following birth asphyxia remain unknown. Here, we studied the short term EEG outcome following experimental asphyxia. We use a model of infant rats that mimics the alterations in systemic

CO2 and O2 levels during and after birth asphyxia. Infant rats (P5-7) were exposed for 45 minutes to asphyxic conditions (20% CO2, 4% O2) and left to recover either in room air for 2 hours (rapid restoration of normocapnia, RRN), or by a graded restoration of normocapnia (GRN, 10% CO2 for 30minutes, followed by 5% CO2 for 30 minutes, and subsequently room air for 1 hour). The impact of asphyxia and recovery on SPW activity was assessed 24 hours post-asphyxia. We recorded SPW activity along the CA1-dentate gyrus axis using multi-site silicon probes under urethane anaesthesia. Rats exposed to RRN showed strong and prolonged suppression of SPW activity. Strikingly, frequency and amplitude of SPW showed no significant changes when asphyxia was followed by GRN. There is a widely recognised lack of therapeutic interventions to effectively ameliorate pathophysiological effects caused by birth asphyxia. Our work suggests that GRN may turn out to be one effective strategy for intervention.

Disclosures: **E. Prokic:** None. **A. Alafuzoff:** None. **J. Voipio:** None. **K. Kaila:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: NIH NS060765

Title: Postnatal erythropoietin modulates excess calpain activation following prenatal hypoxia-ischemia in rats

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Abstract: Preterm infants suffer central nervous system (CNS) injury termed encephalopathy of prematurity from hypoxia-ischemia and inflammation. Caspases and calpain activation are two major mediators of mature CNS injury. Previously it has been shown that Erythropoietin (EPO) limits apoptosis mediated by activated caspases, but its role in limiting calpain activation has not been investigated extensively in immature CNS injury. Here, we examined the impact of calpain degradation in encephalopathy of prematurity in detail by defining the extent and time course of calpain degradation of molecules essential to perinatal neurodevelopment. We hypothesized that excess calpain activation degrades developmentally-regulated molecules essential for CNS circuit formation, myelination and axon integrity, including neuronal potassium-chloride co-

transporter KCC2, myelin basic protein (MBP), and phosphorylated-neurofilament (pNF), respectively, and that post-injury EPO treatment could mitigate CNS calpain-mediated degradation. Using prenatal transient systemic hypoxia-ischemia (TSHI) in rats at E18 to mimic CNS injury from extreme preterm birth, and postnatal EPO treatment with clinically-relevant dosing, we found excess cortical calpain activation, as shown by cleavage of αII-spectrin into 145kDa αII-spectrin-degradation products (αII-SDPs) and p35 into p25. Specifically, cortical SDP ratios were significantly elevated in TSHI animals compared to sham (n=12-15, p=0.007) at P2. To confirm excess calpain activity, we also quantified the degradation of p35 to p25 at P2, and found more p25 in TSHI than sham cortex (n=10-11, p=0.026). Compared to shams at P11 αII-SDP ratios were significantly elevated in cortex following TSHI (n=14, p=0.002, Fig. 2E) concomitant with reduced expression the endogenous calpain inhibitor calpastatin. Postnatal EPO treatment reversed increases in αII-SDP ratios (p=0.016) and loss of KCC2, MBP and NF following prenatal TSHI. Together, these data indicate that postnatal EPO treatment can mitigate excess calpain activity and attenuate degradation of molecules essential to neurodevelopment and the pathogenesis of encephalopathy of prematurity.

Disclosures: L.L. Jantzie: None. **S. Robinson:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

Location: Hall A

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Program#/Poster#: 145.09/J21

Topic: C.20.Perinatal Brain Injury

Title: Decrease of white matter projections in spinal cord after antenatal hypoxia-ischemia in rabbit cerebral palsy model

Authors: *A. DROBYSHEVSKY, K. LUO, Z. SHI, S. TAN;
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Abstract: Newborn rabbit kits after global antenatal hypoxic-ischemic (H-I) injury exhibit motor deficits similar to human infants with cerebral palsy, including muscle hypertonia. We have previously shown that decrease of descending corticospinal projections in internal capsule and cerebral peduncles after antenatal brain injury may play a key role in development of hypertonia. We hypothesized that a decrease of descending corticospinal projections will affect the development of spinal cord from the moment of injury in fetuses till development of hypertonia. Alternatively, direct cell injury in spinal cord after antenatal global hypoxia -ischemia is hypothesized. Rabbit dams underwent global fetal H-I at E22 for 40 min. At P1 fixed neonatal

brains and spinal cord were scanned *ex vivo* on 14T magnet with isotropic 0.15 mm resolution with diffusion tensor imaging. Fractional anisotropy (FA) in brains of hypertonic, non-hypertonic and control kits (n=5,6,9) were compared using Tract Based Spatial Statistics (TBSS) approach. Significant reduction in FA was found in cortico-spinal tract, external capsule, fimbria hippocampi bilaterally in hypertonic group only. There was significant reduction in white and gray matter in spinal cords in only hypertonic group at cervical, thoracic and lumbar levels. Amount of TUNEL and FluoroJade C positive cells was not significantly different between the groups in spinal cord 24 and 72 hours after H-I. We conclude that significant loss of spinal white matter and gray matter in hypertonic kits 10 days after fetal H-I injury is likely to result from degenerative changes or disrupted development due to upstream injury to corticospinal tract and not due to the axonal and cell loss immediately after H-I.

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Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: CIHR

FRQ-S

Heart and Stroke Foundation

Faculté de Médecine de l'Université de Sherbrooke

Foundation of Stars

Title: Neuroprotective effect of hypothermia in a model of neonatal encephalopathy resulting from E. coli-induced inflammation plus hypoxia-ischemia

Authors: *M. CHEVIN[1], C. GUIRAUT[2], G. SÉBIRE[2,3];
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Abstract: Introduction: Neonatal encephalopathy (NE) which affects 2-9/1000 full term newborn leads to life threatening symptoms and can cause permanent brain damage such as cerebral palsy (CP). The two main risk factors of NE are perinatal infection-inflammation and hypoxia-ischemia (HI). The standard treatment is therapeutic mild hypothermia (body cooling of 33-34°C for 72h) providing a partial neuroprotection in moderate to severe NE. Such neuroprotective effect of hypothermia remains unclear when NE result from pathogen-induced inflammation combined to HI. Otherwise, the anti-inflammatory interleukine-1 receptor antagonist (IL-1ra) has a well-proved neuroprotective effect in preclinical models of perinatal inflammation induced by pathogens and/or HI. We hypothesize that the combination of mild hypothermia plus IL-1ra results in additive or synergistic neuroprotection. Objectives: We characterised the impact of hypothermia on the expression of cerebral inflammatory markers (cytokines, chemokines) and on the prevention of cerebral injuries. Material and methods: We used an animal model of rat pups (Lewis) at postnatal day 12 (P12). P12 corresponds to the level of cerebral development of the human full term newborn. Inflammation is induced by injecting the rat pups intraperitonealy with 200 µg/kg of lipopolysaccharide (LPS) from E.coli. Four hours (h) later, the right common carotid artery is ligated, then hypoxia is induced (8% O2, 1 h 30 min). Finally, rat pups are submitted to hypothermia (32° +/- 0.5°C for 4 h). Pups are euthanized at different time points in order to perform histological analysis and cytokine titration. Results: Our results show that hypothermia alleviated brain damage in the LPS+HI-exposed cerebral cortex and hippocampus. This protective effect only occurred in the brain areas affected by ischemic penumbra, but not core injuries; penumbra corresponds histologically to an association of pyknotic and healthy neurons, without destruction of extracellular matrix or ischemic cavitation. This neuroprotective effect did not result from a down-regulation of the neurotoxic response mediated by IL-1β or TNF-α. Conclusion: Our results demonstrate a neuroprotective effect of hypothermia on LPS+HI-induced brain damage in a model of NE. This beneficial effect of hypothermia is not mediated by IL-1β down-regulation meaning that IL-1ra administration might reinforce the hypothermic neuroprotective effect. This project could open new therapeutic avenues to prevent CP.

Disclosures: **M. Chevin:** None. **C. Guiraut:** None. **G. Sébire:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: NIH Grant NS081936

Title: Using neuroimaging biomarkers to study tetrahydrobiopterin pathways critical to hypertonia in antenatal hypoxia-ischemia

Authors: *S. TAN[1], K. LUO[1], Z. SHI[1], A. DROBYSHEVSKY[1,2], J. VASQUEZ-VIVAR[2]; [1]Northshore Univ. Healthsystem, Evanston, IL; [2]Med. Col. of Wisconsin,, Milwaukee, WI

Abstract: Introduction: Previously, there has been a paucity of relevant animal models of cerebral palsy (CP). We were the first to show sustained hypertonia, a pathognomonic feature of CP, in a rabbit model following antenatal hypoxia-ischemia. Furthermore, we have developed sensitive and specific MRI biomarkers to identify which fetuses are critically injured and will develop hypertonia postnatally. This allows the study of early events that are causative of hypertonia. We have previously also shown that maternal tetrahydrobiopterin supplementation ameliorates motor deficits postnatally. **Objective:** Our objective was to investigate tetrahydrobiopterin pathways with hypertonia caused specifically by oxidative stress and not by other pathways such as energy failure or endoplasmic reticulum involvement. **Methods**: Pregnant rabbits at 25 days gestation (79% term) were subjected to uterine ischemia for 40 min under a clinical magnet with MRI monitoring. MRI biomarkers delineated four groups of brain injury: Hypoxic injury alone, Reperfusion-Reoxygenation injury, Mild and No injury. Caesarean section was done at 48 hours after the insult. Cortex, basal ganglia, thalamus, and cerebellum from fetuses located in different regions of the uterus were assayed for biopterin using a fluorescent HPLC method. Statistical significance was defined as $P < 0.05$ (Student t test). **Results:** Biopterin, a surrogate for tetrahydrobiopterin levels, was decreased in the cortex and cerebellum in the Reperfusion-Reoxygenation group compared to other groups. Those fetuses showing only hypoxic injury did not have significant change from Mild or No injury groups. The changes in the basal ganglia and thalamus were not different between the groups. **Conclusions:** Tetrahydrobiopterin levels are decreased 48 hours in the group specifically associated with oxidative stress only, i.e. Reperfusion-Reoxygenation group. This could be due to a secondary decrease in biosynthesis or breakdown of tetrahydrobiopterin from free radicals. Another possibility could be that those fetuses which are unable to increase tetrahydrobiopterin may be vulnerable to the critical pathogenetic event leading to hypertonia.

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Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: NRF Grant 2012R1A2A2A01046132

Title: Administration of zinc plus cyclo-(His-Pro) increases neurogenesis in the subgranular zone of the dentate gyrus in streptozotocin-induced diabetic rat

Authors: *I. KIM[1], B. CHOI[1], J. KIM[1], B. LEE[1], S. LEE[1], A. KHO[1], S. SUH[1], M. SOHN[2]; [1]Dept. of Physiol., Hallym University, Col. of Med., Chuncheon, Korea, Republic of; [2]Inha university, Incheon, Korea, Republic of

Abstract: Diabetes mellitus is intense metabolic disorder with multiple complications that include deterioration of learning and memory, which leads to cognitive impairment and dementia. Although adult hippocampal neurogenesis is reduced in chronic diabetes, the effect of acute diabetes on hippocampal neurogenesis has not been studied. Herein, we investigated the effects of acute diabetes on adult hippocampal neurogenesis in pharmacologically-induced diabetic models. This study also investigated the role of the trace metal zinc in adult neurogenesis. In the present study, we hypothesized that administration of zinc with histidine and proline may increase hippocampal neurogenesis in diabetic rats. To induce type 1 diabetes, rats were injected with streptozotocin (STZ, 50 mg/kg, i.p.). One-month-old male Sprague Dawley rats were given STZ by intraperitoneal injection once per day for two consecutive days. Diabetes was defined as fasting blood glucose levels above 200 mg/dl at two days after the first injection. Zinc plus cyclo-(His-Pro) contains 20 mg of zinc. To test the effects of zinc supplementation on neurogenesis in diabetic rats, 5'-Bromo-2'deoxyuridine (BrdU) was injected twice daily for four consecutive days starting 3 days after the STZ injection. For the experimental group, zinc (30mg/kg) plus cyclo-(His-Pro) was injected by oral gavage for seven days starting at one day after the second STZ injection. For the control group, cyclo-(His-Pro) was injected without zinc for seven days. The present study found that acute streptozotocin-induced diabetes increases progenitor cell proliferation in the subgranular zone of the dentate gyrus at seven days post-injection in diabetic rats. Interestingly, we also found that zinc plus cyclo-(His-Pro) supplement further increased hippocampal neurogenesis in diabetic rats. The present study suggests that supplementation of zinc with cyclo-(His-Pro) promoted hippocampal neurogenesis in early stages of diabetic pathophysiology.

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Poster

145. Perinatal Brain Injury: Acute Therapy

Location: Hall A

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Topic: C.20.Perinatal Brain Injury

Title: Glutamate oxaloacetate transaminase a therapeutic target against periventricular leukomalacia in neonates

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Abstract: BACKGROUND: Periventricular leukomalacia (PVL) is the most common cause of cerebral palsy in premature infants and often a consequence of hypoxia ischemia (HI) leading to excessive neuroexcitotoxic glutamate accumulation.Glutamate oxaloacetate transaminase (GOT-1) catalyzes transfer of the amino group from Glutamate (Glu) to oxaloacetate which will clear toxic extracellular Glu from the ischemic site while also feeding cellular respiration. OBJECTIVE: To delineate the physiologic protective role of GOT and its mechanisms of action in PVL. DESIGN/METHODS: Human premature brain autopsy specimens (23-28 weeks gestation) with PVL and age matched controls were stained for GOT-1. PVL neonate mouse model (HI) was established by temporary bilateral carotid ligation at P5 for 10min, followed by hypoxia exposure 8% for 20 min. Immunostaining for GOT1,neuronal (GAP 43, Doublecortin, NeuN), microglia (Iba1) and astrocyte marker(GFAP).To delineate the physiologic role of GOT-1, neuronal cell culture subjected to oxygen glucose deprivation challenge (OGD) with overexpression of GOT-1 using Lentivirus and suppression of GOT-1 using siRNA were studied. RESULTS: Our mice model of PVL exhibited hind limb paresis, in coordination and feeding problems. MRI and histopathological findings showed both lateral and 3rd ventriculomegally caused by white matter loss and neuronal apoptosis. GOT-1 was significantly over expressed in human premature autopsy brain tissue with PVL and mouse model of PVL as compared to controls. Immunostaining for both human and mice brain showed high colocalization of GOT expression to neurons. Interestingly glutamate receptors are also highly expressed on neurons. In our *in vitro* neuronal cell culture when using GOT Lentivirus overexpression there was a statistically significant increase in neuronal cell viability and a statistically significant decrease in Glu level in OGD as compared to non transfected cells. Using GOT siRNA trasfected neuronal cell culture, there was a statistically significant decrease in neuronal cell viability and statistically significant increase in Glu levels in OGD as compared to non transfected cells. CONCLUSIONS: Based on our *in vitro* findings, GOT may have a significant protective role in neonate mice model with PVL and human neonateswith PVL. As a compensatory mechanism to HI, GOT levels increase in an attempt to decrease serum glutamate levels. This increases the

glutamate gradient between blood and brain, thus decreasing brain glutamate. Recombinant GOT-1 or a small molecule that targets GOT-1 expression and activity could be a potential therapeutic approach for PVL.

Disclosures: **N. Zaghloul:** None. **H.L. patel:** None. **M.N. ahmed:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: Australian National Health and Medical Research Council

Title: Impact of hypoxia-ischaemia and dopamine treatment on Dopamine 2-like receptors in the preterm lamb brain

Authors: ***N. BREW**[1,2], S. INGELSE[2], U. RATNAYAKE[3], F. WONG[2,4,5];
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Abstract: Background: Hypoxic-ischaemic (HI) brain injury is a major concern for preterm infants. Dopamine is a neurotransmitter and inotrope commonly used to raise blood pressure in preterm infants. After HI in term babies reduced dopamine receptor density is associated with adverse outcomes. The effect of dopamine treatment on the immature brain and dopamine receptors remains unknown. Aim: Determine the effect of HI and dopamine treatment on Dopamine-2 receptors (D2R) in the preterm fetal lamb brain. Methods: D2-receptor density was measured with *in vitro* autoradiography using [3H] Spiperone. Treatment groups (99 days of gestation, term =147days) were (A) unoperated control fetuses (n=6), (B) fetuses exposed to a severe HI insult using umbilical cord occlusion and saline infusion (HI-saline, n=5) and (C) HI insult with dopamine infusion (HI-dopamine, 10µg/kg/min, n=4) for 74 hours. The caudate, putamen and globus pallidus were identified on cryostat cut sections using H&E and adjacent sections underwent autoradiography. Results: D2R density was decreased in HI-saline brains compared to controls in the caudate (53.3±10.4 vs. 98.2±13.3 fmole/mg), putamen (90.5±13.9 vs. 150.6±11.6 fmole/mg) and globus pallidus (38.1 ± 2.0 vs. 64.2 ± 14.0. fmole/mg, $p<0.05$). In HI-dopamine brains D2R density in the caudate and putamen (53.2 ± 2.2 and 101.7±6.6 fmole/mg,

respectively) was not different to HI-saline levels. In the globus pallidus of HI-dopamine brains D2R density was not different to controls (53.4±5.0 fmole/mg). Conclusions: HI decreases D2R density of in the basal ganglia of the fetal lamb brain. Dopamine treatment may protect D2R expression in the globus pallidus, and be potentially neuroprotective.

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Poster

145. Perinatal Brain Injury: Acute Therapy

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Topic: C.20.Perinatal Brain Injury

Support: Cerebral Palsy International Research Foundatoion R804-12

Little Giraffe Founation

Title: Neurodevelopmental effects of caffeine on neonatal neurons

Authors: *H.-L. LI[1], I. AHMED[2], T. ADAMS[1], U. ADEN[3];
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Abstract: Apnea of prematurity (AOP), defined as cessation of breathing for more than 20 seconds, is commonly seen in preterm infants. Caffeine is widely used to treat AOP for its safety and effectiveness. Caffeine releases the respiratory arrest by competing with adenosine for binding to A1 and A2 receptors. The plasma concentration of caffeine in preterm infants during AOP treatment is 10-100 times higher than that in infants receiving breast milk from mothers who drink moderate doses of coffee. Caffeine has long been known as a psychostimulant in adult brains. However, the effect of caffeine on developing brains, especially in the high concentrations used for AOP treatment, remains unclear. We first sought to ask 1) whether neonatal brains express A1 and A2 receptors and 2) how these receptors are modulated in response to prolonged caffeine treatment. Using immunostaining and confocal microscopy, we found A1 and A2 receptors are differentially expressed in rodent neonatal brains; A1 receptors are synaptically localized whereas A2 receptors are primarily enriched in soma. The estimated serum concentration of caffeine in preterm infants receiving caffeine therapy is about 25-103 uM. We tested the molecular effects of caffeine within this concentration range in primary

neuronal culture isolated from neonatal rodent brains. Using RT-PCR, we found the expression of A1 receptors was upregulated in neurons treated with 100 uM of caffeine in 5 days, whereas the upregulation of A2 receptor expression was observed using lower doses of caffeine (50 uM). We are currently investigating the underlying mechanisms of upregulation of the expression of A1 and A2 receptors and their consequences on the development of both normal and injured neonatal brains in rodents.

Disclosures: **H. Li:** None. **I. Ahmed:** None. **T. Adams:** None. **U. Aden:** None.

Poster

145. Perinatal Brain Injury: Acute Therapy

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: C.20.Perinatal Brain Injury

Support: Stem Cell Network

Three To Be Foundation

Brain Canada

CIHR Regenerative Medicine Studentship

Title: Metformin promotes sensorimotor recovery and activation of endogenous neural precursor cells following a hypoxic/ischemic insult

Authors: ***P. DADWAL**, N. MAHMUD, L. SINAI, A. AZIMI, M. FATT, F. D. MILLER, C. MORSHEAD;
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Abstract: A Hypoxic/Ischemic (H/I) insult to the immature brain is thought to play a role in the development of cerebral palsy pathology. Our goal is to design therapeutically relevant methodologies to enhance the activation of endogenous subependymal (SE)-derived neural precursor cells (NPCs, composed of stem and progenitor cells) to promote self-repair following a perinatal H/I insult. The widely prescribed drug, metformin (met), used to manage type II diabetes has been shown to promote neural differentiation by activating aPKC-CBP pathway. However, it is unknown if the enhanced differentiation is due to met acting on the neural stem cell and/or the downstream progeny. Using the neural stem cell (NSC) colony-forming assay, we first examined the effect of met in the absence of injury. Postnatal 8 (PND 8) SE-derived NPCs

exposed to met resulted in an 2-fold expansion in the size of the NSC pool, and a 3-fold and 2-fold increase in the numbers of neurons and oligodendrocytes, respectively, upon differentiation, *in vitro*. Next we examined the effects of met following H/I injury. PND 8 mice pups received a unilateral carotid artery ligation followed by 1 h hypoxia. Pups received met at 24 h post-injury via their lactating mothers and for one week (PND 9 – PND 15). To examine the impact of met and H/I injury on endogenous NPCs, a group of mice were sacrificed at PND 12. We observed a 2-fold increase in the size of the NSC pool in met-only pups and a 4-fold increase in H/I injured pups. A separate group of mice were sacrificed at PND 23 (1 week post-met treatment) and most striking, one week of met treatment was able to completely rescue sensorimotor impairments in the cylinder test. To understand the cellular basis for this recovery, we performed lineage-tracking experiments using *Nestin-CreER^{T2}/R26R-YFP* transgenic mice. At two weeks post-injury H/I + met treated pups had significantly more SE-derived YFP+ cells (ranging from 4 to 23 fold increases) in the striatum, motor cortex, corpus callosum, in both the ipsilateral and contralateral hemispheres (relative to the ischemic insult). We examined the fate of SE-derived YFP+ cells and observed increased number of YFP+ neurons and oligodendrocyte (striatum, motor cortex, corpus callosum) in H/I + met treat pups. Interestingly, we observed no difference in the relative percentage of YFP+ neurons or YFP+ oligodendrocytes between H/I and H/I + met treated pups suggesting that the observed recovery following met treatment was due to the increased absolute number of NPCs and not due to preferential differentiation. Hence, met activates endogenous NPCs and promotes endogenous repair of injured postnatal brain.

Disclosures: P. Dadwal: None. **N. Mahmud:** None. **L. Sinai:** None. **A. Azimi:** None. **M. Fatt:** None. **F.D. Miller:** None. **C. Morshead:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

Location: Hall A

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Program#/Poster#: 146.01/J29

Topic: D.02. Auditory System

Support: Andrews University Office of Research

Title: L3 auditory interneuron responses to dual-frequency calling songs in female crickets (Acheta domesticus)

Authors: *J. LEE[1], B. NAVIA[2];
[1]Behavioral Sci., [2]Biol., Andrews Univ., Berrien Springs, MI

Abstract: Female crickets exhibit phonotactic behavior, by selectively walking towards the source of calling songs with specific carrier frequencies (4-5 kHz), syllable periods (SP; 50-70 ms), and other temporal parameters.Such selective behavior is the result of complex processing by a number of neurons. Of the previously identified ascending prothoracic auditory neurons, only L3 has been proposed to be involved in SP selective phonotaxis. Previous tests have shown that females respond more selectivity to calling songs with intensities above L3's threshold than those above L1's threshold and below L3's threshold. Furthermore, L3 responds with selective decrement to 5 kHz calling songs with attractive syllable periods and such decrement has been correlated with selective phonotaxis. In addition, L3 is also tuned to 16 kHz auditory stimulus, which has been suggested to influence aversive phonotactic behaviors. Based on such studies, L3's cellular responses to simultaneous 16 kHz and 5 kHz calling songs will be examined through extracellular recording, thereby further establishing the connection between L3's selective decrement responses and selective phonotatic behavior. We hypothesized that in response to simultaneous 16 kHz and 5 kHz calling songs, L3 will show a decrease in selective decrement. Preliminary results from the current study showed a decrease in decrement pattern in responses to dual frequency auditory stimulus, thereby supporting the hypothesis.

Disclosures: **J. Lee:** A. Employment/Salary (full or part-time):; Andrews University. **B. Navia:** A. Employment/Salary (full or part-time):; Andrews Univerity.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 146.02/J30

Topic: D.02. Auditory System

Support: AU Faculty Research Grant

Title: The effect of varying sound intensities on phonotactic selectivity in Female

Authors: R. GREENE, *B. A. NAVIA;
Dept. of Biol., Andrews Univ., Berrien Springs, MI

Abstract: The intensity of the calling songs to which a female cricket Acheta domesticus is most likely to start responding phonotactically has been reported to be around 55dB at a carrier frequency of 4 - 5 kHz. These reports, however, have not shown selective phonotaxis to occur at such low intensities. Preliminary data from behavioral experiments have demonstrated that i) in response to calling songs with intensities above 75dB, females are more likely to exhibit

selective phonotaxis in response to a full sequence of calling songs with varying syllable periods (30 - 90 ms); ii) in response to calling songs with intensities below 75 dB the same females are less likely to respond selectively to identical stimuli. By testing all three intensities, 85dB, 75dB, and 60dB, at various syllable periods, we intend to find the threshold intensity at which a female cricket will exhibit selectivity in their phonotactic choices.

Disclosures: R. Greene: None. **B.A. Navia:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Topic: D.02. Auditory System

Support: NIH Grant DC010000

HHMI Undergraduate Fellowship

Title: Spike threshold adaptation enhances temporal fluctuation coding in the avian cochlear nucleus

Authors: *S. T. LUBEJKO[1], B. FONTAINE[3], K. M. MACLEOD[2];
[2]Dept. of Biology, Neurosci. and Cognitive Sci. Program, [1]Univ. of Maryland, College Park, MD; [3]Univ. of Leuven, Leuven, Belgium

Abstract: Depending on their intrinsic properties, single neurons operate in functional modes which range on a spectrum from coincidence detection (CD) to broad time scale integration. These properties determine a neuron's input-output functions and how information, particularly temporal information, is transformed or transmitted. In the auditory system, highly specialized CD neurons require rapidly fluctuating inputs to fire. However, we have recently shown that a subset of repetitively firing neurons in the avian cochlear nucleus are also sensitive to the timing of input fluctuations, responding with increased firing rates to larger fluctuations and more reliable firing (Kreeger et al. 2012). The intrinsic properties underlying this sensitivity is not clear, but may arise from the dynamics of spike initiation, specifically spike threshold adaption. We assessed the hypothesis that spike threshold adaptation is related to the operating modes of cochlear nucleus neurons by recording from avian cochlear nucleus angularis (NA), the brainstem area responsible for early sound intensity and spectrotemporal coding. To directly investigate intrinsic mechanisms, we made whole cell patch clamp recordings in brain stem

slices *in vitro* and used direct current injection into the repetitively firing NA neurons. The injected stimuli were white noise ('fluctuating') currents, simulating synaptic bombardment but with controlled mean level and variance. Two neuronal groups were determined: those that responded to increased stimulus variance with increased firing (coincidence detectors, CDs) and those that did not (integrators) as determined from their FI curves. The voltage at which the spike threshold occurred was measured for all the action potentials for each stimulus set. Spike threshold adaptation was widely observed, in that the mean voltage threshold increased with firing rate and mean stimulus level. The CD neurons, however, showed greater adaptation than the integrators, with greater changes in the median threshold and threshold variance. Threshold was inversely correlated with the rate of rise in the voltage and with the interspike interval. We applied a model of sodium channel inactivation to explain the threshold adaptation and whether differences in inactivation parameters could explain the differences between response types. This finding demonstrates that spike threshold adaptation could contribute to the increased activity of coincidence detector neurons in response to a fluctuating stimulus, and more generally contribute to the ability of neurons in NA to encode temporal envelope variations found in complex sound stimuli.

Disclosures: **S.T. Lubejko:** None. **B. Fontaine:** None. **K.M. MacLeod:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Topic: D.02. Auditory System

Support: NIH Grant R01 DC009810

NSF Grant IOS-0920081

Title: Frequency tuning in the songbird auditory cortex: topographical distribution and relation to species-typical vocalizations

Authors: *J. M. MOORE, S. M. N. WOOLLEY;
Psychology, Columbia Univ., New York, NY

Abstract: Tonotopy is a principle organizational feature of the vertebrate auditory system. Increasing evidence supports the hypothesis that avian and mammalian primary auditory cortices are homologous, but the extent to which their functional configurations are shared is unclear.

Primary auditory cortex in mammals is mapped tonotopically with isofrequency columns traversing layers. A similar layout in avian primary auditory cortex has not been shown definitively. Moreover, in both birds and mammals, it is unknown how well tone-evoked cortical responses can explain spiking patterns in response to more complex stimuli, such as spectrotemporally modulated synthetic sounds or vocalizations. Here, we measured the frequency tuning of single neurons throughout the auditory cortex of two songbird species with acoustically distinct songs, the zebra finch (Taeniopygia guttata) and long-tailed finch (Poephila acuticauda). We presented pure tone stimuli and recorded physiological responses extracellularly in awake birds. High resolution mapping of best frequencies in Field L subdivision L2, the thalamo-recipient region, showed a systematic tuning gradient from low-to-high frequencies along the caudodorsal-to-rostroventral axis and along the lateral-to-medial axis. Isofrequency columns extended from L2 dorsally into subdivision L1 and caudal mesopallium and ventrally into subdivisions L and L3. Units in the lateral extents of these regions were heterogeneously tuned and not organized spatially. We also compared topographical patterns of response properties such as best frequency, excitatory and inhibitory bandwidths, and spectrotemporal modulation tuning to song acoustics. Species differences in tuning generally correlated with their disparities in song; some differences existed throughout the cortex while others emerged in specific subdivisions. These results suggest a shared functional architecture in the primary auditory cortex of birds and mammals.

Disclosures: **J.M. Moore:** None. **S.M.N. Woolley:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Program#/Poster#: 146.05/J33

Topic: D.02. Auditory System

Support: NSF IOS #1257891

Title: Organization of auditory cortex in the Nine-banded Armadillo (Dasypus novemcinctus)

Authors: B. MOFFITT, B. SKINNER, *J. J. PADBERG;
Dept. of Biol., Univ. Central Arkansas, Conway, AR

Abstract: This comparative study is one of a series aimed at characterizing the organization of sensory systems in members of Xenarthra, an early superclade in the mammalian class. Here we explore the functional and anatomical characteristics of auditory cortex in the nine-banded

armadillo, (Dasypus novemcinctus), the only extant xenarthran species found in North America. Historically, this armadillo is well known for its armored carapace, its potential as a zoonotic reservoir of Mycobacterium leprae, and its behavioral reliance upon olfaction. Anecdotally, the armadillo has been described as having very sensitive hearing, however, its auditory sensitivity and the range of frequencies it can detect remain poorly described. An early study of evoked cochlear potentials suggests that armadillos are capable of detecting frequencies into the ultrasound range, but may have less sensitive auditory responsiveness than guinea pigs (Peterson and Heaton, 1968). While the general location of auditory cortex has been described using evoked potentials (Royce et al., 1975), the full extent of this field in this animal has not been explored using microelectrode recordings. To this end, we recorded multiunit responses in the auditory cortex of nine-banded armadillo to broadband auditory stimuli in order to determine the functional extent of the field, and later matched the locations of these recordings to series of sections stained for cytochrome oxidase (CO) histochemistry, myelin, or Nissl substance in order to identify the anatomical location. The primary auditory cortex stained intensely for myelin, and was notable for the presence of many large lattice-like features. Adjacent series stained for Nissl showed the inverse of these features, with clusters of cells located inside the myelin lattice. Based on both the electrophysiological responses and staining patterns, the extent of auditory cortex is nearly the entire caudolateral third of the armadillo brain. Experiments using a range of toneburst frequencies will clarify whether the tonotopy of xenarthran auditory cortex more closely resemble those of monotremes, marsupials, or euarchontoglires.

Disclosures: **B. Moffitt:** None. **B. Skinner:** None. **J.J. Padberg:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Topic: D.02. Auditory System

Support: NSF Grant 1355065

NSF IGERT Grant I1144399

Title: Acoustic features contributing to 1/f temporal modulation spectra of vocalization sequences and speech

Authors: *F. KHATAMI[1,2], M. WÖHR[4], H. L. READ[1,2], M. A. ESCABI[1,2,3];
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Connecticut, Storrs, CT; [4]Behavioral Neuroscience, Exptl. and Physiological Psychology, Philipps-University of Marburg, Marburg, Germany

Abstract: In vision, natural scenes exhibit 1/f power spectrum where visual edges are main contributors and cortical neurons are tuned to effectively extract edge related information (Field, 1987). Similarly, in audition, temporal modulations in vocalized sounds exhibit 1/f modulation power spectrum (Voss and Clark, 1975) and central auditory neurons can respond efficiently to such statistics (see discussions, Escabi et al., 2003; Rodriguez et al., 2010). Numerous factors contribute to temporal fluctuations in vocalized sounds including articulatory gestures, vocal tract filtering, and vocal fold vibration. Yet how such factors contribute to 1/f structure is unclear. Vocalized sounds contain a variety of temporal cues, including rhythmic fluctuations (< 20 Hz), onsets and offsets at the beginning and end of vocalizations (e.g., isolated words, species specific calls), and periodic structure such as from vocal fold vibrations (e.g., periodicity pitch) and these vary extensively over several orders of magnitude (from a few Hz for rhythmic information to ~800 Hz for pitch). It is plausible that 1/f structure arises from the combined contribution of such physical cues. We used vocalization sequences from a variety of animals, including nonhuman primates, mice, birds, infant cries, and speech to evaluate the role of vocalization transients, duration and amplitude variation in the sound temporal envelope. First, the modulation spectrum from isolated vocalization and vocalization sequences was compared. Synthetic vocalization sequences were generated based on statistics of the vocalization duration, timing, and amplitude variation of natural sequences and artificially perturbed to investigate contributing factors. In all instances, the presence of onsets and offsets at the beginning and end of isolated vocalizations predicted the observed 1/f modulation spectrum observed in vocalization sequences. Though timing, duration, and amplitude variation shaped the modulation power spectrum these only accounted for small amount of residual variation around the observed 1/f trend. The results demonstrate that acoustic “edges” are largely responsible for 1/f modulation structure found in vocalization sequences including speech. This is similar in principle to visual edges being main contributors to 1/f spectrum of visual images. Since central auditory neurons are particularly sensitive to temporal onsets and are adapted for 1/f structure, the findings imply that temporal edge detection is a major determinant for neural coding of vocalizations sequences.

Disclosures: **F. Khatami:** None. **M. Wöhr:** None. **H.L. Read:** None. **M.A. Escabi:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Topic: D.02. Auditory System

Support: Hong Kong Research Grants Council (HKU7837/11M)

Croucher Foundation

Lam Woo Foundation

Title: Auditory midbrain processing is differentially modulated by auditory and visual cortices

Authors: *P. P. GAO[1,2], J. W. ZHANG[1,2], S.-J. FAN[1,2], D. H. SANES[3], E. X. WU[1,2]; [1]Lab. of Biomed. Imaging and Signal Processing, [2]Dept. of Electrical and Electronic Engin., The Univ. of Hong Kong, Hong Kong, Hong Kong; [3]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The cortex contains extensive descending projections, yet the impact of cortical input on brainstem processing remains poorly understood. In the central auditory system, the auditory cortex contains direct and indirect pathways (via brainstem cholinergic cells) to nuclei of the auditory midbrain, called the inferior colliculus (IC). While these projections modulate auditory processing throughout the IC, single neuron recordings have samples from only a small fraction of cells during stimulation of the corticofugal pathway. Furthermore, assessments of cortical feedback have not been extended to sensory modalities other than audition. To address these issues, we devised blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) paradigms to measure the sound-evoked responses throughout the rat IC, and investigated the effects of bilateral ablation of either auditory or visual cortices (auditory cortex ablation: n=12; visual cortex ablation: n=12; normal control: n=12). Auditory cortex ablation increased the gain of IC responses to noise stimuli (primarily in the central nucleus of the IC), and decreased response selectivity to species-specific vocalizations (most prominently in the external cortex of the IC). In contrast, visual cortex ablation decreased the gain, and induced a much smaller effect on response selectivity. The results suggest that auditory cortical projections normally exert a large-scale and net suppressive influence on specific IC subnuclei, while visual cortical projections provide a facilitatory influence. Meanwhile auditory cortical projections enhance the midbrain response selectivity to species-specific vocalizations. We also probed the role of the indirect cholinergic projections in the auditory system in the descending modulation process by pharmacologically blocking muscarinic cholinergic receptors (normal animals, n=6). This manipulation did not affect the gain of IC responses, but significantly reduced the response selectivity to vocalizations. The results imply that auditory cortical gain modulation is mediated primarily through direct projections and they point to future investigations of the differential roles of the direct and indirect projections in corticofugal modulation. In summary, our imaging findings demonstrate the large-scale descending influences, from both the auditory and visual

cortices, on sound processing in different IC subdivisions. They can guide future studies on the coordinated activity across multiple regions of the auditory network, and its dysfunctions.

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Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Topic: D.02. Auditory System

Support: Action on Hearing Loss 567:UEI:JL

Action on Hearing Loss F44

Royal Society IE40709

Title: In search of the origins of a central auditory deficit in gap-in-noise sensitivity

Authors: ***J. MATTLEY**[1], L. A. ANDERSON[1], J. F. LINDEN[2];
[1]UCL Ear Inst., London, United Kingdom; [2]UCL Ear Inst. & UCL Dept. Neurosci., Physiol. & Pharmacol., London, United Kingdom

Abstract: BXSB/MpJ-Yaa mice are a powerful animal model in which to study the neural mechanisms of auditory temporal processing and gap-detection deficits. These mice have long been used as an animal model for developmental disorders thought to affect auditory temporal processing in humans (Ramus, TINS 2004), such as dyslexia, specific language impairment, and central auditory processing disorder. Approximately 30-50% of BXSB/MpJ-Yaa mice have localised disruptions of neocortical lamination (ectopias) which are considered a hallmark of thalamocortical developmental abnormalities, and which resemble those observed in humans with auditory processing and developmental language disorders. Intriguingly, although the ectopias occur in frontal cortex and not in auditory cortex, ectopic BXSB/MpJ-Yaa mice have greater difficulty than their non-ectopic littermates with behavioural tasks involving detection of brief gaps in noise (Clark et al., Neuroreport 2000). Previous work has shown that neural responses to brief gaps in noise are abnormally weak in the auditory thalamus of ectopic mice (Anderson and Linden, SFN 2012), due to a reduction in the proportion of auditory thalamic neurons with sound-offset responses (Linden and Anderson, SFN 2015). To determine whether these thalamic deficits in gap-in-noise and sound-offset sensitivity arise “bottom-up” from

abnormalities lower in the ascending auditory pathway, we recorded from neurons in the inferior colliculus (IC) in ectopic and non-ectopic BXSB/MpJ-Yaa mice. Extracellular recordings were obtained from urethane-anaesthetised mice during presentations of gap-in-noise stimuli, click trains, tones, and clicks following noise. All recordings were conducted blind to the ectopic status of the animal, which was determined from post-mortem histology. In experiments on 8 ectopic mice (341 recordings) and 7 non-ectopic mice (267 recordings), we found no significant differences in IC responses to brief gaps in noise or noise offsets, even though responses to the same stimuli are abnormal in the auditory thalamus of ectopic animals. In IC, the median "neural gap-detection threshold" (minimum gap duration evoking a significant change in firing rate) was 2.8ms for both ectopic and non-ectopic mice; in auditory thalamus, the median neural gap-detection thresholds were 3ms for non-ectopic mice and 5ms for ectopic mice. Our results indicate that thalamic deficits in gap-in-noise sensitivity in ectopic BXSB/MpJ-Yaa mice may arise above the level of IC, either in auditory thalamus or in auditory cortex.

Disclosures: **J. Mattley:** None. **L.A. Anderson:** None. **J.F. Linden:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Support: National Program on Key Basic Research Projects of China 2011CB933204

Title: Functional connectivity for spectrotemporal processing of neighboring neurons in inferior colliculus

Authors: ***L. SHEN**, Y. YAN, N. GUO, B. HONG;
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Abstract: Natural sounds are rich in spectrotemporal attributes and their processing is important for auditory perception. Inferior colliculus (IC) is sensitive to specific spectrotemporal features and is the first site emerging frequency sweep direction selectivity and envelope periodicity rate-tuning. However, it is still unclear how the neighboring neurons are functionally connected with each other and how this connectivity play roles in spectrotemporal processing. Here, we used tetrodes (inter-tetrode separation < 100 μm) to simultaneously record the responses of neighboring neurons in IC to dynamic moving ripple which had structurally rich time-varying spectrum. Spectrotemporal receptive field (STRF) was obtained using reverse correlation. We

analyzed the cross-covariance function between simultaneously recorded neuron pairs and only pairs with significant correlation with 10 ms delays ($p<0.01$) with respect to an independent Poisson assumption were analyzed further. We found that the activities of many neighboring neurons were temporally correlated. We separated the causal spikes from the activities of the source neuron and the target neuron to obtain causal spikes evoked STRF (causal STRF). We found that the neighboring neurons exhibited three types of functional connectivity: feature integrating, feature extracting and time-delay. In the first case, the target neuron integrated several simple spectrotemporal patterns to shape a complex feature selectivity and the causal STRF resembled the source STRF rather than the target STRF. The function role of this kind of connectivity approximated the "OR" operation. In the second case, only the spikes triggered by certain spectrotemporal features of the source neuron could drive the target neuron and the similarity between the causal STRF and the target STRF was higher. And the function role of this kind of connectivity approximated the "AND" operation. In the last case, the target neuron generally inherited the spectrotemporal properties of the source neuron except with various time-delay. Accordingly, the causal STRF was almost equally similar with the source STRF and the target STRF if the delay was wiped out. These results suggest that the spectrotemporal feature selectivity of IC neurons can be developed and refined via functional connectivity within local circuits.

Disclosures: **L. Shen:** None. **Y. Yan:** None. **N. Guo:** None. **B. Hong:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Topic: D.02. Auditory System

Support: NIH Grant R01 DC000189

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Title: Sound evoked afterdischarge in the auditory midbrain

Authors: ***M. ONO**[1,2], D. BISHOP[1], D. OLIVER[1];
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Abstract: Hearing is normally associated with neural activity caused by sound stimulation. We have discovered a previously unobserved phenomenon where excitatory and inhibitory neurons in the auditory midbrain exhibit a sound-evoked afterdischarge (SAD) that continues long after sound stimulation ceases. To distinguish GABAergic from glutamatergic neurons *in vivo*, we used VGAT-ChR2-EYFP mice in which inhibitory neurons specifically express Channelrhodopsin-2 in all parts of the neuron. When a light stimulus was delivered to the IC from the brain surface, it evoked spikes in GABAergic neurons, but it suppressed firing in glutamatergic neurons that lacked GAD67. To evoke SAD, we used long duration, 30 - 60 s, one-octave noise (60 dB) for 44 GABAergic neurons and 48 presumed glutamatergic neurons. We found that 20% of GABAergic and 17% of glutamatergic neurons continued to fire after the sound termination. The discharge after sound was stronger when the response during sound (RDS) was higher and the sound duration was longer. The minimum sound duration required to induce SAD was around 30 s. The RDS had to be sustained to evoke the SAD since a SAD was not seen when the RDS was transient. The number of spikes in the SAD and RDS responses were positively correlated (R = 0.51). SAD+ neurons had less adaptive firing during sound than SAD- neurons. In response to 30 s sound, both GABAergic and nonGABAergic SAD+ neurons showed more sustained firing during sound than SAD- neurons. Some SAD+ neurons had build-up firing which was not seen in SAD- neurons. The time course of SAD was variable. A peak firing rate occurred 1.0 - 50.1 s after the sound termination. There was no correlation between the peak times and the number of SAD spikes (R = 0.29), but the duration of the decay of the SAD was strongly correlated with the number of SAD spikes. The decays ranged from 0.4 - 235.6 s. SAD was also evoked by discontinuous sound (1 s narrowband noise bursts presented every 2 s, 50 repetitions). Interestingly, there was a gradual increase in interstimulus spikes not seen in neurons lacking SAD. These results suggested a form potentiation that might allow SAD neurons to overcome synaptic adaptation during long duration sounds. These results also suggested that SAD might be a substrate for an auditory afterimage or phantom sound.

Disclosures: **M. Ono:** None. **D. Bishop:** None. **D. Oliver:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Natural Sciences and Engineering Research Council of Canada (Discovery Grant)

Campbell McLaurin Chair for Hearing Deficiencies

Alberta Innovates—Health Solutions

Title: Thalamocortical forward suppression of field excitatory postsynaptic potentials in the auditory cortex

Authors: *C. XIONG, L. KONG, J. YAN;
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Abstract: Auditory forward masking is a common perceptual phenomenon of hearing and the neurophysiological observations of masking in the auditory cortex are that temporally adjacent sounds result in attenuation of neuronal cortical responses to the second sound. The thalamocortical system is the primary input to the auditory cortex, and thus is a likely source of this cortical forward suppression. Recent recordings from our group, with a novel two-stimulus paradigm in the mice mouse auditory cortex, have shown clear suppression of spike rates to the second thalamocortical input. We substituted one or both tones of the conventional two-tone suppression paradigm with electrical stimulation of the ventral medial geniculate body (ESMBGv). We found that tone stimulus strongly suppressed the ESMGBv-induced field excitatory postsynaptic potentials (fEPSPs) and vice versa in the auditory cortex. This cortical suppression occurred consistently at an inter-stimulus interval (ISI) threshold of 80-120 ms, which is notably shorter than ISI thresholds recorded in mice midbrain and auditory nerve fibers. One possibility is that suppression in the auditory cortex has unique cellular properties that induce greater suppression. It remains unclear what the exact mechanisms of cortical suppression are but it is evident that the thalamocortical system can induce suppression autonomously. Here we present recordings of cortical fEPSP showing the suppression of pure thalamocortical inputs. Only present during the suppression time interval was a post-excitatory group "hyperpolarization" effect. These data suggests that thalamocortical circuits have intrinsic mechanism(s) underlying suppression.

Disclosures: C. Xiong: None. **L. Kong:** None. **J. Yan:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

Location: Hall A

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Topic: D.02. Auditory System

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P30 DC08369

T32 DC010775

Title: Nicotine narrows receptive fields and increases gain in A1 via distinct actions at several subcortical auditory regions

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Abstract: Nicotine is thought to enhance sensory-cognitive function by way of increased attentional filtering and processing capacity. The neural basis of this effect is unknown. Previously, we have shown in rat and mouse primary auditory cortex (A1) that systemic nicotine increases responses to characteristic frequency (CF) stimuli and decreases responses to spectrally-distant (2 octaves) non-CF tones, thus appearing to increase response gain within a narrowed frequency receptive field (RF). However, the use of pure tones does not mimic situations when multiple frequency channels are activated simultaneously. Here we recorded local field potentials evoked by notched-noise stimuli (white noise stimuli filtered with a spectral notch centered on the CF of the recording site) in mouse A1, medial geniculate (MGv) and inferior colliculus (IC). We first found that varying notch width can be used effectively to measure frequency RFs. Systemic nicotine (0.7 mg/kg i.p., free base) produced two effects in A1: reduced response amplitude at wider notch widths and increased amplitude at smaller widths, indicating increased gain within narrowed RFs . Surprisingly, muscimol (100um) application in A1 prior to systemic nicotine resulted in qualitatively similar effects, implying a subcortical locus of action. In IC and MGv recordings, systemic nicotine reduced amplitudes at multiple notch widths, demonstrating that nicotine only narrows RFs in these regions. These results suggest that nicotine narrows RFs at various levels in the ascending auditory pathway and enhances gain at least partly due to enhancement in the thalamocortical pathway. Nicotine modulation of RFs plausibly can be the neural basis for nicotine's effects on sensory-cognitive function.

Disclosures: C.E. Askew: None. **I. Intskirveli:** None. **R. Metherate:** None.

Poster

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Action on Hearing Loss 549:UEI:JL

Title: Augmentation of A1 responses is reproduced by a recurrent circuit model combining strong excitation with synaptic depression

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Abstract: Cortical responses to dynamic stimuli display a temporal complexity reflecting the rich repertoire of recurrent cortical circuitry. For instance, primary auditory cortex (A1) responses to the second of a pair of tones are largely suppressed when the inter-tone interval is shorter than 500ms, even though most membrane currents are not sustained for that long (Wehr and Zador, Neuron 2005). This suppression may reflect thalamocortical or intracortical synaptic depression (Loebel et al., Front. Neurosci. 2007). However, in some cells the response to the second tone may be augmented, particularly at intermediate intervals around 300ms. Similarly, some cells in supragranular layers of A1 show augmented responses to the second and later stimuli within a regular train of noise bursts spaced by 300-400ms (Christianson et al., J. Neurosc. 2011) but not at lower or higher inter-burst intervals. In the same layers, an isolated noise burst leads to suppression of neural activity below spontaneous rates for about 300ms followed by a slight rebound above the spontaneous rate; this rebound was hypothesised to be related to the observed augmentation. How might this diversity of responses arise in the absence of long-lived membrane currents? We explored the hypothesis that suppression and augmentation both stem from intracortical synaptic depression. We characterized analytically the response of a network of excitatory and inhibitory neurons with depressing synapses between the excitatory cells, when stimulated by periodic drive of different frequencies, thus extending a study of Ledoux and Brunel (Front. Comp. Neurosci. 2011) on networks with static synapses. Although synaptic depression usually leads to suppressed responses, we found that networks with synaptic depression also exhibited a novel response regime in which low-frequency stimuli could be resonantly amplified. In this regime, with sufficiently strong excitatory recurrence, simulations of network activity showed a long-delay rebound following pulsed stimulation, in agreement with the A1 data. The same mechanism also led to an augmented response to a second input at intervals close to 300ms. This augmentation was driven entirely by network effects, without any mechanism for hyperpolarisation-induced membrane rebound or synaptic facilitation. In the augmentation parameter regime, a network with static and fully replenished

synapses would be unstable. Thus the supragranular layers of A1 might operate in a regime where transient sensitivity to sound onsets is maximised, while runaway activity is prevented by the short-term depression of intracortical synapses.

Disclosures: P.J. Goncalves: None. **J.F. Linden:** None. **M. Sahani:** None.

Poster

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NSF IGERT I1144399

Title: Distinct time-scales for neural discrimination of sound envelope shape in three auditory cortical fields

Authors: A. F. OSMAN[1], C. M. LEE[2], M. A. ESCABI[1,3,2], *H. L. READ[4,1];
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Abstract: Changes in the sound envelope amplitude over time provide perceptual shape cues to identify and discriminate sounds (Iverson and Krumhansl 1993; Irino and Patterson 1996, Drullman, Festen et al., 1994; Geffen et al., 2011). Mammals need cortex to detect many temporal sound cues; however, the underlying cortical circuits and neural coding mechanisms for this ability remain unknown. Sound shapes can be represented with a shape-specific spike rate increase in primary (A1) auditory cortex (Wang et al., 2008). In addition, auditory cortical neurons can represent sound shape with variations in spike-timing (i.e. jitter, Lee et al., 2014). Here we ask whether spike-timing patterns can be used to discriminate sound shape in primary (A1), ventral (VAF) and caudal suprarhinal (cSRAF) auditory fields of the rat. To probe cortical single neuron sensitivities to sound shape, we generated a stimulus set with 55 unique shaped envelope noise sequences. Single neuron response spike trains were recorded from layer 4 neurons in A1, VAF and cSRAF where the physiology and corresponding thalamocortical pathways have been well described (Polley et al., 2007, Storace et al., 2010, 2011, 2012, Higgins et al., 2010). The spike train analysis temporal resolution was varied by convolving spike trains with an exponential kernel having a time constant, tc (van Rossum, 2001). The spike train

distance between responses to different shapes was estimated using the sensitivity index (d-prime; Green and Swets, 1966) for comparing responses across pairs of sounds in our stimulus set (Gai and Carney, 2008). For each pair of spike trains, the slope of time constant, tc, is varied between 1 and 256 ms to determine the tc yielding the maximal discrimination value, i.e. the "best d-prime". In all fields, we find a rank order increase in the response duration with A1 < VAF < cSRAF to any given sound shape (Lee et al., 2015). Similarly, here we find a rank order increase in tc yielding best d-prime with: A1 < VAF < cSRAF (logarithmic means: A1: 33 ms (1.03), VAF: 39 ms (1.02), cSRAF: 44 ms (1.03), $p < 0.001$). A1 neurons discriminate small differences in sound envelope shape (e.g. 2Hz vs 4Hz). Whereas, VAF and SRAF outperform A1 for discrimination of large differences in sound envelope shape (e.g. 2Hz vs 64Hz). This data supports the notion that neural discrimination of shaped periodic sound sequences relies upon the temporal patterns of spiking. Furthermore, distinct response time-scales allow for complementary shape discrimination abilities in primary and non-primary auditory cortices.

Disclosures: **A.F. Osman:** None. **C.M. Lee:** None. **M.A. Escabi:** None. **H.L. Read:** None.

Poster

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HRI

Title: Contributions of synaptic inhibition to forward-masking in awake mouse auditory cortex

Authors: E. A. K. PHILLIPS[1], C. SCHREINER[2], *A. R. HASENSTAUB[3];
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Abstract: Sensory systems continually receive sequences of complex stimuli, and our ability to interpret particular components of such stimuli is dependent on the temporal relationships between many stimulus components. In line with this, neural responses are generally modulated by recent stimulus history. A simple and robust example of history dependence in auditory cortex (AC) is forward-masking, in which recent sound exposure suppresses responses to a

subsequent sound, depending on the spectral and temporal distance between the two sounds. Within AC, synaptic inhibition is thought to enhance forward-masking, but the relative contributions of inhibition versus other potential mechanisms (such as intrinsic adaptation or short-term synaptic depression) remain unclear. This question is complicated by the existence of multiple types of cortical inhibitory interneuron, with markedly different intrinsic and synaptic properties. For instance, while SST+ interneurons tend to receive facilitating synapses, activate GABA receptors with relatively slow kinetics, and have relatively slow intrinsic membrane properties, PV+ interneurons tend to receive depressing synapses, activate GABA receptors with relatively fast kinetics, and have relatively fast intrinsic membrane properties. This suggests that these two networks of interneurons are likely recruited by different temporal patterns of cortical activity and modulate history-dependence in distinct ways. In order to test how different populations of interneurons contribute to history dependence, we recorded from the AC of awake mice (since interneuron activity is differentially suppressed by anesthesia) in which either SST+ or PV+ interneurons expressed archaerhodopsin. We used a two-tone stimulus to elicit forward-masking, and randomly interleaved trials in which inhibition was optogenetically suppressed with trials in which inhibition was unaffected. We found qualitative differences in the tendency of SST+ and PV+ interneurons to modulate responses to masked vs unmasked and preferred vs non-preferred stimuli. Moreover, by comparing trials with equal firing rates to the first tone under the two conditions, we found that these modulation effects are independent of changes in firing to the first tone, counter to intrinsic adaptation models of forward-masking. Results will be discussed in the context of the hypothesis that history-dependent modulation of temporally complex stimuli is not just inherited from subcortical structures, but that it is enhanced within the cortex, and is selectively mediated by cortical interneurons that suppress activity forward in time.

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Poster

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Support: NIH R01DC012947

Title: Entrainment of neuronal oscillations by repetitive 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, NYU Sch. of Med., New York, NY

Abstract: Previous studies have shown that ongoing neuronal oscillations in auditory cortex can entrain to rhythmically-presented auditory stimuli. However, most stimuli used in these studies have been simple tones or noise bursts. Since our auditory environment is composed of a continuous flow of complex, multi-timescale information (e.g. speech/species specific communication) it is important to understand how the brain parses this information into chunks of fused elements to be further processed (e.g. interpreted or counted). The main purpose of our study was to expose a possible neural mechanism for the perceptual parsing and grouping of repeating complex random tone sequences amidst a continuous flow of auditory stimuli. Human psychophysical studies suggest that the recognition of a novel auditory pattern occurs automatically about 1.5 cycles into the pattern if repeated. We hypothesized that if low frequency neuronal activity in primary auditory cortex (A1) plays a role in parsing and grouping, it would entrain to these repeating patterns such that the low excitability phase would segregate each pattern by marking their beginning and end points. In line with human psychophysical findings, we anticipated the onset of this low excitability delta phase concentration to first occur at the end of the second pattern and reoccur after each iteration. To examine pattern-related oscillatory effects in A1, we used linear array multielectrodes to record laminar neuroelectric activity while subjects passively listened to an auditory stimulus stream consisting of pure tones and pure tones embedded in varying amounts of noise presented randomly at a 20 Hz rate. At random intervals we repeated the preceding 11 random sounds 5 times, resulting in the perception of 5 repeating complex auditory patterns. The pattern repetition rate was 1.7 Hz. As predicted, in our initial results we observed an increase in phase concentration at the rate of pattern repeat. However, the onset of the pattern-related effect appeared later than anticipated and the phase of entrainment at the "junction" of patterns was not always the low excitability phase. We interpret these results as an indication that activity in A1 can respond to and track complex auditory patterns even without these "gaps" marking the beginning and end of patterns. However, the observed pattern-related oscillations in A1 may be a consequence of - rather than a mechanism for - the segregation of auditory patterns. Current analyses are focusing on the implications of the laminar differences in the observed entrainment effect as well as variances in the effect timing across hierarchical nodes of thalamocortical information processing.

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Poster

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Title: Sustained spiking patterns discriminate sound envelope shape in ventral non-primary auditory cortices

Authors: *C. M. LEE[1], A. F. OSMAN[2], M. A. ESCABI[3,2], H. L. READ[1,2];
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Abstract: Mammals, including humans and rats, have multiple auditory cortical fields with segregated thalamic pathways but it remains unclear if these cortices support distinct temporal sound cue processing (Storace et al., 2010; Hackett 2010). In many species, onset responses and first spike latencies change with the sound envelope shape in A1 providing one potential means for discriminating this cue (Heil, 2004). In rat, spike-timing precision (jitter) changes with sound envelope shape in A1, ventral (VAF) and caudal suprarhinal (cSRAF) auditory cortical fields providing another potential means for discriminating this cue (Lee et al., SFN 2014). Here we separate onset and sustained responses and examine whether the latter can discriminate sound shape cues in A1, VAF and cSRAF. First we characterize onset and sustained responses to each sound shape by quantifying the peak latency, peak magnitude, and half-amplitude duration of the sound cycle peri-stimulus time histogram (cPSTH) response. We find a rank order increase in PSTH peak delay with: A1 < VAF < cSRAF (means (standard error), A1: -41 (4), VAF: -32 (3), cSRAF: -17 (3) ms, $p < 0.001$) and a rank order increase in duration with: A1 < VAF < cSRAF (logarithmic means and standard error, A1: 19 (1.01), VAF: 27 (1.02), cSRAF: 33 (1.05) ms, $p<0.001$). In addition, the PSTH peak amplitude is greater in A1 than VAF or cSRAF (means A1: 53 (0.5), VAF: 54 (0.4), cSRAF: 40 (0.3) Hz, $p<0.001$). These findings suggest that A1 neurons have primarily a brief robust “onset” response; whereas, VAF and cSRAF neurons have an additional sustained spike response that follows the sound envelop shape. We developed a neurometric discrimination index based on a spike distance (van Rossum, 2001) between spike trains to periodic noise sequences of different shapes. To investigate the role of onset and sustained activity in shape discrimination, onset spikes were removed from the response spike trains. The perturbed spike trains yielded better shape discriminability in rank order with: A1 < VAF < cSRAF as compared to A1. Together, these findings indicate that sound shape is primarily encoded during the onset of the sound in A1; whereas, in ventral fields, shape is also encoded by the sustained response. These distinct primary and non-primary cortical forms of

spike-timing response will have unique contributions to sound envelope shape coding at the next level of sound processing.

Disclosures: **C.M. Lee:** None. **A.F. Osman:** None. **M.A. Escabi:** None. **H.L. Read:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Topic: D.02. Auditory System

Title: Frequency selectivity of neurons in the ferret auditory cortex

Authors: *D. MERRY, T. WELLS, P. ADJAMIAN, A. R. PALMER, C. SUMNER;
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Abstract: Frequency selectivity describes the ability to resolve the individual frequency components in sound, and is a fundamental feature of hearing. It is crucial for most auditory tasks such as identifying sounds, separating auditory objects, and understanding speech. The ability of the auditory system as a whole can be measured through the behaviour of a subject, whereas the ability of neurons to be informative can be measured by observing their spiking activity when presented with an external stimulus. We examine the neural correlates of frequency selectivity using the power spectrum model. Although typically associated with behavioural measurements we instead utilised it to examine multi-unit neuronal response in the ferret's primary auditory cortex (A1). Neuronal responses to stimuli were recorded while the ferret was anaesthetized or awake, and results were compared to previous behavioural studies. In addition, these were compared to peripheral measurements of tuning in the auditory nerve (AN). Awake behaving neuronal tuning in A1 closely resembles behavioural results while the ferret undertakes a frequency specific sound localisation task. These are also broadly similar to prior results from anaesthetised recordings in AN, although AN bandwidths are more homogenous across units of similar characteristic frequency (CF). These would suggest that frequency selectivity is established at the periphery, is maintained to the level of A1, and this ability is reflected in perception. However, units recorded in the ferret A1 under anaesthesia had better frequency selectivity, or sharper tuning, compared with units recorded under awake behaving conditions, and also behaviour and AN. This would imply that the ability to resolve frequency improves along the auditory system, but is then combined using sub-optimal coding to produce a perceptually poorer representation. Further study would be required to understand the factors behind these divergent results.

Disclosures: **D. Merry:** None. **T. Wells:** None. **P. Adjamian:** None. **A.R. Palmer:** None. **C. Sumner:** None.

Poster

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AHL S72_Bajo

Title: Optogenetic silencing of the ferret primary auditory cortex in a behaviorally- and physiologically-relevant model of lesion-induced tinnitus

Authors: J. R. GOLD, F. R. NODAL, A. J. KING, *V. M. BAJO-LORENZANA;
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Abstract: Tinnitus is the subjective perception of a sound in the absence of an environmental source. A key step to facilitate clinical progress is the development of animal models that capture relevant features of the condition in humans - in particular, the links between the patient's percept and the complex network of physiological changes correlated with the condition. Our aims were first to develop a model of trauma-induced tinnitus in the ferret, a species well known for its utility in auditory research, and to interrogate the animal model using longitudinal behavioral and physiological approaches. Second, we wished to investigate a possible causal role for the primary auditory cortex (A1) in tinnitus by optogenetically silencing A1 activity during gap-in-noise behavior (Gold et al., 2015, Behav. Neurosci.) in a mixed group of tinnitus (T) and non-tinnitus (NT) ferrets. In a cohort of ferrets (N=10), a partial, unilateral mechanical lesion of the spiral ganglion (SG) was performed that replicated certain aspects of the peripheral pathology noted in human tinnitus patients. Prior to and following this lesion, animals were tested on an auditory gap-in-noise detection task, to examine whether temporal processing was affected in a manner consistent with the presence of tinnitus. Mixed lesion-mediated effects were seen across the cohort, with a subset of animals showing reductions in psychometric gap detection threshold, slope and asymptote. Within the same (T & NT) cohort, pre- and post-lesion auditory brainstem responses (ABRs) were obtained using clicks, narrowband chirps and masked click stimuli. The data indicated complex lesion-mediated changes, with latency increases and shifts in amplitude-level functions, particularly in late-wave ABR components. Multi-unit

recordings made bilaterally in A1 of a subset of animals showed tonotopic remapping, neural hyperactivity and hypersynchronicity following SG lesion. In a smaller mixed (T & NT) ferret cohort (N=6), the light-sensitive proton pump archaerhodopsin T (ArchT) was expressed in A1 neurons by bilateral viral vector injections (AAV8-CAG-ArchT-GFP), allowing suppression of neural excitability during behavior with 532 λ green laser light via fiber optic implants. Laser illumination modified gap detection behavior differentially according to side of illumination (bilateral, or ipsi/contralesional), acoustic stimulus type, and each ferret's tinnitus status. Our initial results suggest a possible approach to alleviating tinnitus through manipulation of mal-adaptively affected neural circuitry.

Disclosures: J.R. Gold: None. **F.R. Nodal:** None. **A.J. King:** None. **V.M. Bajo-Lorenzana:** None.

Poster

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Support: NIH Grant R01 DC03180

Title: Single-unit responses in lateral belt auditory cortex of the behaving marmoset monkey

Authors: *D. GAMBLE, X. WANG;
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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 data have subdivided core and belt into multiple tonotopic subfields (Kaas & Hackett 2000, Petkov et al. 2006). While previous studies have shown that lateral belt neurons prefer band-pass noise stimuli over pure tones (Rauschecker et al. 1995, Rauschecker and Tian 2004), knowledge of the functional properties of the lateral belt region is still limited. We have studied single neuron responses in the lateral belt region of marmosets under both passive and behaving conditions. The effects of arousal on firing rates were quantified by comparing responses in both conditions. Responses in the passive condition were further split into an 'eyes open' and 'eyes closed' state on the basis of the monitoring via an eye-tracker video camera. Analysis revealed that while arousal could modulate firing rates either up or down,

the eyes-open passive state was usually intermediate between the behaving and eyes-closed passive states. Consistent with previous findings, many lateral belt neurons displayed preferences for restricted spectral bandwidth. However, by using a two-noise stimulation paradigm, we revealed diverse nonlinear interactions extending outside of the single-noise receptive field, suggesting extensive spectral integration in the lateral belt region. We also found that most neurons preferred temporally modulated stimuli, with heterogeneous preferences for amplitude or frequency modulation.

Disclosures: D. Gamble: None. **X. Wang:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Title: Harmonic processing in primary auditory cortex of awake marmoset revealed by intracellular recordings

Authors: *L. GAO, X. WANG;
BME, The Johns Hopkins Univ., Baltimore, MD

Abstract: Numerous natural and man-made sounds, such as species-specific animal vocalizations, human speech and sounds from many musical instruments, contain rich harmonic structures. However, little is known about how our brain processes harmonic sounds. The peripheral auditory system functions as overlapping band-pass filters which segregate complex sounds into narrow frequency channels, whereas the central auditory system functions to reassemble these channels into a coherent representation for perception. Extracellular recording studies in our lab have shown that the primary auditory cortex (A1) of the common marmoset (callithirx jacchus), a highly vocal New World primate species, contains harmonic template neurons which encode spectral and temporal features of harmonic sounds. However, cellular mechanisms for encoding harmonic sounds are still largely unknown. In the current work, we applied a novel intracellular recording technique that we have developed for the awake marmoset to study the subthreshold properties of A1 neurons in response to harmonic complex tones (HCTs). We found that a subgroup of A1 neurons exhibited harmonically related subthreshold response peaks in their frequency tuning profiles. These subthreshold responses showed similar

or sometimes more harmonic peaks as compared to spiking responses. Moreover, the neurons that exhibited preference to HCTs contained the same harmonic peaks at both spiking and subthreshold levels. Many A1 neurons have a single peak in their pure tone frequency tuning. Some of them exhibited preference to HCTs containing the best frequency. Our results indicated that in addition to the harmonic template neurons, many A1 neurons appear to encode some features of harmonic complex tones at both spiking and subthreshold levels.

Disclosures: L. Gao: None. **X. Wang:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Title: Oscillatory mechanisms for the segregation and integration of auditory inputs

Authors: *B. ZOEFEL[1], M. N. O'CONNELL[2], A. BARCZAK[2], R. VANRULLEN[1], P. LAKATOS[2,3];
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Abstract: An auditory scene in everyday life rarely consists of only one source of input. Rather, multiple stimulus streams are mixed and thus, the segregation of simultaneous streams of input is an important task for the auditory system and essential for survival. Another important task that has to be simultaneously performed by the auditory system is the integration of acoustic stimuli into meaningful units that the brain can “understand”. While we have mounting evidence that segregation is performed using neuronal oscillations that track streams in the attentional focus, the neuronal mechanisms underlying integration and parsing of information remain mostly unclear. Recently, it was hypothesized that neural oscillations might be used by the auditory

system to also parse its input (e.g., Giraud and Poeppel, 2012): One oscillatory cycle might act as a “window of integration”, and stimuli falling within this cycle might be perceived as being part of the same stream (i.e. integrated). Vice versa, stimuli falling in different cycles of the oscillation might be perceived as being part of different streams (i.e. separated). Thus, the phase of neural oscillations might determine whether stimuli of two simultaneous auditory streams are perceived as one (integrated) or two (segregated) streams. We investigated this hypothesis by presenting monkeys with tone triplets (ABA paradigm) that can be heard as either a single stream or two separated streams, depending on the frequency separation between A and B tones. The monkeys listened passively to the tone sequences while current source density (CSD) profiles and single/multiunit activity were recorded in primary auditory cortex (A1) and auditory thalamus. In line with our expectation, we observed a phase shift of neural oscillations in A1 when the assumed perception changed from segregation (for large frequency separations between A and B tones) to integration (for small frequency separations), thereby shifting the low excitability phase that is hypothesized to enable parsing. Moreover, neural phases were more consistent for assumedly integrated than for segregated tone triplets, indicating the presence of one integrated stream in the auditory environment rather than two for the segregated percept. These effects were largest in the supragranular layers of A1, in line with previous reports of strongest adaption to auditory stimulation in those layers. Our results indicate that neuronal oscillations are used to facilitate multiple simultaneous operations for the meaningful interpretation of the auditory environment: segregation, parsing and integration.

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Poster

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Title: Isolating neural indices of continuous speech processing at the phonetic level

Authors: *G. M. DI LIBERTO, E. C. LALOR;
Trinity Ctr. for Bioengineering, Trinity Col., Dublin, Ireland

Abstract: That cortical sensory systems are organized in a hierarchical fashion is reasonably well established. While much of the work on this topic has focused on the visual system, increasing effort has been made in recent years to investigate hierarchical organization of the auditory system. In the context of human speech it has been suggested that such an organization could explain how acoustically variable inputs can be perceived as categorical speech units. A method for indexing the neurophysiology of such hierarchical processing in the context of continuous speech has recently been introduced. Specifically, the relationship between continuous speech and low-frequency EEG responses has been shown to be best described when that speech is represented using both its low-level spectrotemporal information and also the categorical labeling of its phonetic features (Di Liberto et al., submitted). Here we outline an experiment aimed disentangling low-level neural activity from that relating to phonetic feature processing. The intelligibility of 10 s speech stimuli was degraded using noise vocoding. Each stimulus was then presented twice with an intervening presentation of the original clean speech version of that stimulus. As such, the second presentation of the vocoded version of the stimulus was primed by the clean speech and was found to be significantly more intelligible on a match-to-sample task. We attempted to predict the EEG responses to the primed and non-primed vocoded stimuli using different representations of the vocoded speech stimulus. When the vocoded speech was represented as its envelope or spectrogram there was little difference in EEG prediction accuracy as a function of priming. However, when the speech was represented as a sequence of time-aligned phonemes or phonetic features, the EEG was significantly more accurately predicted for the primed stimulus than the non-primed. This difference in prediction accuracy represents a dependent measure of speech processing at the phonetic level using non-invasive, low frequency EEG.

Disclosures: **G.M. Di Liberto:** None. **E.C. Lalor:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 146.24/K4

Topic: D.02. Auditory System

Support: DFG, AN 861/4-1

Title: Effects of sequential comparison on the lateralization of processing of different fundamental acoustic parameters

Authors: *N. ANGENSTEIN, A. BRECHMANN;
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Abstract: The perception of complex acoustic stimuli requires the evaluation of basic acoustic parameters like frequency, duration and intensity. Usually, these parameters are not judged in an absolute manner but in relation to preceding sound segments e.g. the decision as to whether a given tone is soft or loud is made relative to a reference. As acoustic information of sound sequences unfolds over time, the evaluation of these sequences requires the storage of information of individual segments until the judgment is finished and it requires a sequential update of this information. Sequential processing is suggested to mainly involve the left hemisphere. With functional magnetic resonance imaging (fMRI) we investigated the effects of sequential comparison on the lateralization of processing in the human auditory cortex (AC) for direction of frequency modulations (FM), intensity and duration. For that we used a method that reveals differential hemispheric contribution to the processing based on an increase in activity by presenting additional noise contralateral to monaurally presented task-relevant stimuli. The categorization of FM tones according to their FM direction strongly involves the right AC. However, the pairwise sequential comparison of this parameter led to an additional involvement of the left AC. For intensity, left-lateralized processing in the AC was observed irrespective of the task (without or with sequential comparison) and irrespective of the stimulus type (tones with and without FM). The categorization of tones according to their duration stronger involved the right AC for FM tones and stronger involved the left AC for tones without FM. For both intensity and duration, additional sequential comparison of tones according to these parameters led to a stronger involvement of auditory areas and a network of brain areas outside the AC in contrast to the categorization of tones according to these parameters. Analogous to the results for FM direction, the stronger activity for comparison of duration compared to categorization was lateralized to the left AC. Together with previous studies on sequential comparison, the results suggests that the left AC is additionally involved when fundamental acoustic parameters have to be sequentially compared. The stronger involvement of the right AC during comparison in contrast to categorization probably has capacity reasons. During comparison areas outside the AC are probably stronger involved because it requires sequential update of information in memory.

Disclosures: **N. Angenstein:** None. **A. Brechmann:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 146.25/K5

Topic: D.02. Auditory System

Title: Effects of speaking rhythm naturalness on the neural basis of speech perception

Authors: *S. HIROYA[1], K. JASMIN[2], S. EVANS[2], S. KRISHNAN[2], M. OSTAREK[2], D. BOEBINGER[2], S. K. SCOTT[2];
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Abstract: Speech communication is important for our daily life. We normally understand spoken sentences in native language without effort. However, neural mechanisms for the perception of speech sounds remain unclear for more than half a century. Recent brain imaging studies of speech revealed a common cortical activation during speech production and perception. This suggests that mirror neuron system may play an important role in speech perception. Manipulation techniques of speech sound naturalness will give us a new possibility to investigate the neural correlates of speech perception. Sinewave speech and noise-vocoded speech based on reducing the frequency information have often been used. However, few studies have investigated the neural mechanisms of temporal information underlying speech perception, i.e., speaking rhythm. English is a stress-timed language which is different from a mora-timed language of Japanese. Thus, Japanese English speaking rhythm is unnatural for native English speakers. We first developed a novel method for decomposing speech signals into a speaking rhythm and phonetic information. For Japanese English speech, replacing speaking rhythm of a native Japanese speaker with that of a native English speaker would improve Japanese English speech naturalness. 16 channel noise-vocoded speech was used for controlling the intelligibility of speech. Seven native English speakers evaluated the naturalness of speaking rhythm. Results showed that naturalness was significantly improved for native English speaking rhythm. Next we performed fMRI scans during passive listening that investigated the neural basis of speech perception. Six types of stimuli such as Japanese rhythm, English rhythm, same phonetic duration, and their spectrally rotated speech were used. We scanned eleven healthy, right-handed English speakers. Result showed that left-lateralized premotor cortex and supplementary motor area (SMA) were more activated for Japanese rhythm than for English rhythm, and the areas overlapped that of vowel production. Moreover, left premotor cortex activation was shown for human articulation-related naturalness and left SMA activation was shown for native language-related naturalness. A series of our studies suggests that greater premotor cortex activation during speech perception would explain speech sound unnaturalness of both frequency and temporal information.

Disclosures: **S. Hiroya:** None. **K. Jasmin:** None. **S. Evans:** None. **S. Krishnan:** None. **M. Ostarek:** None. **D. Boebinger:** None. **S.K. Scott:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Topic: D.02. Auditory System

Support: NIH 2R01DC05660

Title: The cortical representation of sounds with speech-like modulation rates tested with multi-dimensional scaling

Authors: *X. TENG[1], D. POEPPEL[2];
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Abstract: Neural oscillations in auditory cortex can entrain to sounds over a wide range of modulations, although there appear to be preferential rates. Two such discrete regimes have been established and investigated, a low-frequency range (< 8 Hz) and a high-frequency range (> ~ 30 Hz). These regimes arguably reflect specialized temporal coding at two clearly distinct timescales in the auditory system. While cortical oscillations do not appear to be robustly entrained by sounds with intermediate modulation rates (8 – 30 Hz), such sounds are perceivable and still give people a clear sense of temporal structure. One particular question presents itself : how does the auditory system track and code the sounds with mid-range modulation rates? To address this question, we use a match-to-sample task in the context of MEG recording. Participants listen to sounds at five different modulation rates: 4-7 Hz (‘theta’ sound), 8-12 Hz (‘alpha’ sound), 13-20 Hz (‘beta1‘ sound), 21-30 Hz (‘beta2’ sound), and 31-45 Hz (‘gamma’ sound). While undergoing MEG recording, participants executed a 3-IFC match to sample study with modulation rate as the critical variable. We applied a multi-dimensional scaling analysis to the behavioral data – and further elucidate the MDS analysis with the MEG data. We observe that the representation of these different sounds is captured nonlinearly in a two-dimensional space, instead of by a one-dimensional space encoding modulation rate alone. In particular, we observe groupings of theta and gamma, on the one hand, and alpha, beta1, and beta2, on the other, suggesting that there are different coding strategies for the sounds of different modulation rates. MEG analyses of phase locking as well as classification analyses further illuminate the differential encoding. Keywords: Neural oscillations, asymmetric sampling in time, multi-scale, temporal processing, auditory perception

Disclosures: X. Teng: None. **D. Poeppel:** None.

Poster

146. Auditory Temporal, Frequency, and Spectral Processing: Neurophysiology

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Title: Musical experience and interaural correlation processing at various interaural delays

Authors: *M. WANG[1,2], R. NING[1], Y. YANG[1], L. KONG[3];
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Abstract: Interaural correlation processing is known to be based on the neural timing of the auditory system. Recently, it has been found that listeners with musical experience (musical group) usually have more precise or faster neural timing than those with no musical experience (control group). Here we show that participants in musical group behaviorally responded faster to a change in interaural correlation (IAC) than those in control group only at large interaural delays (4 ms and 6 ms). However, the response speed of musical group was the same as those of control group when the interaural delay is smaller (0 ms and 2 ms). We then recorded the cortical response to the interaural correlation change using event-related potentials. Consistent with our behavioral results, participants in the musical group had shorter P2 latency than those in the control group only when the interaural delay is large (4 ms). Thus, musical group showed an advantage over the control group for the processing of interaural correlation change with larger interaural delay. Given that interaural correlation processing at large interaural delay is closely related with speech perception accompanied by noise and reflections, listeners might benefit from their musical experience under noisy and reverberant environments.

Disclosures: M. Wang: None. **R. Ning:** None. **Y. Yang:** None. **L. Kong:** None.

Poster

147. Retinal Ganglion Cells: Classification and Responses

Location: Hall A

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Topic: D.04. Vision

Support: Rebecca Cooper Foundation

Title: The endocannabinoid system exerts a paradoxical effect on visually-evoked responses of mouse retinal ganglion cells

Authors: *D. A. PROTTI[1,2], I. DARWISH[1], J. HUANG[2,3], C. YATES[1];
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Abstract: The retina carries out complex and sophisticated neural computations that involve several distinct physiological mechanisms, including short and long-term plasticity phenomena similar to those described in other parts of the brain. The mechanisms responsible for modifying the strength of retinal synaptic transmission, however, are not fully understood. The endocannabinoid (eCB) system and all of its components (receptors, synthesising and degrading enzymes, and agonists) have been identified in the retina. We have previously found that the exogenous cannabinoid receptor agonist WIN55212-2 decreased the strength of synaptic transmission, whilst the eCB receptor antagonist AM251 enhanced synaptic transmission onto retinal ganglion cells (RGCs). Whether or not eCBs are tonically released in the retina and their potential physiological role, however, is still unknown. This study aimed to establish whether or not eCBs are released in physiological conditions in the retina and, if so, to unravel how they affect retinal processing of visual stimuli. We examined how the eCB system modulates the response strength of RGCs to visual stimulation and their receptive field organisation by doing whole-cell patch-clamp recordings from large ON- and OFF-RGCs in current-clamp mode. In addition, we assessed the effects of modulating the eCB system on RGC excitability by monitoring the activity of sodium channels in RGCs using voltage-clamp conditions. We recorded visual-evoked responses and voltage-gated sodium currents before and after bath application of either Anandamide (15 μM), an endocannabinoid, or URB597 (1 μM), an inhibitor of the enzyme that degrades Anandamide. Bath application of either Anandamide or URB597 reduced the peak amplitude of visual-evoked postsynaptic potentials and the spatial tuning of ON- and OFF-RGCs. Paradoxically, both drugs increased RGC peak spike count of visual-evoked responses. We also found that Anandamide and URB597 increased the occurrence of sodium currents for any given voltage-step and that the current voltage curve of the sodium current was shifted to the left. Finally, accommodation to high frequency visual stimulation was increased by both Anandamide and URB597, in line with the more classical modulatory role of eCBs. We conclude that eCBs are tonically released in the retina and they modulate retinal synaptic transmission, consistent with their function in other central nervous system areas. Their

net effect on retinal output, however, will depend on the balance of their effects at the circuit level and directly on RGCs sodium channels.

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Poster

147. Retinal Ganglion Cells: Classification and Responses

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Topic: D.04. Vision

Support: NINDS Intramural Research Program

Title: Ultraviolet and long-wavelength excitation combine with mid-wavelength inhibition to generate multiphasic spectral patterns in the ganglion cells of larval zebrafish retinas

Authors: *R. F. NELSON[1], V. P. CONNAUGHTON[2];
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Abstract: Larval zebrafish possess 4 cone types with opsin absorbances spanning the spectrum from red to ultraviolet wavelengths. The wavelength processing performed by larval ganglion cells (GCs) on this rich spectral substrate is not yet known. We measured light-evoked GC impulse discharges in the optic nerves of *in vitro*, superfused 5-6 dpf larval eyes. 'Loose-patch' recordings of GC spike discharges were obtained penetrating the optic nerve stump of eyes placed cornea side down in the recording chamber, while stimulating through the sclera with 9 wavelengths of light (330-650nm), each at 7 light intensities (0.5 ND interval). Peri-stimulus time histograms (PSTH) were qualitatively scored for spectral properties. A spectral model based on the integrated ON discharges interpolated spectral sensitivities and extracted the amplitudes of individual cone signals contributing to the spectral patterns. Receptive fields were mapped with 20μm slit stimuli. 89% of 36 characterized cells were spectrally multiphasic (color opponent) types. Spectrally triphasic GCs were the most common type (64%). These GCs were excited by long and short wavelengths and inhibited by mid wavelengths. Green, blue, or green and blue cones could be inhibitory, with the remaining cones being excitatory. Pentaphasic GC's were the next most common (11%). These cells were excited by UV, far red, and mid-spectral stimuli, and inhibited by blue and near red stimuli. Examples of biphasic and tetraphasic spectral patterns were also encountered. In all except the tetraphasic GC's, where UV was inhibitory, ultraviolet stimulation (330,370nm) evoked the greatest discharge of impulses, and the maximum spectral peak. Receptive field space constants of less than 10μm were observed. The cone-

dominated retina of larval zebrafish produces a GC output that is almost entirely color opponent. The common triphasic spectral pattern would appear to send a dominant signal of 'small' and 'not green' to the larval brain. This pattern, also seen in the H3 horizontal cells of adult zebrafish, likely represents the color-opponent output of UV cones. This suggests that UV cone pathways to GCs mature early in zebrafish development.

Disclosures: R.F. Nelson: None. **V.P. Connaughton:** None.

Poster

147. Retinal Ganglion Cells: Classification and Responses

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Topic: D.04. Vision

Support: PIP 0030 CONICET

NIH GM093072-01

NIH Vision Core Grant P30EY02687

Title: SIRT6 deficiency causes neurotransmission defects in the mouse retina

Authors: *D. M. SILBERMAN[1], K. ROSS[2], P. H. SANDE[1], S. KUBOTA[3], R. S. APTE[3], S. RAMASWAMY[2], R. MOSTOSLAVSKY[2];
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Abstract: The retina is one of the major energy consuming tissues within the body and its energetic source for normal functioning comes mainly from glucose metabolism. This necessity for glucose is evidenced by alteration in electroretinogram (ERG) responses and altered neurotransmitter release as described in ischemic and hypoglycemic conditions. Upon activation of photoreceptors, G-coupled glutamate receptors (mGluR) regulate transmitter release by ON bipolar cells ultimately converting visual stimulation to patterns of activity of retinal ganglion cells (RGCs) leading to visual perception in the visual cortex. The synaptic transmission is a highly energy demanding event and it has been shown that a decline in energy supply and a disruption in bioenergy homeostasis play a critical role in multiple neuropathological conditions. It is therefore of physiological significance to have synaptic activity coupled with glucose

homeostasis. SIRT6, a chromatin- bound enzyme member of a conserved family of NAD(+)-dependent deacylases, regulate various metabolic pathways and have emerged as an important sensor of energy status in mammals. SIRT6 has been shown to regulate glycolitic genes as the glucose transporter GLUT1 and has been described as a tumor suppressor factor and as a critical modulator of DNA repair. Given the importance of glucose availability for retina function, the critical role for SIRT6 in modulating glycolysis and the lack of information about this sirtuin in the retina, the goal of this study was to provide a functional characterization of SIRT6 in this tissue. We found that SIRT6 is expressed in the nuclei of cells in all retinal layers and is highly active, as shown by the significant increased levels of acetylation of it substrates, H3K9 and H3K56, in the SIRT6-KO retina compared to the WT. In SIRT6 deficient mice (strain 129/J), the structure of the retina remained unchanged and no significant differences in the thickness of the layers between WT and KO mice were observed. However, SIRT6 deficient retinas exhibited increased apoptosis in the inner layers determined by TUNEL, downregulation of the G0-coupled type 6 metabotropic glutamate receptor (Grm6) gene and upregulation of the glucose transporter GLUT1, indicating that both neurotransmission and glucose levels in the retina might be regulated by SIRT6. Functionally, ERG analysis showed that both scotopic and photopic a- and b-wave amplitudes were severely altered for all the light intensities analyzed in the retina of SIRT6-KO mice, indicating a unique role for SIRT6 as a modulator of retinal function.

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Poster

147. Retinal Ganglion Cells: Classification and Responses

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Program#/Poster#: 147.04/K11

Topic: D.04. Vision

Title: Single cell transcriptomics of defined retinal ganglion cell types

Authors: *L. LABOISSONNIERE[1], J. GOETZ[1], A. MANI[2], G. SCHWARTZ[2], J. TRIMARCHI[1];
[1]Iowa State Univ., Ames, IA; [2]Northwestern Univ., Chicago, IL

Abstract: Retinal ganglion cells (RGCs) comprise a small portion of the vertebrate retina, yet they are the sole communication between retina and brain. This population of neurons is composed of more than 25 different types, as defined by morphology, responsible for responding to different light stimuli and coordinating with visual reflex centers. Despite studies

characterizing the distinct morphologies of RGC subtypes, the transcriptomic profiles of many of these cells have remained elusive. Specifically, there has not been a definitive study that links morphological and physiological properties of ganglion cells to their transcriptome. Our project aims to uncover the genetic networks operating within subsets of retinal ganglion cells identified by different characteristics, including their physiology and morphology. Individual retinal ganglion cells were isolated from adult mouse retinas based upon morphology and physiology. After isolating the RNA and amplifying the cDNA for each individual cell, the resulting cDNA was hybridized to microarrays and analyzed. Gene clusters have been identified that correlate with specific morphologies/electrophysiological properties of ganglion cells. This method has also revealed transcriptomic variation between individual cells, which we have validated using a combination of *in situ* hybridizations and immunohistochemistry. The analysis of single cells allows for in-depth studies regarding the genetic profile responsible for a particular cell's function. Our studies have yielded multiple genetic networks corresponding to subsets of RGCs that we will utilize in the future to better understand the unique roles of these cells and how retinal ganglion cells are specified during development.

Disclosures: L. Laboissonniere: None. **J. Goetz:** None. **A. Mani:** None. **G. Schwartz:** None. **J. Trimarchi:** None.

Poster

147. Retinal Ganglion Cells: Classification and Responses

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Support: NIH grant EY016182

P30EY12576

Title: Delineating retinal ganglion cell subtypes in macaque monkey using large scale morphometric cluster analysis coupled with immunohistochemistry

Authors: D. VAN DER LIST[1], *K. D. MURRAY[2], W. M. USREY[3];
[1]Univ. of California, Davis, Davis, CA; [2]Psychiatry & Behavioral Sci. and Ctr. for Neurosci., Univ. California Davis, Davis, CA; [3]university of California, Davis, Davis, CA

Abstract: All visual information must pass to the central nervous system along sets of axons emanating from a diverse set of retinal ganglion cells (RGCs). In primates, where molecular

markers of RGC subtypes are less well defined, definitive categorization of RGCs has to rely on physiological, connectional and morphological properties. To date, no useful reference database exists that can be harnessed for classifying monkey RGC subtypes. To address this we conducted a large-scale survey of over 300 monostratified RGC's in the adult macaque monkey with the goal of identifying a morphological reference database of functional cell classes using a set of readily quantifiable morphological parameters coupled with antibody staining. All RGC's were filled by direct application of DiI crystals to wholemount retina followed by antibody labeling using several candidate RGC markers including HCN1, SMI-32, Kv1.2 and calbindin. Confocal imaging and Neurolucida software were used to make morphometric measurements of each labeled RGC. A hierarchical cluster analysis was performed using various morphometric features including soma area, dendritic field area and number of branches. In addition, for each cell, eccentricity was recorded at 1mm intervals from the fovea to peripheral edge and depth of stratification within the inner plexiform layer (IPL) was measured. A diverse set of cells were identified using this approach including midget, parasol, wide field, large and giant sparse, broad and narrow thorny cells. Unsupervised hierarchical clustering successfully segregated DiI labeled RGCs into functionally distinct groups that correlated with the known parasol marker, HCN1, along with positively labeled SMI-32 cells. Surprisingly, other purported markers of RGC's, Kv1.2 and calbindin did not correlate with distinct morphological subtypes of RGC suggesting they may label a broader range of RGC 's. These results illustrate that by using a defined set of minimal morphological parameters in combination with antibody staining, we are able to classify RGCs into functionally distinct subtypes in the macaque monkey. The resulting set of morphological features will be an important reference database for future studies identifying RGC subtypes in monkey.

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Poster

147. Retinal Ganglion Cells: Classification and Responses

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Program#/Poster#: 147.06/K13

Topic: D.04. Vision

Title: Classification and characterization of twenty functional subtypes of ganglion cells in the mouse retina

Authors: *A. MANI[1], G. W. SCHWARTZ[2];
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Abstract: Classification of neurons into distinct, functional, morphological, and genetic classes is essential in extending our understanding of the nervous system to the level of individual circuits. We have developed a novel strategy for classifying retinal ganglion cells (RGCs), which form the parallel outputs of the retina, carrying all visual information to the rest of the brain. Far from the textbook camera-like representation of the visual world, in recent years it has been found that intricate computational processing already occurs in the retina. Parallel information channels, carried by the different RGC types, each extract specific features from the visual scene, including contrast, motion, orientation and color. A necessary step in understanding the different computations done in the retina, and characterizing the circuits responsible for them, is classification of RGCs into their different types. Previous attempts to classify RGC types have relied on unique gene expression profiles of subsets of RGCs or on large-scale clustering of dendritic morphology or electrophysiological properties. The different classification schemes have yet to arrive at a definitive, unified typology, and less than half of the ~20 RGC types suggested by morphological studies have been found in functional classifications. Here we present a novel functional classification of RGCs in the mouse retina, using spiking response to a set of light stimuli. We combine clustering and principal component analysis techniques with an efficient method based on a decision tree to probe a large number of parameters of the light response. We have achieved reliable, online classification of 20 RGC types, and our algorithm can be used across labs to standardize RGC typology. Our classification includes all functional RGC types previously identified in mouse, as well as types previously identified only in other species, and types that were previously unknown in any mammalian retina.

Disclosures: A. Mani: None. **G.W. Schwartz:** None.

Poster

147. Retinal Ganglion Cells: Classification and Responses

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Title: Toward a complete functional classification of ganglion cells in the mammalian retina

Authors: *S. RAVI[1], D. AHN[2], M. GRESCHNER[2], A. M. LITKE[3], E. J. CHICHILNISKY[4], G. D. FIELD[1];
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Abstract: Retinal ganglion cells (RGCs) are the exclusive source of visual signals to the brain. Morphological studies suggest that 15-20 types tile the retina with their dendritic fields. This implies that each RGC type provides a uniform sampling of the visual scene. However, functional classifications have found 5 - 12 types, and in some cases RGCs that appear to lie along a functional continuum, in apparent contradiction to the numerous types found in anatomical studies. The purpose of this study is to design a robust classification paradigm that will establish the extent to which visual information is processed in distinct parallel channels. This paradigm will be standardized across animals, so that it can serve as a platform to be used in different labs, animal models, and transgenic lines. We also aim to determine whether looming detectors (LD) and/or orientation selective (OS) cells observed in previous studies sample visual space uniformly. A large-scale multi-electrode-array (MEA) was used to record the action potentials of RGCs in an isolated rat retina preparation. Several visual stimuli were used to drive RGC responses: spatiotemporal white noise, drifting sinusoidal gratings, and full field flashes. Recorded spikes were sorted offline to identify distinct RGCs. A quantitative classification procedure was designed using parameters extracted from responses to visual stimuli. The classification procedure was tested across preparations to ensure reproducibility. The receptive field properties of OS and LD cells were measured to determine their spatial arrangement. Individual recording sessions identified 400-500 RGCs over the MEA, from which we consistently classified 12 functional types, in addition to several others that were less reliably identified. The receptive fields of each type were arranged in a tiling pattern, or “mosaic”, indicating that these RGC types sample space uniformly and that each identified RGC type was not further divisible. Distinct functional channels of LD and horizontal and vertical OS cells in the retina appeared to sample space with a mosaic organization similar to other types of RGCs with center-surround receptive fields.

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Poster

147. Retinal Ganglion Cells: Classification and Responses

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Support: NIH Grant EY017137

Title: Genetic identification of a novel bistratified intrinsically photosensitive retinal ganglion cell

Authors: *D. J. BERG, S. SABBAH, D. BERSON;
Brown Univ., Providence, RI

Abstract: Intrinsically photosensitive retinal ganglion cells (ipRGCs) are rare mammalian photoreceptors that use the photopigment melanopsin. They are essential for such non-image-forming visual functions as circadian entrainment and the pupillary light reflex. ipRGCs comprise six subtypes distinguishable by morphology, physiology, and projections. We used transcriptional profiling (RNAseq) to identify dozens of genes that are much more highly expressed in murine ipRGCs than in conventional ganglion cells. One such gene, *Rbp4*, codes for a retinoid binding protein. It was strongly expressed in cells labeled in a mouse reporter for all known ipRGC subtypes (Ecker et al., 2010), but not in those labeled in another reporter that marks only the M1-M3 ipRGC subtypes. To determine which ipRGC subtype(s) expressed *Rbp4*, we obtained a transgenic Rbp4-Cre mouse (GENSAT) and fluorescently labeled Cre-expressing cells either by crossing with a Cre-reporter mouse or by intraocular injection of flexed viruses. Ganglion cells labeled in these studies appeared to belong to a single type ('Rbp4-RGCs') with intermediate dendritic field size (~165 um diameter) and a bistratified dendritic arbor. One arbor co-stratified with M1 ipRGCs at the outer limit of the inner plexiform layer (IPL), while the other stratified near the ON cholinergic (starburst) sublayer. We assessed functional properties using multiphoton microscopy and selective expression of the Ca^{2+} indicator GCaMP6f(Ai95 reporter) in Rbp4-cre mice. Rbp4-RGCs were strongly excited by increases in illumination either globally or restricted to the receptive-field center, but were strongly suppressed by drifting bars or gratings, suggesting very strong, motion-sensitive surround inhibition. A weak ON response persisted under synaptic blockade, suggesting that Rbp4-RGCs are intrinsically photosensitive. Rbp4-RGCs resemble M1, M3 and M6 ipRGCs in having a purely ON-type synaptically driven responses despite extensive arborization in the OFF sublayer; all such OFF-layer dendrites probably receive ectopic *en passant* contacts from the axons of ON cone bipolar cells. Rbp4-RGC axons innervate the dorsal and ventral lateral geniculate nuclei and superior colliculus, but

spare other major non-image-forming ipRGC targets such as the suprachiasmatic and olivary pretectal nuclei. These findings reveal the existence of a physiologically, morphologically, and molecularly distinct mouse ganglion-cell type that appears to be yet another variety of ipRGC. Its projection patterns suggest a role primarily in form vision rather than in non-image-forming functions such as circadian entrainment or pupillary light reflex.

Disclosures: D.J. Berg: None. **S. Sabbah:** None. **D. Berson:** None.

Poster

147. Retinal Ganglion Cells: Classification and Responses

Location: Hall A

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Topic: D.04. Vision

Support: Weinberg College of Arts and Sciences

Department of Neurobiology, Northwestern University

Title: Intrinsically photosensitive retinal ganglion cells are required for proper dopaminergic amacrine cell development

Authors: *T. MUNTEANU[1], S. PAN[2], P. KOFUJI[3], T. M. SCHMIDT[1];
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[3]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Retinal dopamine is known to play a crucial role in vision and is produced by the dopaminergic amacrine cells (DACs). DAC processes are found in the inner plexiform layer (IPL), where they release both dopamine and GABA. DACs have been found to co-stratify with the melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (ipRGCs). These two cell types interact in a variety of ways. ipRGCs drive light-evoked depolarizations in DACs in a retrograde manner. ipRGCs also respond to dopamine, and their stratification within the IPL closely follows that of DACs. In fact, when DAC stratification is disrupted through a knockout of the semaphorin receptor PlexinA4, ipRGCs co-localize with the aberrant DAC processes. Since ipRGCs depend on DACs for proper development, we examined whether DACs also depend on ipRGCs for proper development. To test this we utilized immunohistochemistry for tyrosine hydroxylase, a marker of DACs, and then counted DACs in a variety of mouse models where ipRGCs are manipulated. When ipRGCs are ablated early in development, we see a reduction in the number of DACs. Surprisingly, this effect is absent in mice lacking melanopsin,

indicating that light signaling through ipRGCs is not likely to play a role in DAC development. Collectively, these results indicate that ipRGCs are required for proper DAC development, possibly through physical contact between these two cell types during early postnatal stages.

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Poster

147. Retinal Ganglion Cells: Classification and Responses

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Title: Optogenetic responses to low-intensity light in dystrophic retinas revealed by blockade of pathological hyperactivity

Authors: ***J. M. BARRETT**, G. HILGEN, E. SERNAGOR;
Inst. of Neurosci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Retinitis pigmentosa (RP) is a hereditary progressive retinal dystrophy that causes visual impairment and eventually blindness. Presently, electrical prostheses are able to restore crude vision, but newer approaches based on optogenetics - genetic expression of light-sensitive proteins in neurons - may potentially offer higher-quality vision. However, alongside photoreceptor loss, retinal degeneration also involves significant inner retinal remodelling that leads to spontaneous, rhythmic bursting activity in retinal ganglion cells (RGCs). Recently, both our lab (Barrett et al, SfN 2014) and others (Toychiev et al, 2013) have shown that reducing spontaneous activity in dystrophic retinas improves the signal-to-noise ratio of RGC responses to photoreceptor, electrical and optogenetic stimulation. We sought to extend these results by investigating whether this approach can improve the contrast sensitivity and spatial acuity of optogenetic responses. We crossed a transgenic mouse line expressing the light-sensitive cation channel channelrhodopsin2 (ChR2) in Thy1-expressing RGCs with the rd1 model of retinal degeneration, creating a strain of mice with retinal degeneration and ChR2-expressing RGCs (ChR2rd1 mice). We recorded RGC activity from ex-vivo ChR2rd1 retinas using a large, high density, 4096-channel, Active Pixel Sensor CMOS multielectrode array (APS CMOS MEA; Berdondini et al 2009). Light stimuli were delivered with a DLP projector, providing 664x664 pixels of patterned light stimulation over a retinal area of 2.67x2.67mm^2 with 4μm resolution and sub-millisecond temporal precision. We were able to evoke ChR2-mediated responses from

hundreds of RGCs per experiment using RGB light with an intensity of around 20μW/mm^2. We presented a variety of stimuli - e.g. gratings of varying phase, spatial frequency and contrast; moving bars; Sloan letters - and used Bayesian classification to decode stimulus properties from the RGC population response (Jacobs et al, 2009). Blocking spontaneous hyperactivity using 40μM meclofenamic acid (MFA) improved the decoder performance in all conditions. Our results show that the principle of blocking spontaneous hyperactivity in the rd1 retina to improve optogenetically-evoked responses extends to a variety of stimulus classes and this enables lower luminance, lower contrast and higher spatial frequency stimuli to be correctly discriminated. This suggests that this technique might improve the quality and usefulness of vision returned by retinal prosthetics.

Disclosures: **J.M. Barrett:** None. **G. Hilgen:** None. **E. Sernagor:** None.

Poster

147. Retinal Ganglion Cells: Classification and Responses

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Topic: D.04. Vision

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Title: Oscillating ON-direction selective ganglion cells induce oscillating eye movements in Nyx^{nob} mice

Authors: ***M. KAMERMANS**[1], K. M. FRANSEN[2], W. DE GRAAFF[4], J. HUISMAN[4], Ö. ÖZYILDIRIM[5], B. BRAAKMAN[5], M. A. MCCALL[3], C. I. DE ZEEUW[5,6], B. H. J. WINKELMAN[5,6];
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Abstract: Retinal motion detection is elemental to image stabilizing eye movement reflexes. In Nyx^{nob} mice a mutation in the X-chromosomal gene encoding for nyctalopin causes defective synaptic input of retinal ON-bipolar cells, leading to congenital stationary night blindness (CSNB) and malfunction of the ON-pathway. Loss of ON-bipolar cell function specifically affects ON-direction selective ganglion cells (ON-dsGCs), which underlie the optokinetic reflex. Analysis of the eye movement response to optokinetic stimulation using sine-wave gratings

showed substantial impairment of the optokinetic reflex and defective gaze holding. Furthermore, eye movements of Nyx^{nob} mice exhibited a prominent horizontal oscillation with an average frequency of 5 Hz. To check whether this pathological eye movement behavior could be caused by abnormal anatomy, we crossed Nyx^{nob} mice with SPIG1-GFP knock-in mice, which show GFP-labeling in a subtype of ON-dsGCs sensitive to downward retinal image movement (Yonehara et al., 2008). Analysis of SPIG1-GFP expression combined with anterograde labelling of ganglion cells with CTB-Alexa 555 showed no apparent miswiring of the central projections of ON-dsGCs to the MTN of the accessory optic system. Electrophysiological recordings in the retina confirmed that ON-dsGCs of Nyx^{nob} mice are unresponsive to light stimulation. Moreover, the membrane potential of ganglion cells showed robust sub-threshold oscillations with a frequency of 5 Hz on average. These oscillations were eliminated or significantly reduced by simultaneously blocking both AMPA and NMDA, using application of DNQX and D-AP5. Intravitreal injection of this cocktail in both eyes of Nyx^{nob} mice also abolished the 5 Hz oscillation in spontaneous eye movements, recorded while the animal was awake, which strongly suggests that synchronous oscillation of dsGCs in the retina is the direct cause of the horizontal eye movement oscillation. This mechanism might also explain the small amplitude horizontal nystagmus observed in CSNB patients (Simonsz et al., 2009).

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Poster

147. Retinal Ganglion Cells: Classification and Responses

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Title: Directional tuning of excitatory synaptic inputs to direction selective retinal ganglion cells revealed by optical quantal analysis

Authors: *V. JAIN, G. B. AWATRAMANI;
Biol., Univ. of Victoria, Victoria, BC, Canada

Abstract: Introduction: Whether excitatory bipolar cell inputs to direction selective ganglion cells (DSGCs) are directionally tuned is a matter of current debate. Patch-clamp analysis of light

evoked currents indicates that they are directionally tuned, implying that 'null' inhibition (known to be mediated by starburst amacrine cells) acts presynaptically at bipolar cell terminals. However, optical imaging of presynaptic bipolar cell activity using Ca2+ and glutamate sensors indicate that inputs to DSGCs are not directionally tuned implying a postsynaptic locus for null inhibition. To resolve this issue, we performed an optical quantal analysis using 2-photon calcium imaging techniques. Methods: We identified DSGCs in the mouse retina based on their directional spiking responses measured extracellularly. Subsequently, DSGCs were loaded with a Fluo-5F through a whole-cell patch-pipette. Changes in Ca2+ in individual dendritic compartments of DSGCs were measured using 2-photon laser scanning microscopy. Results: To estimate presynaptic glutamate release we measured Ca2+ responses mediated by synaptically activated NMDA receptors. In response to moving spots, spatially isolated "Ca2+ hot-spots" (FWHM ~1μm) were observed along the dendrite. These responses were abolished when a NMDA receptor antagonist (50 μM D-AP5) was added to the bath indicating that they represent activity at single glutamatergic synapses between the bipolar terminals and DSGCs. Interestingly, in about a third of synaptic sites responses were strongly directionally tuned. The tuning of the dendritic Ca2+ response matched that of the spiking response. Moreover, DS tuning at single synaptic sites was abolished when GABA receptor antagonists were applied. Conclusions: Optical imaging of synaptic activity unequivocally reveals directional tuning of the inputs to DSGCs. Thus, null inhibition mediated by SACs must act pre-and postsynaptically to shape DS in the retina.

Disclosures: V. Jain: None. **G.B. Awatramani:** None.

Poster

147. Retinal Ganglion Cells: Classification and Responses

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Title: Visual information encoded by the wave of first stimulus-evoked spikes revealed in the retinal ganglion cell layer by large-scale multielectrode array recordings

Authors: *E. SERNAGOR[1], J. M. BARRETT[2], G. PORTELLI[4], G. HILGEN[3], T. MASQUELIER[5], P. KORNPROBST[4];

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Abstract: How a population of retinal ganglion cells (RGCs) encodes the visual scene remains an open question. Going beyond individual RGC coding strategies, a previous study in salamander suggested that the concerted spiking of a RGC pair encodes spatial information (Gollisch and Meister, 2008). Gollisch and Meister suggested that a population code based on such concerted spiking could be a powerful mechanism to transmit visual scenes. Here, we tested this hypothesis in the mammalian retina by recording simultaneous light-evoked responses from hundreds of mouse RGCs using a new generation of large-scale, high density multielectrode array (MEA), the Active Pixel Sensor CMOS MEA consisting of 4096 electrodes covering an active area of 7.12 mm^2. We stimulated the retina using stationary gratings (spatial frequency 0.009 to 0.075 cycles per degree) presented at 8 different phases. In contrast to the previous findings in salamander, we did not find any RGC pair exhibiting a clear latency tuning to the phase of the grating, demonstrating that in mouse, individual RGC pairs may not provide sufficient information to encode visual information (this may be partially due to strong background spontaneous activity in the mammalian retina). However, when we considered the concerted spatio-temporal spiking pattern of a large RGC population at pan-retinal level rather than just in cell pairs, we found that the wave of first stimulus-evoked spikes (WFS) provides an accurate indication of stimulus content. Moreover, we found that it outperforms classical spike count- and latency-based codes by being more efficient and faster. Overall, these novel observations suggest that already at the level of the retina, the WFS provides a reliable and fast strategy to rapidly transmit new visual scenes.

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Poster

147. Retinal Ganglion Cells: Classification and Responses

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Title: Genetically identified Suppressed-by-Contrast retinal ganglion cells in mice reliably signal self-generated visual stimuli

Authors: *N.-W. TIEN[1], J. PEARSON[1], C. HELLER[2], J. DEMAS[2,3], D. KERSCHENSTEINER[1];
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Abstract: Spike trains of retinal ganglion cells (RGCs) are the sole source of visual information to the brain; and understanding how the approximately 20 RGC types in mammalian retinas respond to diverse visual features and events is fundamental to understanding vision. Suppressed-by-Contrast RGCs (SbC-RGCs) stand apart from all other RGC types in that they reduce rather than increase firing rates in response to light increments (ON) and decrements (OFF). Here, we genetically identify and morphologically characterize SbC-RGCs in mice, and target them for patch clamp recordings under 2photon guidance. We find that strong ON inhibition (glycine > GABA) outweighs weak ON excitation, and that inhibition (glycine > GABA) coincides with decreases in excitation at light OFF. These input patterns explain the suppressive spike responses of SbC-RGCs, which are observed in dim and bright light conditions. SbC-RGC responses lack spatial antagonism and inhibition is driven by rectified receptive field subunits, leading us to hypothesize that SbC-RGCs could signal pattern-independent global changes in the retinal image. Indeed, we find that shifts of random textures matching saccade-like eye movements in mice elicit robust inhibitory inputs and suppress spiking of SbC-RGCs over a wide range of texture contrasts and spatial frequencies. Similarly, stimuli based on kinematic analyses of mouse blinking consistently suppress SbC-RGC responses. Receiver operating characteristics show that SbC-RGCs are reliable indicators of self-generated visual stimuli that may contribute to central processing of blinks and saccades.

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Poster

147. Retinal Ganglion Cells: Classification and Responses

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Title: Light adaptation changes the functional properties of direction selective retinal ganglion cells

Authors: *X. YAO[1], G. D. FIELD[2];
[2]Neurobio., [1]Duke Univ., Durham, NC

Abstract: Light adaptation enables vision to operate across an extremely wide range of light conditions. Direction selective retinal ganglion cells (DS-RGCs) respond to motion of objects moving in a preferred direction. ON-OFF DS-RGCs are thought to work at local motion detectors whereas ON DS-RGCs are thought to mediate motion-related behaviors like the optokinetic reflex and object tracking. Yet we know little about whether and how light adaptation alters DS-RGCs response properties. Here we use a large-scale multi-electrode array to record *ex vivo* and simultaneously from large population of DS-RGCs across light levels. Their response properties were probed by using a battery of visual stimuli including drifting gratings, moving bars, full-field light steps, and checkerboard white noise. We observed that direction tuning of superior preferring ON-OFF DS-RGCs was weaker and broader at lower light levels, while other subtypes exhibited more stable tuning. The speed tuning of all DS-RGC types was narrower as at lower light levels, while the peak location remained constant. Furthermore, ON-OFF DS-RGCs changed their response polarity to pure ON at rod-mediated light levels. These findings indicate that direction computations in the retina change across light levels, which likely constrains how rod signals converge onto DS RGCs and is relevant for how the downstream circuitry processes these signals from the retina.

Disclosures: **X. Yao:** A. Employment/Salary (full or part-time):; Duke University. **G.D. Field:** A. Employment/Salary (full or part-time):; Duke University.

Poster

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Title: Spike time patterns in retinal ganglion cell responses convey information about stimulus shape

Authors: *S. V. GIRMAN[1,2], S. WANG[1];
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Abstract: Traditionally it was thought that the modulations of mean firing rate of retinal ganglion cell (RGC) encode all information about the stimulus and that the response magnitude is the only variable which contains stimulus-relevant information. Much of our understanding of how the RGCs encode the visual world is based on studies analyzing the responses solely in terms of mean firing rate. In this study we addressed the question whether all information the RGC spike trains carry can be assessed with the rate-based coding scheme. The simplest way to answer the question is to analyze the iso-rate responses triggered by different stimuli: the responses carry identical information in the mean rate; therefore, if they can be differentiated in any way regarding the stimuli eliciting them, then the answer to the question posed above is "no". Following this approach, we recorded spike activity of RGCs in rat *in vivo* presenting a set of stimuli of different spatial configurations (shapes), while their contrast and temporal attributes were the same. The spatial parameters of the stimuli were adjusted to achieve that, in a given experiment, the stimuli elicited equal responses in terms of mean spike rate. To compare pairwise the PSTH profiles of the responses, we obtained the differential PSTH (dPSTH) by bin-wise subtraction between the two PSTHs. The more the PSTH profiles differ, the more prominent are the time intervals in the dPSTH where contiguous bins contain values of the same sign, either positive or negative, and, correspondingly, the more prominent are the low-frequency components in the Fourier spectrum of the dPSTH. Thus, we suggested quantifying the PSTHs pattern dissimilarity as a ratio of the mean amplitude of the first five harmonics of the dPSTH Fourier transform to that of the middle-spectrum harmonics. This index of pattern dissimilarity (IPD) is invariant to firing rates in the responses; therefore, IPDs obtained for individual cells can be pooled together in order to characterize how dissimilar the PSTH profiles are in the overall set of responses of all recorded RGCs. We found that the distribution of IPD values obtained for response pairs to stimuli of equal strength but having different shapes differed, with high statistical significance, from IPD distribution for the responses to stimuli of the same shape, while magnitudes of the responses under comparison were equal. We concluded that spike temporal patterns of iso-rate responses can be significantly different depending on stimulus shape. Therefore, response temporal patterns of RGCs carry information about stimulus shapes, in addition to information regarding the stimulus strength encoded in the mean spike rate.

Disclosures: **S.V. Girman:** None. **S. Wang:** None.

Poster

147. Retinal Ganglion Cells: Classification and Responses

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Title: Position-dependent characteristics of retinal ganglion cell mosaics across the entire retina

Authors: ***F. FRANKE**[1], M. FISCELLA[1], R. DIGGELMANN[1], A. DRINNENBERG[2], B. ROSKA[2], A. HIERLEMANN[1];
[1]ETH Zürich, Basel, Switzerland; [2]Friedrich-Miescher-Institute, Basel, Switzerland

Abstract: The retina encodes the visual scene by means of roughly 20 parallel channels, each one represented by a specific retinal ganglion cell (RGC) type. The receptive fields of all RGCs of a certain type tile the complete visual scene with small overlap in a mosaic-like fashion. It is thought that each RGC represents the local information about a certain feature in the visual stimulus and that at each retinal position is covered by at least one cell of each type. For some cell types, the visual features they encode seem to be relatively well characterized: direction-selective ON-OFF (DS) cells, e.g., encode the presence of local movement in a certain direction. However, in the retina of many mammalians, the characteristics of individual cells of a specific type, for example their receptive field size (and thus their spatial density), change with retinal position and retinal eccentricity: The further away from the optic nerve, the larger the receptive fields. Why are receptive field characteristics position dependent? One obvious reason is the optical transfer function (OTF) of the eye: The further away from the intersection of the optical axis of the eye and the retina, the larger a point light source will appear on the retina. Thus, the maximal optical resolution of the retina is limited and position dependent. The position dependence of RGC properties is not well characterized and it is not known to which extent it reflects physical properties of the incoming light. To address this question we recorded from a large number of DS RGCs in different regions of the isolated hamster retina by means of high-

density microelectrode arrays (HD-MEA) with 30k electrodes and 1000 read-out channels while the retina was visually stimulated with moving bars. The electrodes of the array record the spiking activities of essentially all RGCs on top of the array and each RGC is recorded from by up to 50 different electrodes. To extract individual RGC spiking activity from the recorded signal mixture, individual spikes were detected by thresholding, and then fed into an automatic spike sorting algorithm. The resulting spiking activity of each cell was used to determine its direction selectivity index, which allowed us to identify the DS cells, as well as preferred direction, peak firing rate, receptive field size, and tuning function width. We then related these characteristics to a simple model of the OTF of the eye. We show that the RGC characteristics are indeed dependent on retinal position, which partially can be explained by the OTF of the eye.

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Poster

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Title: Circuit-based description of retinal ganglion cell computation explains the spike response at millisecond resolution

Authors: *D. A. BUTTS[1], Y. CUI[1], Y. V. WANG[2], J. B. DEMB[2];
[1]Dept. of Biol., Univ. of Maryland, College Park, MD; [2]Ophthalmology and Visual Sci., Yale Univ., New Haven, CT

Abstract: Visual processing depends on computations performed by complex neural circuits, but it is often unclear how these circuits shape processing due to relatively impoverished descriptions of visual computation. In the retina, for example, there is extensive knowledge of the feedforward circuit components underlying retinal output, but model-based descriptions are typically based on linear or mathematically abstract components. Here, we derive a novel description of ganglion cell computation based on retinal circuitry, and constrained by voltage-

clamp and extracellular recordings from the same neuron. We recorded from ON Alpha ganglion cells in the mouse retina, *in vitro*, using a temporal noise modulation (0 - 30 Hz) of a spot (1-mm diam., photopic level), centered on the ganglion cell's receptive field. First, a cell-attached recording measured spikes and then a whole-cell recording measured excitatory currents. We first demonstrate that nonlinear responses of ganglion cells are in part derived from nonlinearities present in the excitatory synaptic input. By fitting a novel nonlinear model for this current, and then extending it to explain the spike train, we offer a nearly perfect prediction of spike response at millisecond resolution. The model could also explain adaptation to stimulus contrast. The model critically depends on divisive suppression that is consistent with presynaptic inhibition of amacrine cells onto bipolar cell terminals, and its interaction with spike refractoriness. Our approach demonstrates a detailed circuit-based model of ganglion cell spike generation, and suggests novel computational components contributing to visual processing by retinal circuitry.

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Poster

147. Retinal Ganglion Cells: Classification and Responses

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Title: The influence of dopamine on the temporal resolution of retinal neurons

Authors: ***M. L. RISNER**, D. G. MCMAHON;
Biol. Sci., Vanderbilt Univ., Nashville, TN

Abstract: Purpose: Dopamine is a key neuromodulator in the retina that reconfigures retinal circuitry based on the prevailing light level. To investigate how dopamine modulates vision our lab has previously generated a mouse model in which dopamine is depleted in the retina by genetically excising the rate limiting enzyme, tyrosine hydroxylase, required for producing dopamine (rTHKO). The rTHKO mouse exhibits deficits in contrast sensitivity measured both behaviorally and in acute retinal ganglion cell (RGC) recordings. The deficits we have observed

in contrast sensitivity of rTHKO are similar to those observed in Parkinson's disease patients. Parkinson's disease patients not only exhibit decreased contrast sensitivity, they also show reduced temporal resolution. The purpose of this study was to investigate temporal resolution of retinal neurons in rTHKO and control (C57Bl/6J) animals. **Methods:** We assessed temporal resolution of the electroretinogram (ERG) b-wave in whole animals and RGCs using *in vitro* multi-electrode array (MEA) recordings. To date we have assessed temporal resolution in 8 control animals and 4 rTHKO using the ERG technique and 2 control and 2 rTHKO animals using the MEA recording method. In both ERG and MEA recordings temporal resolution was probed using a square-wave broad-spectrum white light that varied from 0 to 30 cd/m^2 in luminance and 1 to 40 Hz in temporal frequency. To measure temporal resolution of the ERG b-wave and spike output of RGCs we computed the spectral density power of the first harmonic (F1). **Results:** The F1 of the ERG b-wave was not significantly different between genotypes across temporal frequencies. However, there was a significant difference in the F1 across temporal frequencies between genotypes in ON- and OFF-center RGCs. rTHKO OFF-center RGCs exhibited a decrease in the F1 across most temporal frequencies (1-34 Hz). rTHKO ON-center RGCs showed a decrease in the F1 at low and higher temporal frequencies (1, 15-40 Hz). **Discussion:** The divergent results between electrophysiological methods measuring the ERG b-wave and RGC spike output suggests temporal resolution is decreased by knocking out dopamine but only for RGCs. These results suggest that dopamine effects the response integration time of RGCs either through receptors native to RGCs or through synapses between bipolar cells and RGCs.

Disclosures: M.L. Risner: None. **D.G. McMahon:** None.

Poster

148. Visual Thalamus

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Title: Naturalistic visual stimuli modulate neural oscillations in the rodent ventral lateral geniculate nucleus of the thalamus

Authors: *U. M. CIFTCIOGLU[1], K. R. DING[1], V. SURESH[1], A. S. GORIN[2], F. T. SOMMER[3], J. A. HIRSCH[1];

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Abstract: There are two main routes from the eye to the brain in mammals. The pathway from the retina to the superior colliculus (SC) is directly involved with sensorimotor tasks and the pathway from the retina to the lateral geniculate nucleus of the thalamus (LGN) is associated with form vision. However the LGN is not a homogeneous structure and comprises a dorsal and a ventral subnucleus, the dLGN and the vLGN respectively. While the dLGN projects directly to the neocortex, the evolutionarily older vLGN connects with subcortical structures that coordinate movement, the SC, for example. The dLGN, which has been studied in depth, is much larger than its ventral counterpart, whose small size creates a challenge for electrophysiological studies *in vivo*. In mice, however, the two subnuclei are roughly the same size and are thus equally accessible. We have taken advantage of this species difference to explore the structure and function of the vLGN in the whole animal, using techniques of whole-cell recording with dye-filled electrodes *in vivo* in addition to computational analyses. Recently, we compared the two divisions of the murine LGN and found that neurons in the vLGN had receptive fields several times larger than those of relay cells in the dLGN, along with broader dendritic arbors. These findings were in keeping with the idea that the vLGN does not play a dominant role in form vision. Our next step was to compare neural coding in the vLGN with that in sensorimotor structures it connects to, using the literature about the SC as a starting point. In particular, experiments in both awake (Brecht et al., 2004) and anesthetized animals (Stitt et al., 2013; Sridharan et al., 2011) show that neurons in the SC encode visual stimuli in two ways, as a conventional rate code and also by means of gamma oscillations. We began our analysis using full field stimuli of different luminance values. Approximately a third of the cells in the vLGN responded both with an increase in firing rate and the initiation of gamma oscillations. The strength and frequency of the oscillations remained fairly constant once the threshold luminance value was reached. To determine if these oscillations might play a role in natural vision, we recorded responses to movies and found that gamma oscillations occured during some image sequences. The strength of the oscillations decreased with increasing stimulus contrast. These oscillations might help coordinate activity between the vLGN and subcortical visual motor networks in the execution of motor tasks.

Disclosures: U.M. Ciftcioglu: None. **K.R. Ding:** None. **V. Suresh:** None. **A.S. Gorin:** None. **F.T. Sommer:** None. **J.A. Hirsch:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.02/K28

Topic: D.04. Vision

Support: Science and Technology Development Fund (STDF) Grant 5168

Title: Optimizing electrical stimulation parameters in a lateral geniculate nucleus (LGN) model

Authors: *S. ELDAWLATLY, A. JAWWAD;
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Abstract: Restoring vision to the blind is no longer impossible thanks to recent advances in neural interfaces. Successful demonstrations of retinal implants motivate the development of more sophisticated and effective visual prostheses. However, when initial visual processing sites such as the retina or the optic nerve are completely damaged, the thalamic Lateral Geniculate Nucleus (LGN) makes the next logical interfacing site for visual prostheses. One of the main challenges in developing thalamic visual prostheses as well as other visual prostheses is optimizing the parameters of electrical stimulation. We propose a Kalman-based optimal encoder whose function is to determine the optimal electrical microstimulation parameters required to induce a certain visual sensation. In this approach, two Kalman filters are first trained: the first one relates LGN neuronal responses to visual stimuli while the second filter relates LGN neuronal responses to electrical stimuli. For a given visual stimulus, we use the first filter to predict the corresponding responses of the underlying population. The predicted responses are then decoded using the second filter to determine the electrical stimulus needed to elicit the responses predicted by the first filter. We demonstrate the performance of the proposed approach in analyzing synaptically-coupled LGN neuronal population using a probabilistic point process model. In this model, spiking of LGN neurons is modeled using a Generalized Non-linear Model (GNM) with stimulus-driven suppression which has been demonstrated to better fit LGN neuronal responses compared to other models (Butts et al., 2011, J. Neurosci., 31:31). We modeled a population of ON-center and OFF-center cells in which each ON-center cell has a corresponding OFF-center cell covering the same receptive field. Corresponding ON-center and OFF-center cells were synaptically-coupled through inhibitory interneurons. Visual stimuli driving the model were generated using a Gaussian distribution with zero mean presented at 30 Hz. We examined the performance of the proposed optimal encoder for different electrical stimulation parameters (quantization levels, stimulation duration, stimulation frequency and number of stimulation electrodes). The results demonstrate that we can achieve a significant similarity between LGN neuronal responses obtained using electrical stimulation and the responses obtained using the corresponding visual stimuli with a mean correlation of 0.61 ($P < 0.01$, $n = 54$). These results indicate the efficacy of the proposed optimal encoder in driving LGN neurons to induce visual sensations.

Disclosures: **S. Eldawlatly:** None. **A. Jawwad:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.03/K29

Topic: D.04. Vision

Support: NIH Grant EY012716

Title: Early maturation of trn gabaergic feedback inhibition to dlgn

Authors: *P. W. CAMPBELL[1], T. A. SEABROOK[2], G. GOVINDAIAH[2], W. GUIDO[2];
[2]Anatom. Sci. and Neurobio., [1]Univ. of Louisville, Louisville, KY

Abstract: The thalamic reticular nucleus (TRN) is a key modulator of thalamocortical activity. GABAergic neurons of TRN provide the main source of inhibitory feedback to relay cells in primary sensory thalamic nuclei such as the dorsal lateral geniculate nucleus (dLGN). Despite their role in gating retinogeniculate signal transmission little is known about how and when these inputs innervate the developing dLGN. To address this we used a GAD65 transgenic mouse in which GABAergic projections to dLGN can be visualized with GFP. We found that reticulothalamic fibers begin to innervate dLGN at early postnatal ages (P2-4). During the first postnatal week there is a rapid increase in innervation so that by P7-9 about 85% of dLGN is occupied by a dense plexus of TRN GABAergic fibers that are distributed throughout the nucleus. We also found that retinal signaling regulated the timing of TRN innervation. In transgenic mice lacking retinofugal projections (*math5 -/-*, Wang et al., 2001), reticulothalamic innervation was highly accelerated showing a two-fold increase in the rate of innervation at early ages. To assess whether these early projections form functional connections with dLGN cells, we conducted *in vitro* whole cell recordings in acutely prepared thalamic slices in transgenic mice that express channelrhodopsin in somatostatin (SST)-containing neurons of TRN (SST-Cre x ChR2). Blue light stimulation of SST containing fibers in dLGN evoked postsynaptic inhibitory activity in dLGN relay cells. Weak inhibitory responses were present as early as P2, a time when TRN terminals first arrive in dLGN. These responses matured rapidly over the first three postnatal weeks, growing in strength 50-fold, showing reliable responses to even high rates of stimulation (50 Hz) and displaying strong paired-pulse depression. By contrast, feed-forward inhibition, evoked by the electrical activation of optic tract and intrinsic interneurons, emerged at much later ages (P9-10). Thus in dLGN, feedback inhibition from TRN develops at early postnatal ages, at a time when retinal synapses onto dLGN cells are actively pruning to form adult-like patterns of connectivity. The presence of feedback inhibition in dLGN occurs well

before innervation and circuit formation of other nonretinal modulatory systems including descending input from cortical layer VI and ascending cholinergic input from brainstem.

Disclosures: **P.W. Campbell:** None. **T.A. Seabrook:** None. **G. Govindaiah:** None. **W. Guido:** None.

Poster

148. Visual Thalamus

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Topic: D.04. Vision

Support: .BFU2013-45343-P

XUGA-Educación-Grupos consolidados

Title: Spatial changes on LGN receptive fields by corticofugal input manipulations

Authors: ***C. RIVADULLA**, J. AGUILA, J. CUDEIRO;
Dept Medicine-Neurocom, Univ. De Coruna and INIBIC, Coruña, Spain

Abstract: Ascending sensory pathways in the brain are paralleled by descending feedback pathways, but there has been little consensus as to the role of the feedback connections in perception. The cortical feedback to the thalamus can modify how LGN neurons behave under visual stimulation, and work carried out mainly in anaesthetized animals has shown how the feedback thus affects the spatial and temporal characteristics of the visual signals to be transferred to the cortex. We have explored the effect of inactivation of V1 on LGN cell responses in the awake monkey. Transient inactivation of cortical neurons was achieved by mean of repetitive Transcranial Magnetic Stimulation (rTMS). We have recorded from 75 parvocellular cells in the LGN of two monkeys (Macaca mulatta) during visual stimulation with both, static and drifting gratings of several sizes before and after rTMS (figure-8 coil, 0.7Hz during 4 min; parameters able to induce a depression of cortical activity; (1)). 75% of the cells showed a decrease in firing rate during cortical blockade. This effect was stimulus dependent. However, the most striking result concerned the spatial location of LGN cells receptive fields (RFs). 33% of the cells (n=25) showed a significant shift in RFs position; 4.53 degrees in average. We were able to stablish a relationship between the retinotopy of the recorded cells and the direction of the shift. Since rTMS was always applied at the same cortical location, we conclude that the relative retinotopy between the affected area of the cortex and the recorded

LGN cells was critical for predicting the shift. Even when our inactivation method affects several millimeters of the cortical surface and, admittedly, we are altering an extensive part of the cortical feedback, our results are robust and suggest that RFs in awake animals are more dynamic than previously thought and are modulated by the cortico-thalamic input. (1) Ortuño, et al, Bursting thalamic responses in awake monkey contribute to visual detection and are modulated by corticofugal feedback. Front Behav Neurosci. 2014; 8: 198.

Disclosures: **C. Rivadulla:** None. **J. Aguila:** None. **J. Cudeiro:** None.

Poster

148. Visual Thalamus

Location: Hall A

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Program#/Poster#: 148.05/K31

Topic: D.04. Vision

Support: National '973' Programs 2011CBA00400

Title: Negative afterimages to a flashed spot may originate as early as in the visual thalamus

Authors: *H. LI, X. LIU, Y. LU, X. LI, I. M. ANDOLINA, W. WANG;
Chinese Acad. of Sci., Inst. of Neurosci., Shanghai, China

Abstract: The mismatch between the perceived and the physical stimulus is typically regarded as evidence for a visual illusion. The removal of a flashed spot often generates the perception of a negative afterimage. However, where the perception of such a negative afterimage is initiated in the brain remains unclear. It is well-known that ON or OFF centre neurons in dorsal lateral geniculate nucleus (dLGN) of visual thalamus respond vigorously to the removal of flashing spot stimuli with opposite signs. Considering recent progresses demonstrating visual thalamus in primates engages heavily in behavioral tasks and higher cognitive functions, we hypothesize the neuronal responses to the removal of a flashed dark or light spot in dLGN may provide the initial foundation for the generation of negative afterimages. In the current study using human psychophysical tests, electrophysiological single-unit and whole-cell recordings of cat dLGN, we found evidence to support such a hypothesis. Specifically, ON centre neurons exhibited higher firing rates with longer response duration in response to the removal of a dark spot than that of OFF centre neurons to the removal of a light spot. These results matched the observation of an asymmetry between opposite sign negative afterimages in our psychophysical experiment, as dark flashed spots generated perceptually stronger and longer afterimages than light flashed spots. Furthermore, we found that dLGN responses also showed a clear size tuning to the

removal of a flash spot of various sizes, comparable with our psychophysical test that the observed size of a negative afterimage was determined by the stimulus size. These findings suggest that the negative afterimage of a flashed spot could initiate at least as early as in the dLGN.

Disclosures: **H. Li:** None. **X. Liu:** None. **Y. Lu:** None. **X. Li:** None. **I.M. Andolina:** None. **W. Wang:** None.

Poster

148. Visual Thalamus

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Topic: D.04. Vision

Support: NIH Grant R01EY024173

KSEF

Title: Pretectal modulation of retinogeniculate signal transmission

Authors: ***S. P. MASTERSON**, G. GOVINDAIAH, N. ZHOU, P. S. MAIRE, W. GUIDO, M. E. BICKFORD;
Anatom. Sci. and Neurobio., Univ. of Louisville, Louisville, KY

Abstract: A projection from the pretectum (PT) to the dorsal lateral geniculate nucleus (dLGN) that utilizes gamma amino butyric acid (GABA) was previously described in cats, but the impact of PT-dLGN projections on geniculate circuitry remains poorly understood. We combined anatomical, physiological and optogenetic techniques to examine this projection in mice. Injections of cholera toxin subunit B conjugated to Alexafluor 488 (CTB-488) into the dLGN of C57/BK6 mice labeled PT cells via retrograde transport that were primarily distributed in the ipsilateral and contralateral posterior pretectal nucleus (PPT) and nucleus of the optic tract (NOT). Similar injections were placed in the dLGN of mice in which GABAergic cells express the red fluorescent protein tdTomato (GAD2-cre x Ai9 reporter mice). This revealed that GABAergic PT-dLGN cells are located in both the ipsilateral and contralateral PT. Biotinylated dextran amine (BDA) injections were placed in the PT to label PT-dLGN terminals for ultrastructural analysis. Postembedding immunocytochemical staining for GABA was used to identify the GABAergic PT-dLGN terminals and determine the GABA content of their postsynaptic targets. This revealed that GABAergic PT-dLGN terminals are relatively large

profiles that contact the dendrites of both relay cells and interneurons. To label and activate GABAergic PT-dLGN terminals, we injected a cre-dependent viral vector into the PT of GAD2-cre mice, or a non-cre-dependent virus in the PT of mice that express green fluorescent protein in dLGN interneurons (GAD67-GFP mice). These viral injections induced the expression of tdTomato and the channel rhodopsin variant ChIEF (a cation channel activated by blue light) in PT cells and their terminal projections. Whole-cell recordings were then obtained from relay cells or interneurons within the dLGN maintained *in vitro* and ChIEF-expressing PT-dLGN terminals were activated with short pulses of blue light. At holding potentials of 0 mV (with an internal solution that contained cesium), both dLGN relay cells (16 of 28) and interneurons (12 of 18) responded to activation of PT-dLGN with small amplitude IPSCs (10-120pA). In current clamp experiments at the resting membrane potential, activation of PT-dLGN terminals (50Hz) suppressed retinogeniculate EPSPs (activated via electrical stimulation of the optic tract) in 50% of the cells tested (n=10). This suggests that the PT could simultaneously suppress some visual signals (via direct relay cell inhibition) and enhance others (via disinhibition). Thus, the mouse PT may provide a powerful substrate for modulating the gain, and specificity, of retinogeniculate signal transmission.

Disclosures: **S.P. Masterson:** None. **G. Govindaiah:** None. **N. Zhou:** None. **P.S. Maire:** None. **W. Guido:** None. **M.E. Bickford:** None.

Poster

148. Visual Thalamus

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Topic: D.04. Vision

Support: NHMRC Project Grant APP1042893

NHMRC Fellowship (JAB) APP1077677

Title: Modifications to the dorsal stream visual cortex following early-life lesions of the inferior pulvinar

Authors: *J. A. BOURNE, W. C. KWAN, I. C. MUNDINANO;
Aust. Reg. Med. Inst., Monash University, Australia

Abstract: Previous studies from our laboratory revealed that the medial portion of the inferior pulvinar (PIm) receives retinal input that normally regresses during postnatal development. PIm

also projects to the middle temporal cortex (area MT) with this connection being responsible for the early maturation of area MT. MT distributes information primarily to the dorsal stream areas of the visual cortex. Following removal of the primary visual cortex (V1), the retina-PIm-MT pathway remains unpruned, which could be the anatomical substrate for the preserved vision observed in infants rather than adults ensuing V1 lesions. To study the influence of thalamocortical input from PIm on the organization and maturation of the visual cortex, 4 neonatal (14-21 days) marmoset monkeys (Callithrix jacchus) received unilateral PIm ablations. Ablations were induced by NMDA (100 nl, 0.12M) infusions employing an MRI-guided stereotaxic surgery. The consequence of the lesion were followed up by Diffusion Tensor Imaging (DTI) analysis 6, 12, 18 and 36 weeks post-lesion. Voxel-based morphometry and region of interest analysis revealed a significant reduction of fractional anisotropy values in ipsilateral area MT, dorsal stream associated areas and posterior parietal association cortex. DTI results were further qualified with tracing experiments, and immunohistochemistry revealed morphological changes. Data presented here demonstrate that thalamocortical input from PIm in early life is critical for the correct development and maturation of dorsal stream visual areas.

Disclosures: **J.A. Bourne:** A. Employment/Salary (full or part-time):; Monash University. **W.C. Kwan:** A. Employment/Salary (full or part-time):; Monash University. **I.C. Mundinano:** A. Employment/Salary (full or part-time):; Monash University.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.08/K34

Topic: D.04. Vision

Support: NIH R01 EY009593

Title: Do oscillations in the retinogeniculate pathway encode information about the stimulus?

Authors: ***A. S. GORIN**[1], U. M. CIFCIOGLU[2], V. SURESH[2], K. R. DING[3], F. T. SOMMER[5], J. A. HIRSCH[4];
[1]Neurosci. Grad. Program, [2]Neurobio., [3]Neurosci., [4]Neurobiology, Neurosci. Grad. Program, USC, Los Angeles, CA; [5]Redwood Ctr. for Theoretical Neuroscience, UC Berkeley, Berkeley, CA

Abstract: Traditionally, sensory stimuli are thought to be encoded by spike firing rate. However, stimulus features can be encoded by other schemes, such as oscillatory activity. What features

might oscillations encode? In the frog visual pathway, looming stimuli evoke gamma oscillations in retinal ganglion cells that trigger escape behavior (Arai et al, 2004). In the cat, gamma (40-80 Hz) oscillations originating in a subset of retinal ganglion cells are transmitted by thalamic relay cells to cortex, multiplexed with stimulus-locked information in the low frequency band (<30 Hz)) (Koepsell et al, 2009). While oscillations in the cat thalamus can as much as triple the amount of information sent downstream, it is not clear what visual features these oscillations encode. To investigate this question, we turned to the rodent- a model system in which experiments are economical and that offers the potential for a wide range of genetic manipulations. We used whole-cell recording *in vivo*, a technique that allows us to resolve both retinal inputs (EPSCs) and thalamic spikes during visual stimulation; Events were detected and EPSCs and spikes sorted using a support vector machine. The occurrence of retinal-based oscillations in each record was assessed using multitaper spectral analysis (Bokil et al, 2010; Thomson, 1982) and their strength was measured using the oscillation score index (Muresan et al, 2008). We found intrinsic oscillatory activity in the gamma frequency band in the EPSC and spike trains in some murine relay cells, similar to observations in cat. Furthermore, our data indicate that oscillation strength is inversely correlated to stimulus contrast, especially for naturalistic stimuli. Two interpretations arise from this result: (1) retinal oscillations are generated by distributed networks and thus might signal information about spatially extensive features and/or context. Sensitivity may increase when contrast is low; (2) retinal oscillations may form a complementary second information channel, reducing redundancy and improving the robustness of signal transmission. Our next steps will be to estimate the information rates conveyed by spike times relative to intrinsic oscillations vs the stimulus and, ultimately, to use genetic tools to determine if these oscillations are specific to particular subtypes of ganglion cells.

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Poster

148. Visual Thalamus

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Topic: D.04. Vision

Support: NIH grant EY024173

KSEF Research and Development Excellence Grant

Title: Parallel tectothalamic pathways in the mouse lateral posterior nucleus

Authors: P. S. MAIRE, S. P. MASTERSON, N. ZHOU, *M. E. BICKFORD;
Anatom. Sci. and Neurobio., Univ. Louisville Sch. Med., Louisville, KY

Abstract: The superior colliculus (SC) contains a unique population of cells with wide dendritic fields (wide field vertical, WFV), and either bilateral (type I) or ipsilateral (type II) projections to the thalamus (Fredes et al., JCN 2012). We combined anatomical, physiological, and optogenetic techniques to examine the synaptic connections of type I and type II WFV cells in the mouse lateral posterior nucleus (LPN). Bilateral and ipsilateral WFV-LPN projections were confirmed using injections of two different viruses in the right and left LPN to label WFV cells via retrograde transport. Virus injections in the right and left SC demonstrated that ipsilateral WFV projections fill the LPN, while contralateral WFV projections are confined to a caudal/medial subdivision. Next unilateral virus injections were placed in the SC to induce the expression of tdTomato and the channel rhodopsin variant ChIEF (a cation channel activated by blue light) in WFV cells and their terminal projections. Whole-cell recordings, with biocytin-filled pipettes, were obtained from neurons in the ipsilateral or contralateral LPN maintained *in vitro*, and ChIEF-expressing WFV-LPN terminals were activated with short pulses (1-10ms) of blue light. WFV-LPN responses could be elicited in the presence of 1 μM TTX when paired with 100 μM 4-aminopyridine, and were abolished in the presence of glutamate receptor antagonists. Activation of ipsilateral WFV-LPN terminals (type I + type II) with trains of light pulses (1-20 Hz) evoked excitatory postsynaptic potentials (EPSPs, n = 38) with amplitudes that depressed at high frequencies, or with amplitudes that were frequency-independent. Activation of contralateral WFV-LPN terminals (type I) evoked frequency-independent EPSPs (n = 4). A Scholl analysis (as in Krahe et al., J Neurosci 2011) of biocytin-filled LPN neurons (n = 65) was used to calculate a direction of orientation index (DOI; high values denoting symmetry), which ranged from 0.03-1.00 (mean = 0.53). Dendritic diameters (DD, 201-436 μm) were also measured (mean = 201 μm). The arbors of LPN cells that responded to activation of ipsilateral WFV-LPN terminals (n = 13) were more oriented (mean DOI = 0.46, mean DD = 289) than the arbors of LPN cells that responded to contralateral WFV-LPN (n = 4; mean DOI = 0.71, mean DD = 268 μm). Our preliminary results suggest that a subset of LPN neurons with symmetric dendritic arbors receive convergent input from type I WFV cells, while a second subset of LPN neurons with more selective dendritic arbors receives input from ipsilateral type II WVF cells. This organization may contribute to the reliable initiation of appropriate action in response to diverse visual signals.

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Poster

148. Visual Thalamus

Location: Hall A

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Topic: D.04. Vision

Support: NIH Grant EY012716

Title: Developmental regulation of cholinergic input to the visual thalamus

Authors: *G. SOKHADZE, G. GOVINDAIAH, W. GUIDO;
Anatom. Sci. and Neurobio., Univ. of Louisville, Louisville, KY

Abstract: Sensory nuclei of the thalamus receive extensive projections from cholinergic neurons of mesopontine brainstem as well as the basal forebrain. Such input modulates sensory transmission during different behavioral states including arousal, attention, and the sleep/wake cycle. Despite playing such a crucial modulatory role, little is known about how and when these cholinergic circuits develop in the thalamus. In the visual system two key targets include the dorsal lateral geniculate nucleus (dLGN), a primary sensory relay that transmits retinal information to the cortex; and the thalamic reticular nucleus (TRN), an area that receives indirect visual input but provides GABAergic feedback inhibition to dLGN cells. To examine cholinergic innervation, we utilized a transgenic ChAT-Cre mouse and crossed it with a reporter line (Ai9) to express tdTomato fluorescent protein in cholinergic neurons and their processes. At early postnatal ages, cholinergic axons course along the optic tract and begin to innervate the dorsolateral shell region of dLGN. Their progression through dLGN is slow and diffuse, finally innervating the entire nucleus after 3-4 weeks of age. In dorsal areas of TRN that project to dLGN, cholinergic innervation follows a similar time course. These fibers appear during the first postnatal week and become denser with age, occupying the entire region by 3 weeks of age. By contrast, cholinergic innervation of ventral TRN, in areas that project to primary somatosensory nuclei, is more advanced. To assess when cholinergic projections to TRN form functional connections, we conducted *in vitro* whole cell recordings in acutely prepared thalamic slices in transgenic mice that express channelrhodopsin in choline acetyl-transferase-containing neurons (ChAT-Cre x ChR2). Blue light stimulation of ChAT containing fibers in TRN evokes biphasic (excitation followed by inhibition) postsynaptic activity. The emergence of light evoked activity corresponded to the timing of cholinergic innervation, with responses appearing in ventral areas earlier than in more dorsal locations. We also found that retinal signaling affects cholinergic innervation of dLGN, but not TRN. In mutant mice lacking retinofugal projections (math5 -/-, Wang et al., 2001), the density of cholinergic inputs to dLGN is greatly reduced. Moreover, the spatial distribution is perturbed with cholinergic arbors showing a dense patchy clustering. These results suggest that cholinergic innervation of thalamus happens in a coordinated manner, and

that a disruption of sensory input may affect the timing and pattern of cholinergic innervation in primary relay nuclei.

Disclosures: **G. Sokhadze:** None. **G. Govindaiah:** None. **W. Guido:** None.

Poster

148. Visual Thalamus

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Topic: D.04. Vision

Support: NIH Grant EY012716

Title: Developmental remodeling of intrinsic interneurons in the mouse dorsal lateral geniculate nucleus

Authors: ***N. CHARALAMBAKIS**[1], G. GOVINDAIAH[2], W. GUIDO[2];
[2]Anatom. Sci. and Neurobio., [1]Univ. of Louisville, Louisville, KY

Abstract: The dorsal lateral geniculate nucleus (dLGN) of the mouse thalamus has become a powerful model system to understand visual circuit development. Key elements of these circuits include the two primary cell types of dLGN, relay cells and intrinsic interneurons. Studies have focused primarily on relay neurons, and little is known about the development of the other principal cell type, intrinsic interneurons. Here, we used a transgenic mouse line (GAD67-GFP) in which green fluorescent protein (GFP) is expressed within dLGN interneurons. Such cell-type specific visualization allowed us to readily target interneurons for *in vitro* whole cell recordings with biocytin-filled electrodes, and test whether their form and function changes with postnatal age. At birth, interneurons begin to migrate into the dorsolateral tier of dLGN, and by the end of the first postnatal week they are evenly distributed throughout the nucleus. Between P0-7 there is a six-fold increase in the density of interneurons in dLGN. In contrast, the cell density and positioning of relay cells is well established by early postnatal ages. Confocal reconstructions of 32 biocytin-filled interneurons reveal substantial age-related changes in morphology. Overall, interneurons display type-2 morphology, with 2-3 primary dendrites that arise from opposite poles of spindle-shaped soma. Between postnatal weeks 1-3, there is a three-fold increase in soma size and a four-fold increase in dendritic field. At all ages dendritic fields are quite large, and by 3 weeks of age a given interneuron occupies as much as a third of the nucleus. Initially (P5) branching is simple and sparse. Between P8-17, interneurons undergo a period of exuberant branching, showing a four-fold increase in the total number of branches and branch order, with

some interneurons exhibiting 12-14th order branching. By P21 branch number and complexity begins to prune, as dendritic trees assume a more simple and elongated architecture. These changes are accompanied by age-related increases in resting membrane potential, input resistance, and a nine-fold increase in spike firing frequency. Thus compared to relay cells that take on adult-like features by the end of the first postnatal week, interneurons seem to mature more slowly. Such differences suggest that relay cells and interneurons are governed by separate developmental programs.

Disclosures: **N. Charalambakis:** None. **G. Govindaiah:** None. **W. Guido:** None.

Poster

148. Visual Thalamus

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Program#/Poster#: 148.12/K38

Topic: D.04. Vision

Support: ERC Grant

Title: Visual responses under naturalistic patterns of illumination in the mouse dlgn

Authors: *R. STORCHI;
Univ. of Manchester, Manchester, United Kingdom

Abstract: Introduction: Every day we experience a wide array of gradual and abrupt changes in background light intensity (irradiance) that encompasses several log units. Experimental evidence supports the notion that firing rate activity in the early visual system is defined by the instantaneous level of irradiance as well as by irradiance changes integrated over multiple timescales. In spite of this evidence most visual experiments focussing on neuronal responses to spatial stimuli are conducted at steady irradiance levels, therefore ignoring the effect of irradiance history. We addressed this gap by electrophysiological recordings in dorsal LGN of awake and anaesthetized mice. Results Neurons in dLGN widely differed in their relative sensitivity to instantaneous irradiance and irradiance changes. This irradiance profile was largely invariant upon presentations of uniform screens and artificial or naturalistic images. Critically, at matching levels of instantaneous irradiance, each unit could provide substantially more or less information depending on irradiance history and its irradiance profile. Conclusion Our results show that dLGN neurons exhibit a great diversity of sensitivities to irradiance history. Their irradiance profile shape responses to spatial stimuli and therefore represents an additional dimension to take into account in order to understand how spatial stimuli are encoded in real life

situations. Key Words: Irradiance; Mutual Information; Generalized Linear Models; Neuronal Variability; Overdispersion; Melanopsin; ipRGCs;

Disclosures: R. Storchi: None.

Poster

148. Visual Thalamus

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Support: NIH Grant DC008794

NIH Grant EY022338

Title: Synaptic properties of the thalamocortical projections from lateral posterior nucleus to secondary visual cortex in mice

Authors: *Y.-W. LAM, C. MO, S. SHERMAN;
Univ. of Chicago, Chicago, IL

Abstract: Little is known of the synaptic properties of the pathway from the lateral posterior nucleus (LP), a higher order thalamic nucleus, to visual cortical areas. Studying the thalamocortical pathway from LP is difficult using conventional experimental techniques. We therefore attempted to study the synaptic properties of the thalamocortical pathways from LP to the visual cortices using optogenetic techniques. We stereotactically injected the adeno-associated virus carrying genes for hsyn-ChR-YFP or CamK2-ChR-YFP fusion protein into LP of 5-6 week old mice. The animals were sacrificed 3 to 5 weeks after injection and their brains were sliced coronally for whole-cell recordings. Expression of the fusion channelrhodopsin protein was much stronger in V2 than V1 and therefore, in our initial experiments, we recorded from layer 4 neurons of V2 and stimulated them optically using a UV laser at a wavelength (355 nm) that we found, at high intensity, readily activates their LP inputs. In these cells, photostimulation usually evoked biphasic responses that consisted of fast monosynaptic EPSCs followed by strong polysynaptic IPSCs, suggesting that the thalamocortical projection from LP can strongly suppress layer 4 neurons through disynaptic feed-forward inhibition. When evoked EPSCs were isolated, these monosynaptic EPSCs showed paired-pulse depression, suggesting driver properties for the synapses in this pathway. In this regard, the properties of LP input to V2 seems similar to those we have previously defined for higher order thalamic input to secondary

cortical areas, namely, the input from POm to S2 and from MGNd to A2. Supported by NIDCD grant DC008794 and NEI grant EY022338.

Disclosures: Y. Lam: None. **C. Mo:** None. **S. Sherman:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.14/K40

Topic: D.04. Vision

Title: Rapid and strong gamma oscillations in response to snakes in the monkey pulvinar

Authors: *Q. V. LE, J. MATSUMOTO, Q. V. LE, R. V. BRETAS, T. ONO, H. NISHIJO; Univ., Toyama, Japan

Abstract: Gamma oscillations (30-80Hz) have been suggested to be involved in high-level perception, and might play an important role in detecting threat-relevant objects. In the present study, we analyzed gamma oscillations of pulvinar neurons in the monkeys during delayed non-matching to sample task (DNMS), in which monkeys were required to discriminate 4 kinds of stimuli (snakes, monkey faces, monkey hands and simple geometrical patterns). Of 745 neurons recorded, 115 neurons responded to visual stimuli. Of these 115 neurons, 91 neurons were tested with all stimuli and were used to analyze gamma oscillations. Gamma oscillations were analyzed in three periods around the stimulus onset (pre500: from 500 to 0 ms before the onset; Post0-200 and Post300-500: from 0 to 200 ms, and from 300 to 500 ms after the onset, respectively). The results indicated that, although there was no significant difference in ratios of SC neurons showing gamma oscillation and frequencies of gamma oscillations among these periods, the snake images induced a significant increase of mean strength of gamma oscillations in the Post0-200 and the facial images induced an increase in the Post300-500. In the period Post0-200, strength of gamma oscillations in response to snakes was significantly larger than those to the other stimuli, while in the period Post300-500, strength of gamma oscillations in response to the faces was significantly larger than those to the snakes. These results provided neurophysiological evidence of involvements of gamma oscillations in representation of threat relevant events, including snakes in the early phase.

Disclosures: Q.V. Le: A. Employment/Salary (full or part-time):; full time. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship

even if those funds come to an institution.; No. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); No. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); No. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); No. F. Consulting Fees (e.g., advisory boards); No. Other; No. **J. Matsumoto:** None. **Q.V. Le:** None. **R.V. Bretas:** None. **T. Ono:** None. **H. Nishijo:** None.

Poster

148. Visual Thalamus

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.15/K41

Topic: D.04. Vision

Support: CIHR Grant MOP-119498

Vanier Canada Graduate Scholarship

Title: Y-cell responses to texture stimuli predicted by a nonlinear receptive field model based on bipolar cell subunits

Authors: *A. GHARAT[1], C. BAKER, Jr[2];
[1]Integrated Program in Neurosci., [2]Ophthalmology, McGill Univ., Montreal, QC, Canada

Abstract: A prime aim of visual neuroscience is to estimate neuronal receptive field models that can predict responses to arbitrary stimuli. Y type cells of the retina and the LGN may be an important early stage for second-order processing (Demb et al., 2001b; Rosenberg et al., 2010). A linear receptive field model with static output nonlinearity (LN model) cannot account for nonlinear responses of Y-cells to high resolution texture stimuli. Recent studies have shown that retinal Y-cells receive rectified synaptic inputs from bipolar cells (Demb et al., 2001a). A model with spatial pooling of rectified inputs from several small subunit receptive fields (corresponding to bipolar cells) might better predict responses of Y-cells to arbitrary high resolution texture stimuli. We recorded single-unit responses of cat LGN Y-cells to synthetic naturalistic texture movies and to grating stimuli (drifting and contrast reversing). We estimated both a linear receptive field (LN) model as well as a nonlinear (LNLN) subunit model of Y-cells with regularized gradient descent optimization using STRFlab software (http://strflab.berkeley.edu/). The estimated subunit (LNLN) model explains a significant fraction of response variance to texture movies, while the quasi-linear (LN) model performs poorly. The subunit model also

predicts the "Y-cell signature" spatial frequency tuning to grating stimuli (Hochstein & Shapley, 1976) while the linear model fails to predict tuning to contrast reversing high spatial frequency gratings. These results demonstrate that the subunit model captures nonlinear spatial summation across the receptive fields of Y-cells, and better predicts responses to naturalistic arbitrary stimuli.

Disclosures: A. Gharat: None. **C. Baker:** None.

Poster

148. Visual Thalamus

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

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NIH Grant P30EY008126-24

Title: A comparison of the synaptic input to visual areas v1 and v2 from primate pulvinar

Authors: *B. MOORE[1,2,3], K. LI[4], J. A. MAVITY-HUDSON[3], V. A. CASAGRANDE[3,4,5]; [2]Vanderbilt Brain Inst., [3]Cell and Developmental Biol., [4]Psychology, [5]Ophthalmology and Visual Sci., [1]Vanderbilt Univ., Nashville, TN

Abstract: Several puzzles remain about the flow of visual signals between the primary and secondary visual areas (i.e., V1 and V2) in primates. Although it is generally agreed that the lateral geniculate nucleus (LGN) provides the main sensory message to V1, newer data from our lab (Purushothaman et al., 2012) has shown that signals from lateral pulvinar (PL) to V1 can strongly gate output signals from V1 to V2. In V2, axons from V1 and PL both terminate in layer 4 and the boutons from PL are significantly larger than those arriving from V1 (Marion et al.,

2013). The latter results suggest that visual driving signals to V2 might not come directly from V1 but instead arrive via a loop through the pulvinar. The question remains exactly how does the pulvinar relate to visual processing in V1 and V2. As a first step in understanding how pulvinar interacts with the local networks of V1 and V2 we examined and compared the synaptic targets of PL in each cortical area. The retinotopic maps in PL and inferior pulvinar (PI) of adult bush babies (Otolemur garnettii) were identified using extracellular recording. Once the pericentral area of the dorsal map in PL was identified, biotinylated dextran amine (BDA) was pressure injected. After a 20 day survival period, the animals were perfused, tissue sectioned on a vibratome and the axons visualized in V1 and V2 using streptavidin-HRP/tetramethylbenzidine/diaminobenzidine-cobalt stain. Ultrathin sections containing labeled axons were then immuno-labeled with antibodies to GABA and visualized with 15nm gold particles. Results showed that in both V1 layer 1 and V2, layer 4 pulvinar axons made asymmetric single synapses with non-GABAergic dendrites on both spines and shafts. No evidence of multiple synapses was found in either area. Except for bouton size differences, our results did not identify any major differences between pulvinar axons in V1 and V2. These results suggest that pulvinar axons in both V1 and V2 can directly activate excitatory postsynaptic cells. Given the similarity in the synaptic morphology of pulvinar axons in these areas and the synaptic morphology described by Anderson and Martin (2009) for V1 to V2 axons, more detailed analysis will be required to determine the relative potency and separate functional roles of these pathways.

Disclosures: B. Moore: None. **K. Li:** None. **J.A. Mavity-Hudson:** None. **V.A. Casagrande:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.17/L1

Topic: D.04. Vision

Support: McKnight Foundation Scholar Award

Pew Charitable Trusts Biomedical Scholar Award

NIH/U01 U01NS090562

Title: Molecular dissection of parallel visual pathways in primate and mouse

Authors: *O. S. DHANDE[1], B. K. STAFFORD[1], R. EL-DANAF[1], P. L. NGUYEN[1], K. A. PERCIVAL[2], B. J. HANSEN[3], N. C. BRECHA[4], W. R. TAYLOR[2], E. M. CALLAWAY[3], A. HUBERMAN[1,3,5,6];
[1]Dept. of Neurosciences, Univ. California San Diego, La Jolla, CA; [2]Casey Eye Inst., Oregon Hlth. Sci. Univ., Portland, OR; [3]Salk Inst., La Jolla, CA; [4]Neurobio., UCLA, Los Angeles, CA; [5]Dept. of Ophthalmology, Univ. California, San Diego, La Jolla, CA; [6]Div. of Biol. Sciences, Univ. California, San Diego, La Jolla, CA

Abstract: Visual information is conveyed from the eye to the brain by functionally distinct parallel pathways. The output neurons of the eye are retinal ganglion cells (RGCs). There are an estimated 30 RGC types, each encoding a different and specific set of visual features such as contrast, direction, luminance, or self-motion and projecting to specific targets in the brain. A major goal of neuroscience is to understand the nature of these signals and how they contribute to central visual processing (Dhande and Huberman, Curr Opin in Neurobiol, 2014). In mice, the identification of molecular markers expressed by distinct RGC types has lead to a significant advance in understanding the organization of eye-to-brain parallel pathways and in some cases the contribution of individual parallel channels to specific behaviors. Although the primate retina also includes a rich diversity of RGC subtypes (Dacey et al., Neuron, 2003), the visual features encoded by most primate RGCs and how these signals impact visual processing within the brain remain a mystery. To bridge the gap between molecular and functional understanding of primate RGCs, we screened for molecular markers expressed in specific RGC types in mouse (Wang et al., in preparation), in the macaque monkey retina. We discovered that the transcription factor Satb2 (Special AT-rich sequence-binding protein 2), which is unique to On-Off direction selective RGCs in mouse is expressed by a quasi-mosaic of RGCs in the macaque. The density of Satb2 RGCs scales with retinal eccentricity in macaque and has a peak density of ~ 160 cells/mm2 parafoveal, suggesting that i) Satb2-expressing RGCs may represent a single RGC subtype and ii) that these cells may contribute to central vision. Next, to determine whether the Satb2 RGCs in primate project to the dorsal lateral geniculate nucleus (dLGN) and thus may be involved in the generation of "sight", we injected a modified rabies virus expressing GFP into the dLGN to infect the axon terminals of dLGN-projecting RGCs and then stained the retinas for Satb2. Indeed, Satb2-expressing RGCs synapse in the dLGN. We also confirmed the morphology of the Satb2 RGCs is uniform from cell to cell and are now exploring their physiological responses to specific light stimuli, including directional stimuli. These experiments represent our first step towards the goal of achieving a thorough understanding of the functional diversity and evolutionary conservation of parallel visual pathways in primates and other species.

Disclosures: O.S. Dhande: None. **B.K. Stafford:** None. **R. El-Danaf:** None. **P.L. Nguyen:** None. **K.A. Percival:** None. **B.J. Hansen:** None. **N.C. Brecha:** None. **W.R. Taylor:** None. **E.M. Callaway:** None. **A. Huberman:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.18/L2

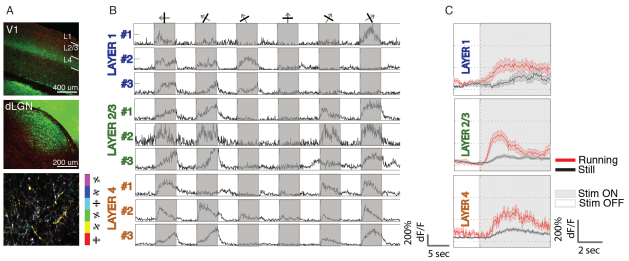
Topic: D.04. Vision

Support: NERF Funding from imec,KU Leuven,VIB

Title: Diversity and layer specificity of thalamic inputs to mouse visual cortex

Authors: *K. Z. SOCHA-JANIAK, C. AYDIN, V. BONIN;
Neuro-Electronics Res. Flanders, Leuven, Belgium

Abstract: Neuronal activity in the dorsal lateral geniculate nucleus (dLGN) is functionally diverse with distinct neurons conveying information about specific features of the visual scene (Piscopo et al., 2013). This activity likely reflects the response properties of retinal ganglion cells and their inputs to dLGN (Cruz-Martin et al., 2014). While the properties of dLGN neurons have been studied extensively, little is known about how distinct dLGN populations convey distinct information to cortex. We combined chronic cellular imaging and a head-fixed locomotion assay to characterize the response properties of dLGN axons projecting to distinct layers of the primary visual cortex (V1) of mice. We performed stereotaxic injections of an adeno-associated viral vector to express the calcium indicator GCaMP6f in dLGN populations (Fig. 1A). We then used 2-photon microscopy to image the fluorescence calcium signals of dLGN axons in response to visual stimulation. We measured responses to a broad range of visual stimuli including full-field drifting sinusoidal gratings of different directions, spatial and temporal frequencies. We identified visually responsive boutons (Glickfeld et al., 2013) and investigated the boutons' tuning properties to stimulus attributes. We obtained strong GCaMP6 expression in dLGN cell bodies and their axonal projecting to cortex across layers (Fig. 1A). Consistent with a recent study of dLGN inputs to superficial V1 (Cruz-Martin et al., 2014), responses of dLGN axons were tuned to different stimulus orientations and directions (Fig. 1B). However, far from being confined to layer 1, orientation and direction selective boutons were found within all layers of V1. We observed that locomotion modulates visual responses of V1-targeting dLGN axons (Fig. 1C). Our results are consistent with recent studies (Erisken et al., 2014), showing the influence of locomotion on visual responses already at early processing stage in the visual system. Currently, we investigate how visual and movement-related outputs from dLGN are distributed and vary with depth in V1.



Disclosures: **K.Z. Socha-Janiak:** None. **C. Aydin:** None. **V. Bonin:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.19/L3

Topic: D.04. Vision

Support: DFG EXC 307

European Research Council (project acronym: PERCEPT)

Title: Dissecting corticothalamic feedback during active behavior in the mouse visual system

Authors: ***S. ERISKEN**[1,2], A. VAICELIUNAITE[1], L. BUSSE[1];
[1]Univ. of Tuebingen, Tuebingen, Germany; [2]Grad. Training Ctr. of Neurosci., Tuebingen, Germany

Abstract: Causal manipulation of neural circuits has led to promising advances in understanding the neural substrates of visual perception and behavior. From local computations to long-range communication across areas, e.g. shaping of thalamic activity by cortical feedback, a broad range of circuit functions can be interrogated through activation and silencing of distinct neural cell-types. Such circuit-mapping approaches are particularly powerful if used to control not just action potential firing but rather neurotransmitter release from selective terminals. Designer receptors exclusively activated by designer drugs (DREADDs), are one set of such tools and are activated by biologically inert CNO. CNO, however, is typically delivered systemically, which limits its selectivity. To target specific brain areas and selectively manipulate transmission at specific terminals it is thus desirable to implement methods for local intracranial CNO injections. Here, we describe a method for minimally disruptive intracranial delivery of CNO during simultaneous extracellular recordings in awake behaving mice. In mice headfixed on a spherical treadmill, we recorded activity with Neuronexus linear probes and injected CNO with a glass

pipette into the same structure. To position the tip suitably close to recording sites, pipettes were pulled with ~1.5 cm long tips, and connected to a 10 uL Hamilton syringe via a glass coupler and PEEK tubing. The system was filled with silicone oil and the syringe placed in a syringe pump. We drew a drop of CNO solution on parafilm into the pipette. After electrode insertion, we put a layer of aCSF on the brain surface before we inserted the pipette to avoid leakage. During recording we delivered CNO slowly (500 uL/hr) via 25-50 uL injections at 1-10 s intervals. These injections did not cause significant distortions, as ongoing LFPs did not exhibit injection-related artifacts that were evident with higher-pressure injection methods. We are currently using this technique to investigate influences of feedback from primary visual cortex (V1) in modulating the dorsolateral geniculate nucleus (dLGN) during switches of behavioral state. L6 corticothalamic cells in V1 influence dLGN by both direct excitatory and indirect inhibitory feedback, via the reticular nucleus (TRN). By injecting CNO in dLGN or TRN of Ntsr1-Cre mice expressing Cre-dependent DREADD receptors, we can selectively silence the excitatory or inhibitory feedback at corticothalamic synapses while not disturbing L6 control over cortical gain. This approach promises to precisely dissect the influence of corticothalamic feedback with pathway-specificity and minimal damage.

Disclosures: **S. Erisken:** None. **A. Vaiceliunaite:** None. **L. Busse:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.20/L4

Topic: D.04. Vision

Title: Estimating the response properties of the human lateral geniculate nucleus using a spatiotemporal population receptive field model

Authors: *K. DESIMONE[1], K. A. SCHNEIDER[2];
[1]Psychology, York Univ., North York, ON, Canada; [2]York Univ., Toronto, ON, Canada

Abstract: The population receptive field (pRF) model has been widely adopted in the imaging community as a quantitive approach for estimating the response properties of populations of neurons. The initial formulation of the pRF model represented the spatial tuning of each voxel in terms of a two-dimensional gaussian (Dumoulin & Wandell, 2008). This spatial pRF model has characterized the retinotopic organization of a number of cortical areas (Dumoulin & Wandell, 2008; Amano et al., 2009) and a number of patient groups (Levin et al., 2010; Hoffmann et al., 2012). More recently, the spatial pRF model has been used to estimate the retinotopic

organization of multiple subcortical nuclei (DeSimone et al., in revision), including the lateral geniculate nucleus (LGN). We sought to extend the spatial pRF model to include a temporal component for characterizing the functionally and anatomically distinct layers of the LGN. Using a flickering visual stimulus, we found evidence that the magnocellular and parvocellular layers of the LGN may be functionally differentiated using a spatiotemporal pRF model.

Disclosures: K. DeSimone: None. **K.A. Schneider:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.21/L5

Topic: D.04. Vision

Support: Whitehall Foundation Research Grant

Title: Corticogeniculate feedback enhances temporal precision of LGN neurons in the ferret

Authors: *J. M. HASSE, F. BRIGGS;
Physiol. & Neurobio., Geisel Sch. of Med. At Dartmouth, 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 infected through their axon terminals. 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 coupled to a fiberoptic cannula such that we can record responses of ChR2-expressing corticogeniculate neurons to both visual stimulation and optogenetic stimulation (via blue LED activation). 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.

Disclosures: **J.M. Hasse:** None. **F. Briggs:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.22/L6

Topic: D.04. Vision

Support: NIH grant EY013588

NIH grant P30EY12576

Title: Contribution of retinal mechanisms to nonlinear receptive field properties in the lateral geniculate nucleus

Authors: ***T. FISHER**[1], H. ALITTO[1], D. RATHBUN[1,2], W. M. USREY[1];
[1]Univ. of California at Davis, Davis, CA; [2]Inst. for Ophthalmic Res., Eberhard Karls Univ., Tübingen, Germany

Abstract: Extraclassical surround suppression and gain control in the lateral geniculate nucleus (LGN) are generally accepted to result from a combination of mechanisms involving feedforward and feedback circuits. Although extraclassical suppression and contrast gain control have been separately characterized for retinal ganglion cells and LGN neurons, a direct one-to-one comparison of these nonlinear response properties between synaptically coupled neurons has not been performed. This comparison would provide much needed information for understanding the cellular and circuit mechanisms underlying nonlinear processing in the LGN. To achieve this goal, we made simultaneous recordings from the retina and LGN, identified monosynaptically connected cell pairs via cross-correlation analysis, and quantified extraclassical suppression and gain control from responses to drifting grating stimuli that varied in size or contrast. Our results reveal a dynamic interplay between stimulus parameters and the communication of spikes between the retina and LGN. Additionally, our results reveal stimulus-dependent interactions between feedforward and feedback pathways. These results provide key insight into the contributions made by retinal and non-retinal inputs to visual responses in the LGN and provide important information needed to develop a comprehensive model of visual processing in the LGN.

Disclosures: **T. Fisher:** None. **H. Alitto:** None. **D. Rathbun:** None. **W.M. Usrey:** None.

Poster

148. Visual Thalamus

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Program#/Poster#: 148.23/L7

Topic: D.04. Vision

Support: NIH Grant EY013588

NIH Grant EY015387

Title: The influence of contrast on area summation in the lateral geniculate nucleus of the alert macaque monkey

Authors: ***D. ARCHER**, H. J. ALITTO, W. M. USREY;
Univ. of California, Davis, Davis, CA

Abstract: Nonlinear extraclassical suppression is a fundamental receptive-field property for neurons in the early visual system, including the retina, LGN, and primary visual cortex (V1). Previous studies examining area summation in V1 of macaque monkeys have shown that the size of the classical receptive field and the magnitude of non-linear suppression depend on stimulus contrast (Sceniak et al., 1999). Interestingly, LGN neurons do not show this effect in a preparation that involves silencing the cortex pharmacologically (Sceniak et al., 2006). To gain insight into whether corticogeniculate feedback is necessary for contrast to influence size tuning and extraclassical suppression in the LGN, we made single-unit recordings from cells in the LGN of two alert, fixating macaque monkeys. Area summation response functions were generated from neuronal responses to drifting sinusoidal gratings varying in size and contrast centered over the receptive field of the recorded cell. Response functions were fit with a difference of Gaussians (DOG) model to estimate the spatial parameters of the central excitatory and surround inhibitory subunits at various contrasts. Our results reveal a range of effects of stimulus contrast on area summation, with a subset of LGN cells demonstrating a clear influence similar to the contrast-dependent modulation found in macaque V1. When considered in the context of previous reports, results from the current study suggest that visually responsive corticogeniculate neurons may contribute to contrast-dependent area summation in the macaque LGN.

Disclosures: **D. Archer:** None. **H.J. Alitto:** None. **W.M. Usrey:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.24/L8

Topic: D.04. Vision

Support: NIH Grant EY013588

NIH Grant P30EY12576.

Title: Behavioral modulation of visual responses and network dynamics in the lateral geniculate nucleus

Authors: *H. J. ALITTO, W. M. USREY;
Univ. of CA, Davis, Davis, CA

Abstract: The main function of the lateral geniculate (LGN) nucleus is to transmit visual information from the retina to primary visual cortex (V1). The LGN also serves as a gate, selecting when and what retinal information is transmitted to the cortex. How this is accomplished is not entirely understood, but it is likely to involve direct and indirect modulation from a variety of cortical and subcortical regions, including, the thalamic reticular nucleus (TRN), the cholinergic brainstem, the hypothalamus, basal forebrain, and V1. This modulation affects visual responses during sleep and arousal, as well as when cognitive processes such as decision making and spatial attention are engaged. To understand how spatial attention affects the network dynamics of the LGN, we measured local field potentials and spike-field interactions with well isolated LGN units while macaque monkeys performed a spatial attention task. Recordings were made using either single electrodes or multielectrode recording arrays (V-probe, Plexon) that spanned multiple layers of the LGN and, in some cases, the TRN. During the spatial attention task, monkeys fixated on a central location while two sine-wave grating patches (typically 2^{o} in diameter, located ~4^{o} -8^{o} from fixation) were presented peripherally: one positioned over an LGN receptive field, the other positioned at a separate location. Animals were cued, in block format, to detect an increase in contrast either at or away from the spatial position of the LGN receptive field and were rewarded for making a saccade toward the grating where the contrast change occurred. For a subset of cells, these spatial attention conditions were compared to a non-attention condition where the monkey was rewarded for maintain fixation and no stimulus change occurred. Preliminary analysis indicates the firing rate of LGN neurons is invariant to changes in spatial attention; however, there is apparent modulation of the local field potentials in the in alpha and beta bands. Ongoing analysis is aimed at determining if these

changes are systematically influenced by spatial attention or may represent an inherent instability in network dynamics that is more strongly influence by other factors, such as fluctuations in task engagement, motivation, or arousal.

Disclosures: H.J. Alitto: None. **W.M. Usrey:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.25/L9

Topic: D.04. Vision

Support: NIH EY013588

NIH P30EY12576

Title: Retinogeniculate communication during thalamic bursts

Authors: H. J. ALITTO[1], D. L. RATHBUN[2], *W. USREY[1];
[1]Ctr. for Neurosci., Univ. of California, Davis, Davis, CA; [2]Inst. for Ophthalmic Res., Eberhard Karls Univ., Tübingen, Germany

Abstract: Visual information from the retina is transmitted to primary visual cortex through the lateral geniculate nucleus (LGN) of the thalamus. At a basic level, retinal information is processed by LGN neurons in two distinct modes: tonic and burst. During tonic mode, LGN neurons respond to excitatory input with regularly spaced action potentials, the rate of which is proportional to the strength of the excitatory input. By contrast, during burst mode, LGN neurons respond to excitatory input with irregular trains of action potentials at a rate that is roughly independent of the strength of the excitatory input. Burst mode occurs when LGN neurons are sufficiently hyperpolarized that T-Type Ca+2 channels are deinactivated. The subsequent activation of the T-Type channels leads to a Ca+2 plateau potential that can trigger a short sequence of high frequency, Na+ based action potentials (a burst). Given the mechanisms underlying burst mode, it is generally thought that individual spikes in a burst are less dependent on retinal activity than their tonic counterparts. However, previous experiments using S-potentials as a surrogate for retinal inputs onto an LGN neuron indicate that nearly all LGN spikes, tonic and burst, are directly triggered by retinal action potentials. To investigate the retinal contribution to LGN burst spikes, we made simultaneous recordings from monosynaptically connected RGCs and LGN neurons in the cat while cells were excited with a

variety of stimuli. LGN bursts were identified using previously established statistical criteria: a preceding interspike interval (ISI) >100 msec followed by at least one ISI <4 msec. Our results show that retinogeniculate communication changes as a function of visual stimulation and LGN response mode. In particular, the retinal contribution to burst spikes was approximately half of that for tonic spikes. This difference increased as the preceding ISI increased beyond the minimum value (100 msec) towards the longest recorded values (>400 msec). Interestingly, although LGN activity was less dependent on retinal activity during burst mode, retinal spikes were more effective in driving thalamic activity during burst events. These results provide direct insight into how retinogeniculate communication is influenced by nonvisual aspects of thalamic circuitry.

Disclosures: H.J. Alitto: None. **D.L. Rathbun:** None. **W. Usrey:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.26/L10

Topic: D.04. Vision

Support: ERC

Wellcome Trust

Title: Thalamus conveys diverse contextual information to layer 1 of visual cortex

Authors: *D. R. MUIR, M. M. ROTH, F. IMHOF, S. B. HOFER;
Univ. of Basel, Basel, Switzerland

Abstract: The pulvinar is a complex of higher-order visual nuclei in the thalamus. It is heavily interconnected with many cortical and subcortical areas, and has been shown to exert strong influence on visual cortical activity. The pulvinar may be important for attentional gating of visual information and integration of visual and behavioral information, but knowledge about its function is still very limited. Specifically, it is unknown what type of information this thalamic hub conveys to different cortical areas. The homologue of the pulvinar in the mouse is the lateral posterior nucleus (LP). We quantified information conveyed by LP to visual cortex, and compared it to the information arising from the dorsolateral geniculate nucleus (dLGN), the first-order visual thalamic nucleus that receives its primary input from the eye. By using *in vivo* two-photon imaging of axons labelled with genetically-encoded calcium indicators, we recorded

responses of thalamic axon terminals from LP or dLGN in layer 1 of mouse visual cortex. During imaging, head-fixed mice were free to run either in the dark or through a simple virtual corridor with variable visual patterns on the walls. In some sessions, the visual stimulus was uncoupled from the animals' locomotion to separate the visual from motor signals, and to examine the role of thalamo-cortical interactions in sensory-motor integration. We found that both LP and dLGN transmit a wide range of sensory, behavioral and contextual signals to layer 1 of visual cortex. Both dLGN and LP boutons carried information about the animals' running speed as well as visual flow speed of the virtual corridor. However, LP boutons carried significantly more running speed information in the absence of visual stimulation. Visual flow and running speed were signaled largely by separate subsets of boutons, and visual and motor signals also differed in their timing. Only LP boutons carried prominent signals representing discrepancies between expected and actual optic flow during locomotion. These results suggest that LP is engaged in sensory-motor integration, for instance to enable animals to differentiate self-generated from external visual motion. In summary, we found that layer 1 of visual cortex receives diverse contextual information from the higher order thalamic nucleus LP, including purely visual, purely motor, and combined sensory-motor interaction signals.

Disclosures: **D.R. Muir:** None. **M.M. Roth:** None. **F. Imhof:** None. **S.B. Hofer:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.27/L11

Topic: D.04. Vision

Support: NEI R01EY017699

Title: Functional and anatomical organization of the dorsal pulvinar in humans

Authors: ***M. J. ARCARO**, M. A. PINSK, S. KASTNER;
Princeton Univ., Princeton, NJ

Abstract: The primate pulvinar is thought to facilitate communication between cortical regions. In general, directly connected cortical regions are also indirectly connected via the thalamus. Previously, we found that cortical connectivity within the ventral and posterior pulvinar reflects cortical distance even for functionally similar areas that are spatially distributed across temporal cortex (Arcaro et al. 2014 SfN). Tracer studies in non-human primates have shown that cortical connectivity greatly differs between the dorsal and ventral pulvinar (Baizer et al. 1993). Still,

little is known about the functional organization of the dorsal pulvinar and its relation to cortical connectivity. Using neuroimaging, we investigated functional response properties and cortical connectivity in the human dorsal pulvinar. Retinotopic mapping, basic response properties, and attentional modulation were measured. Functional connectivity was assessed during states of rest and movie viewing. Functional connectivity was assessed using correlation analyses. Anatomical connectivity was assessed using probabilistic tracking analyses on diffusion imaging data. Functional response properties differed between the dorsal and ventral pulvinar. Retinotopic representations were mainly found within the ventral pulvinar. Visually-evoked responses were typically weaker in the dorsal pulvinar. Attention effects were specific to contralateral space in the ventral pulvinar, but bi-lateral in the dorsal pulvinar. Activity patterns in the dorsal and ventral pulvinar were consistent across repeated movie presentations. Consistency was greatly reduced within the dorsal, but not ventral, pulvinar when movies were temporally scrambled, suggesting that the dorsal pulvinar is more sensitive to coherent structure in stimuli. During rest and movie viewing, activity in the dorsal pulvinar was strongly coupled with frontal and parietal regions that are part of the dorsal attention network. Cortical connectivity with the dorsal pulvinar reflected both cortical distance and functional similarity. There was a broad topography of anatomical connections between parietal cortex and the dorsal pulvinar with posterior areas most strongly connected with lateral portions and anterior areas most strongly connected with medial portions. We also found evidence that connectivity between functionally dissociable parietal regions converges within the dorsal pulvinar. Overall, our results suggest that the dorsal pulvinar is well situated to facilitate communication between cortical regions that are functionally related as well as regions that are in close anatomical proximity.

Disclosures: **M.J. Arcaro:** None. **M.A. Pinsk:** None. **S. Kastner:** None.

Poster

148. Visual Thalamus

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 148.28/L12

Topic: D.04. Vision

Support: NIH Grant EY018251

Title: Hour-long adaptation in the awake early visual system

Authors: ***C. R. STOELZEL**[1], J. HUFF[1], Y. BERESHPOLOVA[1], J. ZHUANG[1], X. HEI[1], J.-M. ALONSO[2,1], H. A. SWADLOW[1,2];

[1]Dept. of Psychology, Univ. of Connecticut, Storrs, CT; [2]Dept. of Biol. Sci., State Univ. of New York, New York, NY

Abstract: Sensory adaptation serves to adjust awake brains to changing environments on different time scales. However, adaptation has been traditionally studied under anesthesia and for short time periods. Here, we demonstrate, in awake rabbits, a novel type of sensory adaptation that persists for more than one hour and acts on visual thalamocortical neurons and their synapses in the input layers of the visual cortex. Following prolonged visual stimulation (10 - 30 minutes), cells in the dorsal lateral geniculate nucleus (LGN) show a severe and prolonged reduction in spontaneous firing rate. This effect is bi-directional and prolonged visual response suppression is followed by prolonged increase in spontaneous activity. The reduction in thalamic spontaneous activity following prolonged visual activation is accompanied by increases in (a) response reliability, (b) signal detectability, and, (c) the ratio of visual signal/spontaneous activity. In addition, following such prolonged activation of an LGN neuron, the monosynaptic currents generated by thalamic impulses in layer 4 of primary visual cortex (V1) are enhanced. These results demonstrate that, in awake brains, prolonged sensory stimulation can have a profound, long-lasting effect on the information conveyed by thalamocortical inputs to visual cortex.

Disclosures: C.R. Stoelzel: None. **J. Huff:** None. **Y. Bereshpolova:** None. **J. Zhuang:** None. **X. Hei:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.01/L13

Topic: F.02. Animal Cognition and Behavior

Support: Office of Naval Research N00014-13-1-0253

Title: A specialized region in dog temporal cortex for face processing

Authors: *D. D. DILKS[1], P. COOK[1], S. K. WEILLER[1], H. P. BERNS[1], M. SPIVAK[2], G. S. BERNS[1];
[1]Dept. of Psychology, Emory Univ., Atlanta, GA; [2]Comprehensive Pet Therapy, Atlanta, GA

Abstract: Recent behavioral evidence suggests that dogs, like humans and monkeys, are capable of visual face recognition. But do dogs also exhibit specialized cortical face regions similar to

humans and monkeys? Using functional magnetic resonance imaging (fMRI) in six dogs trained to remain motionless during scanning without restraint or sedation, we found a candidate region in the canine temporal lobe that responded significantly more to movies of human faces than to movies of everyday objects. Next, using a new stimulus set to investigate face selectivity in this predefined candidate dog face area, we found that this region responded similarly to images of human faces and dog faces, yet significantly more to both human and dog faces than to images of objects and scenes. Such face selectivity was not found in dog primary visual cortex. Taken together, these findings: 1) provide the first evidence for a face-selective region in the temporal cortex of dogs, which cannot be explained by simple low-level visual feature extraction; 2) reveal that neural machinery dedicated to face processing is not unique to primates; and 3) may help explain dogs' exquisite sensitivity to human social cues.

Disclosures: D.D. Dilks: None. **P. Cook:** None. **S.K. Weiller:** None. **H.P. Berns:** None. **M. Spivak:** None. **G.S. Berns:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.02/L14

Topic: D.04. Vision

Support: FWO G093214N

IUAP

PF

Title: Neural encoding of shapes in the middle superior temporal sulcus (mid-STS) body patch neurons

Authors: *S. KUMAR, R. VOGELS;
Neurosci., KU Leuven, Leuven, Belgium

Abstract: In monkeys (Macaca mulatta), fMRI defined body category-selective regions ("body patches") are more strongly activated by images of bodies compared to inanimate objects, however the quantitative characteristics of the stimulus selectivity of body patch neurons still remain unclear. Mid-STS body patch neurons respond well to silhouettes, suggesting that mainly shape determines the response of these neurons. Here, we attempt to unravel the shape selectivity of mid-STS body patch neurons by finding their discriminative shape features and quantifying

their interactions by employing adaptive stimulus sampling and deriving feature-based mathematical models of these single neurons. We varied shape, including body silhouettes, parametrized with elliptical Fourier features and sampled the stimulus space of these neurons adaptively varying both local and global shape. This adaptive stimulus sampling procedure produced a large number of stimuli with a wide range of responses, which were used to derive models of the shape selectivity of each neuron, using multi-start non-linear least squares optimization with 5-fold cross-validation, providing a safeguard against overfitting. Shape tuning was quantified by 4 dimensions including curvature, orientation and relative position XY values of decomposed shape contour elements with approximately constant curvature. Statistically significant (n = 51 neurons, $p < 0.001$) parsimonious (penalized for complexity) models were reliable predictors of the response of single mid-STS neurons to complex test stimuli widely varying in shape, mean goodness of fit (r) being 0.61 (range = 0.31 - 0.88). The fitted models of most cells contained more than 3 subunits, each corresponding to a contour element at a different relative position along the outer contour of the shape. This suggests that midSTS body patch neurons receive information from a multitude of low level inputs that respond to contour features similar to those reported for area V4 neurons by Pasupathy et al. NN 2002. We observed an inhibitory influence of one or more contour subunits in the majority of fitted models, while excitatory influences being slightly higher in magnitude than inhibitory (mean E/I index = 0.55). Some neurons exhibited strong non-linear interactions between their subunits. These results suggest that at least part of the neural encoding of complex shapes in the mid-STS body patch can be described with linear and nonlinear interactions of multiple local contour features.

Disclosures: **S. Kumar:** None. **R. Vogels:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.03/L15

Topic: F.02. Animal Cognition and Behavior

Title: Distinct patches for gaze following and the passive vision of faces in the macaque posterior superior temporal sulcus can be distinguished by specific local field potential fingerprints

Authors: *H. RAMEZANPOUR, I. CHONG, P. W. DICKE, P. THIER;
Cognitive Neurol., Hertie Institue For Clin. Brain Res., Tübingen, Germany

Abstract: Rhesus monkeys are able to follow their conspecific's head gaze ("sender") in order to shift their focus of attention to potential objects of interest. These are identified by the sender's gaze vector to establish joint attention, a key step in developing the theory of the other's mind. Although previous studies have shown that the posterior part of the superior temporal sulcus (pSTS) plays an important role in gaze following, how gaze information is encoded in the pSTS and its relationship to general face processing remains unclear. To answer these questions, we trained a rhesus monkey on a task requiring spatial shifts of attention based on either following the head gaze of monkey portraits or alternatively on matching their identities to the same targets. The monkey was required to single out one of four small targets by making a saccade to the target that was pointed out by the head gaze direction or matched to one of the four learned identities. In each trial the color of the fixation point indicated which of the two tasks had to be performed. A separate task required the passive viewing of face and non-face stimuli while maintaining a small fixation point. Single unit responses recorded from the right pSTS of the monkey showed a clear topographical organization of response preferences in these tasks. Among others, we were able to identify a small patch congruent with the gaze following patch (GFP) previously reported by Marciniak et al. (Elife, 2014) based on fMRI. In the GFP, neurons showed a clear response only to gaze following. On the other hand, face selective neurons responding to the passive vision of faces were found in a patch roughly 7mm away from the GFP, probably corresponding to the middle face patch (MFP) first delineated by Tsao et al. (Science, 2006). Here we report the clearly different features of local field potentials (LFPs) recorded from these two patches. The spectral analysis of LFPs recorded from the GFP exhibited a dominance of beta frequency oscillations (12-30Hz) as soon as the first facial information became available, continuing until the monkey made a saccade to the target indicated by the gaze cue in gaze following trials. On the other hand, LFPs recorded from the MFP were dominated by a broad range of high frequency gamma oscillations (30-100Hz). These clear differences in the frequency domain are in full support with the notion that the pSTS accommodates distinct neural architectures in distinct patches, i.e. the passive processing of facial attributes in face patches versus spatial shifts of attention based on directional facial information in the GFP.

Disclosures: **H. Ramezanpour:** None. **I. Chong:** None. **P.W. Dicke:** None. **P. Thier:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.04/L16

Topic: D.04. Vision

Support: Swartz grant # 2013-36

Title: Recordings from macaque face patches ML and AM reveal a mechanism for view-invariant computation

Authors: *L. CHANG, D. TSAO;
Div. of Biol., CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena, CA

Abstract: The macaque temporal lobe contains six patches of face-selective cortex. Previous experiments have explored face representation in the middle face patches using cartoon images and found that face cells are tuned to subsets of feature dimensions (Freiwald, Tsao and Livingstone, 2009), but it remains unclear how this result applies to realistic faces and other face patches. Furthermore, neurons in anterior face patch AM show face view-invariant tuning to individual identities (Freiwald and Tsao, 2010). What are the special tuning properties underlying view-invariant identity representation in AM? To address these questions, we generated a parametric real face space. We started with a database of 200 frontal faces (FEI face database), and for each face, used an "active appearance model" (Cootes, Edwards and Taylor, 2001) to extract significant facial features describing both the shape and shape-free appearance; we then computed the top 25 principal components for shape and for appearance. Thousands of faces were randomly drawn from the 50-d face space and presented during recording from face patches. We found that middle face patch neurons are better tuned to shape dimensions than appearance dimensions, while for neurons in anterior patch AM the opposite is true. Using linear regression, we decoded intensity variations at different locations of faces based on population responses. Two types of facial images were used for decoding: the original images presented to the monkey and the same set of images morphed to a standard shape template. Interestingly, we found using middle face patch population, the intensities around the eyes can be nicely decoded for the original images but not for shape-normalized images, while for AM population, the decoding is much better after shape normalization. This result suggests that AM cells are warping the face representation they receive from middle patch cells to a standard template. This provides a mechanistic explanation for how identity can be represented in a way that is invariant across small changes in view. The warping process we observed in AM neurons can be implemented in two different ways: 1) IT cortex may dynamically morph the facial images to match facial features like eyes to that of a standard face template; 2) Templates of "facial features" at different positions and scales could first be used to filter the facial image, then the outputs of the templates could be pooled together by nonlinear operations (like "max") to extract position- and scale-invariant feature values (Riesenhuber and Poggio, 1999). We are now exploring these possibilities with new experiments.

Disclosures: L. Chang: None. **D. Tsao:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.05/L17

Topic: D.04. Vision

Support: Simons foundation grant 325023

Institutional start-up

Title: Multiple object representation in the middle face patch

Authors: *P. BAO, L. CHANG, D. TSAO;
Biol., Caltech, Pasadena, CA

Abstract: In the natural environment, primates can recognize objects in the presence of the other objects and electrophysiological studies show that IT neurons reduce their response to a preferred object when presented with a non-preferred object (Zoccolan et al., 2005). It still remains unclear how the reduced responses can account for recognition invariant to the presence of multiple objects. To address this question, we performed electrophysiological recordings in the macaque middle face patch (ML). In the first experiment, to test how responses to preferred stimuli are affected by non-preferred stimuli, one face and one non-face object were presented horizontally or vertically around the fixation point. We also varied the relative contrast energy of two stimuli, while maintaining the whole contrast energy constant. In the horizontal configuration, we found when the face was in the visual field contralateral to the recording hemisphere, neurons performed a MAX operation regardless of contrast energy; when the face was presented in the ipsilateral visual field, neurons performed weighted averaging, with weight determined by contrast energy. In the vertical configuration, regardless whether the face was above or below the fixation, neurons always performed a MAX operation. In the second experiment, to test how neurons code multiple preferred objects, two faces were presented in a vertical or horizontal configuration as in the first experiment. Each face was randomly and independently sampled from a parametric real face space defined by three shape and three appearance dimensions (Chang & Tsao, 2014). In the vertical configuration, responses evoked by two-face stimuli were modulated by both faces. Moreover, the shapes of the tuning curves for each face in the two-face condition were similar, but lower in gain, than those for the single-face conditions. When two faces were presented in the horizontal configuration, the tuning curve to the ipsilateral face was flat, while the tuning curve to the contralateral face was similar to that for a single contralateral face. These two experiments reveal that neurons in the middle face patch can perform either MAX or averaging depending on the spatial configuration of the stimuli, and moreover, single cells participate in coding identity of multiple faces.

Disclosures: **P. Bao:** None. **L. Chang:** None. **D. Tsao:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.06/L18

Topic: D.04. Vision

Support: Conte Center Grant P50 MH942581A

Title: Functional connectivity of category-selective patches in macaque IT cortex

Authors: *J. S. GUNTUPALLI, D. Y. TSAO;
Dept. of Biol., Caltech, Pasadena, CA

Abstract: fMRI of the macaque brain reveals six face-selective regions in the IT cortex. Electrophysiological recordings targeting these patches show that most neurons in these patches are also face-selective and the representation of faces by the neural population in these patches evolves along the posterior-to-anterior axis gradually building viewpoint invariance. Electrical stimulation within face patches revealed strong and specific connectivity among these patches suggesting that they form a network with strong connections. Similar category-specific regions for scenes and color have also been found using fMRI. Using resting state fMRI data, we found that a similar specific connectivity can be seen non-invasively among the face patches. Scene and color patches also show resting state functional connectivity within the respective networks but neither as strong nor as specific as face patches. Moreover, we found similar functionally-connected networks that do not overlap with any known category-selective regions in IT cortex. We targeted nodes within these networks for electrical stimulation to further explore the connectivity of these regions, and understand the relationship between electrical stimulation- and resting-state fMRI-defined connectivity. Overall, our results show that resting state fMRI connectivity provides a useful guide to to explore IT cortex, especially regions not yet identified as selective for any particular category.

Disclosures: **J.S. Guntupalli:** None. **D.Y. Tsao:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

Support: NSF CAREER BCS 0847798

Feodor Lynen Research Fellowship from the Humboldt Foundation

Title: Location, location, location: face recognition critically depends on the information processing of individual face patches

Authors: *S. MOELLER[1], D. Y. TSAO[2];
[1]Dept. Animal Physiol., Tuebingen, Germany; [2]Caltech, Pasadena, CA

Abstract: We know that objects are processed by the inferior temporal cortex (IT); however, we do not know how object vision is organized within IT. There is evidence that objects are coded in a distributed fashion by large parts of IT; but face processing seems to be concentrated into six separate nodes, the face patches. While these face patches are only strongly connected within themselves, it is so far not clear whether face patch activity is necessary or sufficient for face perception or whether the activity of the surrounding cortex also supplies useful information about faces. We trained two monkeys on a delayed match to sample task, showing two images in sequence separated by a delay, followed by saccade targets, one to report images of the same identity and one to report images of different identity. We used fMRI (Siemens Tim Trio, 1 mm resolution, MION contrast agent) to localize face patches in both monkeys, and targeted 5 patches for perturbation experiments. In those experiments we perturbed the targeted face patch by electrical microstimulation (e.g.: 200 μA bipolar pulses @150 Hz for 200 ms) during the presentation of the second image. Microstimulation inside each of these 5 face patches resulted in a strong bias to report faces as different, but did not affect (most) non-face object identification. This bias appeared as a strong performance decrease in same-identity trials (performance change in % points by face patch: MF -55.35; ML -74.63; AF -44.59; AL -68.58; AM -84.65). Stimulation outside of the face patches did not affect face identification behavior. Interestingly, stimulation in the face patches also affected identification of non-face objects whose overall shape was consistent with a face (e.g., apples) albeit weaker than face identification. The magnitude of the stimulation effect on face, but not apple and citrus fruit identification significantly correlated with the stimulation site's face selectivity; showing that the large effect on faces required precise targeting to the core of a face patch, while the milder effect on face-compatible objects did not. Perturbing the activity of each individual node in the face patch network strongly disrupts face recognition behavior. Perturbed face patch activity cannot be overridden by un-perturbed activity of non-face selective IT cortex. Together this shows face

identification critically depends on information processing in the face patches but not the distributed activity of larger areas of IT cortex. The effects also indicate that while faces are encoded by face-selective regions, activity in these regions can also affect the percept of a large number of face compatible non-face objects.

Disclosures: S. Moeller: None. **D.Y. Tsao:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

Support: NSF Grant 1157121

Title: Dynamic relations between the left and right fusiform cortex in the spatially cued detection of faces

Authors: *M. MENG[1], B. GUO[1], J. GOOLD[1], H. LUO[2];
[1]Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [2]Peking Univ., Beijing, China

Abstract: A fundamental issue in understanding brain functions lies in the relationship between the left and right cerebral hemispheres. It is known that human face perception appears to be dominant in the right fusiform cortex, although face-selective responses have been identified bi-laterally. Moreover, activity in the left fusiform cortex was found to correlate with the perceived face semblance in non-face stimuli, whereas the right selectively responded to only faces (Meng et al., 2012). However, the question remains unknown of how the two hemispheres coordinate to form unified face representations. One possibility is that synchronized oscillatory activity across hemispheres may enable the coordination (Fries, 2005; Doron, Bassett, & Gazzaniga, 2012). To test this possibility, we combined an fMRI study with a novel experimental design that enables time-resolved sampling of brain responses across trials. This design has been successfully used to measure rhythmic behavioral effects, such as reaction time and response accuracy, in spatial and object-based attention (e.g., Fiebelkorn, Saalmann, & Kastner, 2013; Song et al., 2014). In each trial, an uninformative cue was presented in the left or right hemifield. Participants were asked to make speeded responses of whether a face or a house was presented, at either the cued or un-cued location. Critically, the cue-to-target stimulus onset asynchrony (SOA) varied trial-by-trial in small steps of 20ms, from 200ms to 1080ms. Bilateral fusiform face areas (FFA) and parahippocampal place areas (PPA) were localized with separate fMRI scans. On average, across

all SOAs, activity in the left FFA depended on whether the target had been shown in the contralateral hemifield or ipsilateral hemifield, whereas activity in the right FFA invariantly corresponded with the face/house target. More interestingly, the spatial cue led to significant theta-band (5Hz) oscillations of the activation patterns in the FFA as a function of SOA, complementing the results of another SFN2015 abstract, in which we report similar rhythmic effects of visual priming in the FFA and PPA. Together, our results suggest: 1) asymmetric response functions of visual hemifield in the left and right FFAs, and 2) reliable neural correlates of the theta-band oscillation induced by the spatial cue shown to each hemifield. These findings shed light on how the left and right cerebral hemispheres may work dynamically in tandem.

Disclosures: **M. Meng:** None. **B. Guo:** None. **J. Goold:** None. **H. Luo:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

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Support: Canada Graduate Scholarship: Master's CIHR

NSERC Discovery Grant

Title: Rapid, accurate detection of faces but not houses suggests a visually coarse but context sensitive subcortical face processing mechanism

Authors: ***L. M. CABRAL**, B. STOJANOSKI, R. CUSACK;
Western Univ., London, ON, Canada

Abstract: Recent neuroimaging and behavioural investigations into face processing have suggested a role for subcortical structures. Two conflicting views have emerged about the nature of the representations: subcortical structures are responsible for 1) coarse representations such as those involved in orienting to faces and 2) more fine grained representations at the level of face identification. The aim of the current experiment is to examine 1) the level at which faces are represented and 2) whether context influences the extent to which subcortical areas are recruited. We hypothesized that a rapid subcortical mechanism would facilitate face detection at fast reaction times, but that no such mechanism would be present for houses. In one block, participants (N=22) performed a face detection task, pressing a button for intact faces, but not for scrambled distractor stimuli. In another block, they performed the same task for houses. Block

order was counterbalanced. The distractor stimuli were matched for lower level visual features, but were unrecognizable. Presentation was monocular. Stimuli were randomly presented to the temporal or nasal visual field. Each participant's reaction times were divided into five bins, where bin each spanned an equal percentile range. At slower reaction times, detection accuracy was generally similar. However, at the fastest reaction times, participants were more accurate at identifying faces than houses ($p < 0.05$), supporting the idea that a rapid, subcortical mechanism facilitates face detection. No difference was found across visual fields. A second experiment (N=22) was conducted to examine the precision of the subcortical route. Less scrambled distractor stimuli were used, requiring a finer visual discrimination. In experiment 2, there was no subcortical advantage; at fast reaction times faces were not detected more accurately than houses. Finally, we tested whether the subcortical route was automatically recruited, or whether it depended on the task context. A subset of participants (N=11) completed an experiment requiring fine (cortical) visual discriminations and then completed an experiment requiring coarse (subcortical) discrimination. Under these conditions, even for coarse discriminations, faces were not detected more accurately than houses. This suggests that when participants had recently performed a task that recruited a cortical route, subcortical structures no longer effectively contributed to face processing. These results support a rapid, subcortical mechanism that exists to detect faces, which is only effectively engaged in coarse visual discriminations, and is sensitive to the task context.

Disclosures: **L.M. Cabral:** None. **B. Stojanoski:** None. **R. Cusack:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

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Topic: D.04. Vision

Support: KAKENHI 25120009

KAKENHI 26120535

Title: Information representation in monkey area TE for global categorization of faces and for upright versus inverted face categorization

Authors: ***N. MATSUMOTO**[1], Y. SUGASE-MIYAMOTO[1], K. KAWANO[2], M. OKADA[3]; [1]AIST, Tsukuba, Japan; [2]Kyoto Univ., Kyoto, Japan; [3]Univ. Tokyo, Kashiwa, Japan

Abstract: Sparse representation might be useful for improving memory storage in an artificial neural network. Some researches indicate that information is sparsely represented in the brain. We have reported that face-responsive neurons in monkey area TE represent information about a global category, namely human faces vs. monkey faces vs. simple shapes and about an upright vs. inverted face category. To examine whether the global categorization and the upright vs. inverted face categorization are sparsely represented in area TE, we analyzed activities of 119 face-responsive neurons in area TE of two rhesus monkeys (Macaca mulatta), performing a fixation task. The test stimuli were colored pictures of monkey faces (4 models with 4 expressions), human faces (3 models with 4 expressions), and inverted pictures of these faces. The population activity vectors consisting of mean spike counts across trials to each stimulus were computed in a 50-ms time window slid by 5 ms from 25 ms to 350 ms after each stimulus onset. Sparse logistic regression (SLR) was applied individually in each window to the population vectors for the upright human vs. monkey faces (GL), for the upright vs. inverted human faces (HUI), and for the upright vs. inverted monkey faces (MUI). In the 115-165 ms time window, when the Euclid distance between the gravity centers of human population vectors and of monkey vectors was the largest, the number of neurons selected to categorize GL, HUI, and MUI was 2, 2, and 5, respectively. Averaged spike counts revealed that the two neurons selected for GL by SLR also showed a significant effect of GL and that the two neurons selected for HUI by SLR showed a significant effect of HUI (one-way ANOVA, $p<0.01$). On the other hand, three out of the 5 neurons selected for MUI by SLR did not show a significant effect of MUI (one-way ANOVA, $p>0.01$). Each of the 3 neurons did not support to differentiate MUI, but a combination of one of the 3 neurons and one of the remaining two neurons did categorize MUI. In all time windows, the number of neurons selected in common for HUI and MUI was significantly larger from the number for GL and HUI (Wilcoxon signed-rank test, $p<0.01$) and for GL and MUI ($p<0.01$). The number selected in common for GL and HUI was not significantly different from the number for GL and MUI ($p>0.01$). The results suggest that only a small number of neurons in area TE are necessary to categorize human vs. monkey and upright vs. inverted faces and different members of the neuronal population contribute to categorize GL and HUI or MUI, and that HUI and MUI have contributing members in common.

Disclosures: **N. Matsumoto:** None. **Y. Sugase-Miyamoto:** None. **K. Kawano:** None. **M. Okada:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.11/L23

Topic: D.04. Vision

Support: NIH IRP

Title: The face inversion effect in rhesus macaques

Authors: O. B. TOMEO, *N. LIU, S. W. LI, L. G. UNGERLEIDER;
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Abstract: Face perception plays a critical role in social communications and interactions. Given the similarities between monkeys and humans in the neural circuitry underlying social cognition, the rhesus macaque could provide an ideal animal model to study face processing. Although human and monkey neuroimaging studies have demonstrated similar face patch systems, behavioral studies exploring face processing in monkeys, unlike those in humans, have yielded inconsistent results. Here, we sought to clarify face-processing mechanisms by re-examining in macaques the face inversion effect, a phenomenon well documented in humans, which refers to greater difficulty in recognizing inverted faces compared to inverted non-face objects. Head-fixed rhesus macaques were trained to perform an oculomotor delayed match-to-sample (DMS) task. Stimuli were from six categories (faces: macaque, chimpanzee, human and sheep; objects: shoes and cars). Faces were forward facing and emotionless. All stimuli were contrast-normalized and grayscale. Faces were cropped with an oval mask to isolate central face information and exclude peripheral features that could be used to cheat. Subjects were first trained on the DMS task with simple shapes until achieving 85% accuracy, and then performed the test for each category equally per day. Reaction time (RT), accuracy and pattern of eye movement were recorded; the efficiency score (RT/accuracy) was used to account for the trade-off between RT and accuracy. We found that scan patterns were similar when viewing macaque, chimpanzee and human faces but not sheep faces. However, better efficiency scores for recognizing upright stimuli than the inverted ones were only found for macaque and chimpanzee faces but not for human faces, sheep faces or non-face objects. These results thus revealed a face inversion effect only for conspecific (macaque) and heterospecific (chimpanzee) faces, implying that macaques process both macaque and chimpanzee faces holistically. Moreover, our data support the idea that the effect is specific for stimuli for which the individual has developed expertise.

Disclosures: O.B. Tomeo: None. **N. Liu:** None. **S.W. Li:** None. **L.G. Ungerleider:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

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Topic: D.04. Vision

Support: Cedars-Sinai Medical Center

Grant/Other Support: Caltech Conte Center for the Neurobiology of Social Decision Making from NIMH

Title: The effects of crowding on the tuning and latency of neurons in human and monkey amygdala

Authors: *J. MINXHA[1], J. K. MORROW[4], C. P. MOSHER[4], A. N. MAMELAK[5], K. GOTHARD[4], R. ADOLPHS[2,3], U. RUTISHAUSER[5,3];
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Abstract: The electrophysiological characteristics of primate amygdala neurons have been explored in a variety of visual tasks. While previous work in this area has shed some light on the properties of the cells in the amygdala, the electrophysiological response has typically been measured with respect to the stimulus onset, leaving the relationship with eye movements largely unexplored. To evaluate the link between cell responses and eye movements, we recorded from single neurons in the amygdalae of eight neurosurgical patients who were implanted with depth electrodes for the purpose of monitoring their seizures. In addition, we also recorded from the amygdalae of 3 rhesus macaque monkeys as they performed the same task as the human subjects. We recorded eye-movements as both monkeys and humans freely scanned an array of images arranged in a circle around a fixation point. The images depicted items from six categories: fractals, flowers, human faces, monkey faces, cars, and fruits. The competing visual targets elicited a sequence of fixations and saccades (scan path) that resembled more closely the natural pattern of eye movements. Aligned at fixation onset, the response of neurons was highly sensitive to which images were fixated. Although we found neurons responding to fixations on images of all six categories, the greatest number of cells responded to fixations on human and monkey faces. The majority of these face-selective cells were tuned to conspecifics in both the monkeys (n=78 cells, 1% fractals, 1% flowers, 47% monkey faces, 4% human faces) and humans (n=112 cells, 20% human faces, 14% monkey faces, 2% fractals, 2% flowers). The response latency of image-selective neuron in humans was on average 60 ms longer than in monkeys (relative to fixation onset). Furthermore, this response latency depended on the number of images presented simultaneously to the subject. In humans, the response latency was 348 ms for a single image and 256 ms for an array of 8 images. In monkeys, the response latency was 320 ms for a single image and 150 ms for an array of 8 images. This difference in both species

might be due to the allocation of attention that precedes fixation on the target image. Together, this data shows both similarities and differences between neural responses in the human and monkey amygdala. In both species, (a) image-selective neurons were sensitive to the number of visual targets available; b) cells were sensitive to the context in which visual targets are presented; c) higher tuning preference for the faces of conspecifics. Neurons in the human amygdala, however, responded systematically later compared to the neurons in the monkey amygdala.

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Poster

149. Visual Processing: Representation of Faces and Bodies

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Support: A post-doctoral fellowship from the Autism Science Foundation

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Caltech Conte Center for the Neurobiology of Social Decision Making from NIMH

Title: Parametric encoding of emotion and ambiguity in the human amygdala

Authors: *S. WANG[1], R. YU[3,4], M. TYSZKA[2], S. ZHEN[4], S. SUN[4], R. HURLEMANN[5], I. B. ROSS[6], A. N. MAMELAK[7], R. ADOLPHS[1], U. RUTISHAUSER[7,2];
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Abstract: The human amygdala plays a key role in processing facial emotion, as evidenced by lesion, functional neuroimaging, and human electrophysiological studies. Here for the first time we combine all three approaches to elucidate the amygdala's role in encoding emotions expressed by faces. We employed a face morph task with fear-happy morphs (ranging from 30% fear/70% happy to 70% fear/30% happy as well as 2 anchor faces of fear and happy without

ambiguity). In each trial, subjects judged whether the emotion was ‘fear’ or ‘happy’. Behavioral performance varied systematically as a function of morph levels: anchors were reliably identified whereas the most ambiguous intermediate stimuli variably resulted in a fear or happy judgment. This task is thus well suited to differentiate sensory representations from decisions. BOLD-fMRI analysis (N=19 healthy subjects) showed that our faces activated the amygdala reliably within a region of interest specified both anatomically and functionally by an independent localizer task. Both emotion identity (morph level) and emotion uncertainty (ambiguity level) modulated BOLD-fMRI amygdala activity, with happier faces and unambiguous faces resulting in the largest BOLD response. To investigate the detailed response characteristics at the cellular level, we next recorded single neurons from the human amygdala in epilepsy patients implanted with depth electrodes (235 units, recorded in 14 sessions from 9 neurosurgical patients). The response of 33 neurons (14.0%, binomial $P<1e-7$) correlated with the gradual change of emotion identity (linear regression of average firing rate across trials vs. morph levels, $P<0.05$; 21 and 17 neurons increased and decreased their activity as a function fearfulness, respectively). A separate population of 36 neurons (15.3%, binomial $P<1e-9$) signaled levels of ambiguity (trial-by-trial linear regression of average firing rate in a 1.5s time window 250ms after face onset vs. ambiguity levels, $P<0.05$). Of these, the majority of neurons (30/36) had the largest firing rate for the anchors. Population level decoding showed that during ambiguous trials, amygdala single neurons correlated with the subjective decision (fear or happy) given the same stimuli. Lastly, three patients with focal bilateral amygdala lesions who performed our task were more likely to identify stimuli as fearful compared to healthy controls and the neurosurgical patients. This confirms the amygdala’s causal role in our task. Taken together, our results reveal a significant functional role of the amygdala in making decisions about facial emotions.

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Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

Support: DFG (Th425/12-1)

Title: Following the eye gaze of others activates a patch of cortex that is not part of the "face patch" system

Authors: *K. MARQUARDT, H. RAMEZANPOUR, P. W. DICKE, P. THIER;
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Abstract: Human observers follow another person´s eye gaze to objects and locations of interest to the other one, thereby establishing "joint attention", a first and major step towards developing a theory of the other´s mind. Previous fMRI studies agree that a small patch of cortex in the posterior superior temporal sulcus (STS) is specifically implicated in eye gaze following. This "gaze following patch" (GFP) is located in the same general region known to be part of the core face processing system that consists of several distinct patches in the STS and ventral visual cortex, the "face patch" system. Hence, the GFP might actually correspond to one of the "face patches". In order to test this hypothesis, we carried out an fMRI experiment in which we tried to clarify the spatial relationship of the GFP and the face patch system in the same subjects. In order to identify the GFP, we resorted to the paradigm introduced by Materna et al. J. Cogn. Neurosci. 2008. Subjects were asked to make saccades to distinct spatial targets based on information provided by a human portrait presented to the observer. Depending on the instruction, subjects either had to rely on the seen eye orientation to identify the correct target or, alternatively, they had to use the color of the eyes, changing from trial to trial but always matching to one of the targets, in order to make a saccade to the target having the same color. In other words, the only difference between the two tasks was the information, subjects had to exploit in order to solve the task and the performance levels achieved in both were actually not different. We localized the face patch system in a separate experiment in which subjects passively viewed images of faces as well as a variety of biological and non-biological non-face stimuli while maintaining fixation of a central cue. In correspondence with previous works (e.g. Tsao et al., PNAS, 2008) passive viewing of faces elicited BOLD activity within a distributed cortical network that included the occipital face area (OFA), the fusiform face area (FFA), as well as patches in the posterior superior temporal sulcus (pSTS) and the inferior frontal gyrus (IFG). In accordance with Materna et al., gaze following as assessed by the gaze following vs. identity matching BOLD contrast activated a distinct GFP in the pSTS, with its activation maximum separated by more than 12mm (euclidean distance) from the nearest member of the "face patch system". This segregation suggests a distinct function of the GFP beyond the elementary processing of facial information accommodated by the face patch system. On the other hand, it would of course be fully compatible with members of the face patch system serving as major input to the GFP.

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Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

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Topic: D.04. Vision

Support: DFG (TH 425/12-1)

Title: Role of dorsolateral prefrontal cortex in the cognitive control of gaze following

Authors: *M.-S. BREU, H. RAMEZANPOUR, P. DICKE, P. THIER;
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Abstract: Non-verbal communication is indispensible for successful social interactions. In humans, the face and the eyes are major sources of information on the other´s mental state. It is the direction of gaze that informs us about objects another person might be interested in. By following the other´s gaze to this object, we establish "joint attention". And by mapping our own object-associated needs, interests and aspirations onto the other, we develop a theory of her/ his mind. Human gaze following is a fast and almost reflex-like behavior, yet, it can be controlled, if not opportune, for instance in an attempt to disguise interest in the other one. As shown by fMRI studies, the major substrate of human gaze following is a well defined region in the posterior superior temporal sulcus ("pSTS") (Materna et al., J. Cogn. Neurosci., 2008; Marquardt et al., SfN 2015), arguably also the target of the executive control of gaze following. In order to identify the source(s) of the putative control signal(s), we carried out an fMRI-experiment, in which humans subjects were exposed to gaze following cues which - depending on the prevailing instruction - were used to establish joint attention or not. More specifically, the experimental subjects saw the portrait of a female ("sender"), looking with her eyes at one out of five targets in front of her. Subjects had to follow the gaze of the sender with a saccade to the target. Alternatively, they had to ignore gaze and to look at the target whose color matched the sender´s (variable) eye color. The two trial types were presented randomly interleaved. The instruction to choose the target based on gaze direction or on eye color was presented before a particular trial started. In accordance with previous work, gaze following activated the right pSTS region. Moreover, we could identify a circumscript region in the right dorsolateral prefrontal cortex, being part of Brodmann area 46, that was specifically activated in color matching trials in which gaze following had to be suppressed. This result suggests that area 46 plays a central role in the context-dependent control of human gaze following.

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Poster

149. Visual Processing: Representation of Faces and Bodies

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Topic: D.04. Vision

Support: Feodor Lynen Scholarhip from the Alexander von Humboldt Foundation

Title: Neural signatures of eye gaze and face identity representations in MEG data

Authors: *J. LI[1], M. FANG[2], Q. LI[5], R. CICHY[3], D. PANTAZIS[4];
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Abstract: Human face perception is a core cognitive function, crucial for social interaction, communication and cooperation. It involves two orthogonal functions: one is to identify a person's identity by analyzing the invariant features of a face while neglecting it's changeable features, the other what the person is feeling, or attending to by analyzing the state of the changeable features irrespective of identity. While the locations of brain networks involved in either function have been well described, the description of temporal dynamics within those regions has remained challenging. Neural responses to identity and gaze processing overlap in time, making singling each of them out problematic using non-invasive methods in humans that have only low spatial resolution. To address this issue we investigated the encoding of gaze or identity as a function of task context: We reasoned that a task requiring attention to either identity or gaze would specifically boost function-specific neural activity, allowing dissociation of identity and gaze processing. We recorded magnetoencephalography data (MEG) while participants conducted either a gaze or identity 1-back repetition detection task on a set of images (3 individuals with 3 eye gaze directions each, i.e. 9 images). Trials were ordered in blocks by task. We then used multivariate pattern classification to decode facial identity and gaze direction dependent on task. In line with our prediction, we found that eye gaze was discriminated by visual representations during the eye gaze task for several hundred milliseconds after the stimulus, while decoding fluctuated around baseline for the identity task. Unexpectedly, we did not find the reverse effect for identity: identity was discriminated by visual representations in the eye gaze task but not in the face identity task. Taken together, these results indicate that while eye gaze representations are readily captured by MEG, face identity representations are more challenging to measure. Our results also suggest that face identity representations might highly dependent on the attended area of the face.

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Poster

149. Visual Processing: Representation of Faces and Bodies

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Topic: D.04. Vision

Support: NEI grant 1R01EY02391501A1

Title: Task differentially modulates the spatial extent of category-selective regions across anatomical locations

Authors: *L. BUGATUS[1], K. WEINER[1,2], K. GRILL-SPECTOR[1,2];
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Abstract: The human brain contains separate functional networks consisting of multiple regions across occipital, temporal, and prefrontal cortices that are involved in processing faces and words. Prior research shows that cognitive tasks performed by subjects while viewing these stimuli modulate neural responses. However, it is unknown if task effects vary by (a) macroanatomical location, (b) stimulus category, and (c) hemisphere. To address these gaps in knowledge, we examined how different tasks affect representations of faces and words across the cortex. We scanned 12 subjects using fMRI at 3T. Subjects viewed stimuli from five categories (faces, bodies, houses, cars, and pseudo-words) while performing one of three tasks. In the oddball task, subjects detected a randomly presented phase-scrambled image. In the working memory task, subjects indicated when a stimulus repeated after an intervening image (2-back). In the selective attention task, subjects indicated when an attended image of two superimposed images was upside down. We examined how these tasks affected category-selective representations in three anatomical regions: lateral occipitotemporal cortex (LOTC), ventral temporal cortex (VTC), and ventrolateral prefrontal cortex (VLPFC). We report three main findings. First, multivoxel pattern (MVP) analyses revealed differential effects of task across anatomical regions: in LOTC and VTC, MVPs for a category were similar across tasks (LOTC: $r = 0.45 \pm 0.08$; VTC: $r = 0.5 \pm 0.08$), while in VLPFC, MVPs for a category differed across tasks ($r = 0.09 \pm 0.09$). Second, quantifying the consistency of category-selective voxels ($t>3$) across tasks revealed a triple-interaction among hemisphere, category, and task ($F(5,672) = 5.55$, $p < 0.001$): in LOTC and VTC, the same voxels displayed category-selectivity across tasks, whereas in VLPFC, different voxels displayed category-selectivity in different tasks, with word-selective voxels lateralized to the left and face-selective voxels lateralized to the right VLPFC. Third, we observed selectivity differences for faces and words across tasks ($F(2, 230) = 11.73$, $p < 0.001$): for faces, the highest selectivity was during working memory, while for words, the highest

selectivity was during selective attention, except for left VLPFC in which it was highest during working memory. Together, these results reveal a complex interaction of stimulus, hemisphere, and task, affecting representation of category-selective responses. In turn, this suggests that the multiplicity of face- and word-selective regions may be related to task-relevant functional differences of separate components of these functional networks.

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Poster

149. Visual Processing: Representation of Faces and Bodies

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Support: NIH Grant R01 EY023384

Title: The representation of semantic content and attentional state in medial temporal lobe during visual processing of natural scenes: an ECoG study

Authors: Z. SABRA, J. BREEDLOVE, L. BONILHA, *T. NASELARIS;
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Abstract: Brain areas within the medial temporal lobe (MTL) are critical for a variety of cognitive functions, most notably declarative memory. MTL is also anatomically and functionally connected to areas within the ventral visual stream. However, the role of MTL in ventral stream visual processing is currently unclear. MTL may act primarily as a source of purely cognitive, top-down signals that modulate visual processing; it may also be possible that it has a more purely sensory role in the ventral stream representation of the semantic content of scenes. To address this issue, we recorded electrocorticographic (ECoG) potentials from hippocampus and surrounding MTL cortical areas with the goal of answering a simple question: Is variation in neural activity in MTL driven more by changes in attentional state or by changes in stimulus content? Two patients implanted bilaterally with depth electrodes in MTL viewed a stream of natural scenes that prominently featured a face, a building, or a car. Subjects were instructed to fixate a small dot at the center of each image while attending to one of the stimulus categories (i.e., attend to either face, building, or car). Image presentation was divided into blocks of 50 images. The attended category was fixed during each block, while the stimulus category was randomly interleaved. Subjects demonstrated compliance by performing a simple identification task. Image and inter-stimulus interval durations were randomized. For each

attentional/stimulus condition (for example, attend face/view car) we estimated a distinct finite impulse response function (FIR). We then quantified the amount of variation in the FIR due to changes in the attended category, as well as the amount of variation due to changes in the stimulus category. We found that variation in the FIR due to changes in attended category was significantly larger than variation due to changes in stimulus category for more than 70% of the electrode contacts in MTL. These preliminary results suggest that variation of activity in MTL is driven more by variation in attended category than by variation in stimulus category.

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Poster

149. Visual Processing: Representation of Faces and Bodies

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Support: the US National Institutes of Health Intramural Research Program of the National Institute of Mental Health

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Title: Increased distinctiveness of visual face representations during memory retrieval compared to perception

Authors: *S.-H. LEE[1,2], B. A. LEVY[2], C. I. BAKER[2];
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Abstract: Despite the high similarity of human faces, we can easily discriminate and recognize face identity, and can retrieve how people look. Here, we asked how individual face information is represented in the visual cortex during perception and retrieval. To address this question, we performed an event-related functional magnetic resonance imaging (fMRI) experiment, comprising separate perception, learning and retrieval sessions. During the perception session, which took place inside the scanner, participants were presented with fixed pairings of six auditory cues (pseudowords) with six face images (e.g. ‘greds’- man1, ‘drige’-man2), and six auditory cues with six shoe images. During the learning session, which took place on a separate day outside the scanner, participants were trained to memorize the pseudoword-image associations for about one hour. Finally, one day after the learning session, participants were

scanned again and instructed to retrieve each image in response to auditory presentation of the paired pseudoword cue. To test the veracity of the retrieved visual information, participants were asked to perform forced-choice tests, in which they heard one of the pseudoword cue and chose the paired category or image, after the retrieval scan session. Every participant showed near perfect performance in the forced-choice test (> 95% correct). We focused on the patterns of response in face-selective cortical areas. Using multivoxel pattern analyses, we found that anterior face-selective areas showed more discriminable patterns of response to individual faces during retrieval compared to those elicited during perception whereas those areas did not show any significant difference between perception and retrieval for individual shoe images. To determine whether the increased discrimination reflected a difference between perceived and retrieved face information and not an effect of learning, we conducted a similar fMRI experiment in which the second session was also perception and not retrieval. Importantly, there was no difference in face discrimination between the first and second perception sessions in anterior face-selective areas. Taken together, these results suggest that retrieval of face information generates more discriminative neural responses for individual faces than that evoked by perception of the very same faces.

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Poster

149. Visual Processing: Representation of Faces and Bodies

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Support: ERC Grant facessvep 284025

Belgian National Foundation for Scientific Research (FNRS)

Title: Temporal dynamics and quantification of face-selective responses to natural images

Authors: T. L. RETTER, *B. ROSSION;
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Abstract: Despite a wealth of research in human vision, the quantification and temporal dynamics, i.e., the onset, evolution, and duration of complete face-selective responses to briefly visible images remains largely unknown. Here, we present various nonface object images at a fast periodic rate (one image every 80 ms, i.e., 12.5 Hz), with natural face images inserted every

3, 5, 7, 9, or 11 stimuli (240 to 880 ms, i.e., 4.17 to 1.14 Hz) during electroencephalographic (EEG) recording. This fast periodic visual stimulation allows for quantification of complete face-selective responses in the frequency domain, identified at the various pre-defined face stimulation rates objectively and directly (without subtraction). These responses are the largest over the right occipito-temporal cortex and are significantly reduced below 400 ms of face onset asynchrony (i.e., at 240 ms), indicating that the duration of a face-selective response exceeds that amount of time. In the time domain, face-selective responses emerge shortly after 100 ms following stimulus onset and, despite a brief stimulus duration, they possess a succession of components with slightly different spatio-temporal signatures over the occipito-temporal cortex until shortly after 500 ms. These observations clarify a number of key issues related to investigations of human face categorization, such as the quantification of face-selective responses in the human brain, the distinction between evoked EEG responses to slow and fast, periodic repetitive stimulation (i.e., "ERPs" vs. "SSVEPs"), and the exact projection of a fixed onset delay to each successive temporal response. More generally, they go a long way towards understanding the temporal dynamics of face-selective responses to natural images in the human brain, paving the way for investigating its neural basis and relationship to face categorization behavior.

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Poster

149. Visual Processing: Representation of Faces and Bodies

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Title: Face-evoked visual field potential generators in the macaque temporal lobe

Authors: *Y. KAJIKAWA[1], C. E. SCHROEDER[2,1];
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Abstract: When we communicate with others, we look at their faces that trigger a sequence of sensory activation in occipital and temporal lobes. fMRI studies in humans show face selective

cortices in those regions: the fusiform face area (FFA), the occipital face area (OFA) and the superior temporal sulcus (STS). Electrophysiologically, viewing face evokes visually evoked potential (VEP) that contains occipito-temporal positive peak at 100 ms (P1) followed by occipito-temporal negative peak at 170 ms (N170). N170 is the largest peak and is widely used to study the mechanisms of face perception and to index social cognitive dysfunction. In contrast, the face-evoked VEP has not been studied well in macaques. We trained macaque monkeys to perform audiovisual (AV) vocalization discriminations. They pulled a lever to initiate trials, and in each trial, monitored a repetition of AV vocalizations (brief videos) to detect oddballs (e.g., change from a "bark" to a "coo") in either auditory or visual modalities. Upon detection of an oddball, monkeys had to respond by releasing the lever quickly to obtain reward. The behavioral requirement ensured that animals pay attention to both modalities. Stimulus sequences contained 3-6, 500 ms audiovisual movie clips of a conspecific vocalization interleaved with 600~1200 ms of silence with a static image. During the tasks, scalp potentials over the temporal lobe were recorded along with intracortical electrophysiological activity, recorded using linear array electrodes straddling the auditory cortex, and/or underlying superior temporal sulcal regions. Face-evoked VEPs on the macaque scalp started with a vertex positive peak near 70 ms (P1) followed by larger vertex negative peak at 110 ms (N1). Face-evoked field potentials with peaks near 110 ms, like the scalp N1, were recorded in the auditory cortex, the superior temporal polysensory area (STP), and the inferior temporal cortex (IT). Current source density analysis revealed local contributions to the scalp N1 that were consistent in IT, sporadic in STP, and weak or absent in the auditory cortex. These results indicated that face-evoked field potentials concurrent with scalp potentials are present in wide cortical regions of the temporal lobe. However, cortical activity generating face-evoked VEP is largely confined to the cortices in the superior temporal sulcus.

Disclosures: Y. Kajikawa: None. **C.E. Schroeder:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.22/L34

Topic: D.04. Vision

Support: MOE AcRF Tier 1

Title: Attention shifts and Microsaccades in dynamic bubbled faces

Authors: *H. XU, H. YING;
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Abstract: We recently showed that adapting to dynamic bubbled faces generated significant facial expression aftereffect, but adapting to static bubbled faces did not (Xu et al., VSS 2013, Luo & Xu, APCV 2013). It thus renders the question whether attention plays a role in the above difference. Microsaccade, a kind of unconscious eye movement typically occurs during prolonged visual fixation reflecting covert attention (Engbert & Kliegl, 2003), has been suggested to indicate attention shifts. Five subjects were tested in our study. The task was to judge the emotions (happy vs. sad) of 580 faces from three categories: static full faces, static bubbled faces, and dynamic bubbled faces. We recorded their judgment accuracy of these faces and reaction time. Simultaneously we recorded their eye movement by eye tracker (EyeLink II), and analyzed their microsaccades patterns in particular. We found that although there was no significant difference between judgment accuracy in the three face categories, the subjects spent more time on judging bubbled faces, and more frequent microsaccades toward bubbled faces, with larger amplitude of microsaccades to static bubbled faces. It thus suggests attention shifts in bubbled faces are different than in full faces, and the attention difference in dynamic and static bubbled faces. Our findings may shed light on the mechanism of attention in incomplete face perception.

Disclosures: H. Xu: None. **H. Ying:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.23/L35

Topic: D.04. Vision

Support: National Eye Institute of the National Institutes of Health Grant F32EY023479

Title: Word and text processing in developmental prosopagnosia

Authors: *C. RUBINO[1], J. C. CORROW[1], J. J. S. BARTON[2], S. L. CORROW[1];
[1]Ophthalmology and Visual Sci., [2]Ophthalmology and Visual Sciences, Med. (Neurology), Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Word and text processing in developmental prosopagnosia Cristina Rubino Jeffrey C Corrow Jason J S Barton Sherryse L Corrow Human Vision and Eye Movement Laboratory,

Departments of Medicine (Neurology) and Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, Canada Objective The recent 'many-to-many' hypothesis asserts that multiple cortical regions participate in processing multiple object types[1]. Studies of subjects with impaired face or word recognition provide an opportunity to test predictions of this hypothesis, that prosopagnosic subjects would have minor impairments in word processing, while alexic subjects would have subtle impairments in face perception. In this study, we evaluate whether subjects with developmenta*l* prosopagnosia have deficits in processing aspects of written text. Methods In a first experiment we evaluated the word-length effect for reading time in subjects with developmental prosopagnosia (n=9). In a second experiment we assessed their sorting of written text by word content across variations in font or handwriting style, and their sorting of text by handwriting or font style across variations in word content. Results In Experiment 1, prosopagnosic subjects had normal word-length effects. In Experiment 2, sorting time and accuracy for processing word content across stylistic variations was normal, When sorting text by stylistic properties two of the nine showed a mild reduction in accuracy for handwriting but not for computer font. Interpretation Subjects with developmental prosopagnosia have efficient single-word reading and can extract word identity across stylistic variations. Only a minority demonstrate mild difficulty processing stylistic properties of text, which contrasts with the frequent impairment of this process recently reported in acquired prosopagnosia. This may suggest a difference in either severity or selectivity between the acquired and developmental forms of prosopagnosia. Key Words: face, agnosia, words Footnotes [1] Behrmann M, Plaut DC. Distributed circuits, not circumscribed centers, mediate visual recognition. Trends Cogn Sci. 2013;17:210-219

Disclosures: C. Rubino: None. **J.C. Corrow:** None. **J.J.S. Barton:** None. **S.L. Corrow:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

Support: NIH Grant F32-EY023479

NIH Grant R01-DC009823

CIHR Grant MOP-102567

Canada Research Chair

Marianne Koerner Chair in Brain Sciences

Title: Tone deafness in developmental prosopagnosia - is there a common cause?

Authors: *S. CORROW[1], S. BUSS[3], H. LI[3], G. SCHLAUG[3], J. J. S. BARTON[2]; [1]Ophthalmology and Visual Sci., [2]Neurology/Ophthalmology and Visual Sci., Univ. of British Columbia, Vancouver, BC, Canada; [3]Neurol., Beth Israel Deaconess Med. Ctr. and Harvard Med. Sch., Boston, MA

Abstract: OBJECTIVE: Developmental prosopagnosia is a disorder of face recognition that is believed to reflect impairment of high-level visual mechanisms. Recent work has shown differences between those with prosopagnosia and control subjects in matter connectivity of the inferior longitudinal fasciculus. Amusia (e.g. tone deafness) is another developmental disorder which has been shown to have grey matter abnormalities in several non-primary perisylvian regions and is also associated with white matter connectivity in the arcuate fasciculus (Loui et al, 2009). Based on anecdotal reports of some subjects, we hypothesized that there may be instances in which these two disorders overlap. **METHOD:** Subjects with developmental prosopagnosia were compared to healthy control subjects on two measures. The Montreal Battery of Evaluation of Amusia examined melodic organization (scale, contour, and interval), temporal organization (rhythm and meter), and memory (DP n=7, Control - normative data from 160 healthy adults). In addition, subjects completed a pitch discrimination threshold task (DP n=8; Control n=10). For details of these test batteries, see Loui et al (2009) and Fujii and Schlaug (2014). **RESULTS:** On pitch discrimination, as a group, subjects with developmental prosopagnosia were impaired relative to controls [$p<0.05$], and at the individual level, 5 of 8 subjects with developmental prosopagnosia exceeded the mean for control subjects by at least 10 standard deviations. On the Montreal Battery of Evaluation of Amusia, the prosopagnosic sample was impaired relative to normative controls on their overall score [$p<0.01$] as well as specific scores for pitch interval perception [$p<0.001$] and tune memory [$p<0.05$]. At the individual level, 2 of 7 subjects with prosopagnosia were impaired on their global score and several were impaired specifically on contour (1), interval (2), scale (3), meter (1), and memory (1). **CONCLUSION/DISCUSSION:** At least some cases of developmental prosopagnosia have not only impaired face recognition but also show deficits found in congenital amusia, particularly impaired pitch discrimination. One possible explanation is that focal cortical abnormalities and connectivity profiles of brain regions in different domains could be abnormal, resulting in a co-occurance of amusia and prosopagnosia. Whether this is a primary white matter disconnection or secondary to a cortical migration disorder is not yet clear. Our future work will include high resolution MRI to reveal neural substrates that are common between both disorders to determine the anatomic substrate of this behavioral observation.

Disclosures: S. Corrow: None. **S. Buss:** None. **H. Li:** None. **G. Schlaug:** None. **J.J.S. Barton:** None.

Poster

149. Visual Processing: Representation of Faces and Bodies

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 149.25/L37

Topic: D.04. Vision

Support: NIH Grant F32EY023479

CIHR Grant MOP-102567

Title: Getting lost: Topographic skills in acquired and developmental prosopagnosia

Authors: *J. CORROW[1], S. L. CORROW[2], E. LEE[2], F. BURLES[3], R. PANCAROGLU[1], B. DUCHAINE[4], I. GIUSEPPE[3], J. J. S. BARTON[2];
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Abstract: Previous studies report that acquired prosopagnosia is frequently associated with topographical disorientation. However, whether this is associated with a specific anatomic subtype of prosopagnosia, how frequently it is seen with the developmental variant of this disorder, and what specific topographical function is impaired to account for this problem are not known. We studied ten subjects with acquired prosopagnosia from either occipito-temporal or anterior-temporal lesions and seven with developmental prosopagnosia. Subjects were given a battery of tests assessing cognitive processes important for topographical orientation, including house and scene recognition, the road map test, a test of path integration from optic flow, and cognitive map formation and use. House and/or scene recognition were frequently impaired after either occipito-temporal or anterior-temporal lesions in acquired prosopagnosia. Subjects with occipito-temporal lesions were also impaired in cognitive map formation: an overlap lesion analysis identified right fusiform and parahippocampal gyri as a likely correlate of this deficit. Path integration was intact in all participants, and only one subject with acquired prosopagnosia had mild difficulty with directional orientation on the road map test. Only one subject with developmental prosopagnosia had difficulty with cognitive map formation, and none were impaired on the other tests. We conclude that topographical disorientation in acquired prosopagnosia reflects impaired place recognition, with a contribution from poor cognitive map formation when there is occipito-temporal damage. Topographical impairments are less frequent in developmental prosopagnosia.

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Poster

150. TrpA1

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 150.01/L38

Topic: D.08. Pain

Support: Rustaveli GNSF Grant 31/40

Title: Behavioral study of trpa1 and trpv1 channels inactivation by nsaids

Authors: ***M. G. TSAGARELI**[1], I. NOZADZE[2], N. TSIKLAURI[2], G. GURTSKAIA[2], E. ABZIANIDZE[2];
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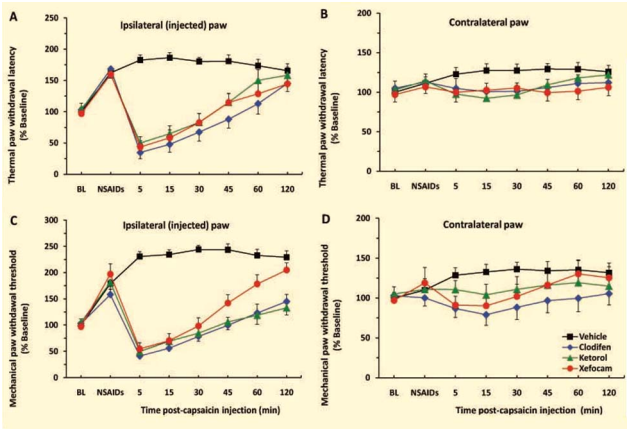
Abstract: Transient receptor potential (TRP) cation channels have been extensively investigated as targets for analgesic drug discovery. Because some non-steroidal anti-inflammatory drugs (NSAIDs) are structural analogs of prostaglandins, we examined three widely used non-steroidal anti-inflammatory drugs (NSAIDs) (clodifen, ketorolac, and xefocam) on the activation of TRPA1 and TRPV1 channels using thermal paw withdrawal (Hargreaves) test and mechanical paw withdrawal (von Frey) test in male rats. Thermal withdrawal latencies and mechanical thresholds for both hind paws were obtained with 5, 15, 30, 45, 60, and 120 min intraplantar post-injection of TRPA1 agonists' cinnamaldehyde (CA) from cinnamon and allyl isothiocyanate (AITC) a natural compound of mustard oil, and TRPV1 agonist capsaicin a natural compound of chili pepper, or vehicle. Twenty minutes prior to the start of the experiment, ketorolac or xefocam were pre-injected in the same hindpaw and animals were examined by these two tests. After pretreatment of all three NSAIDs in the ipsilateral (injected) hindpaw that produced strong antinociceptive effects, CA, AITC, and capsaicin caused significant decreases in latency of the thermal withdrawal reflex compared with vehicle or the contralateral hindpaw. The same findings were observed for the paw withdrawal threshold. In approximately 30 min the effects of CA, ITC, and capsaicin returned to baseline. The findings in this study are different from our previous results, where TRPA1 agonists CA and AITC and TRPV1 agonist capsaicin produced hyperalgesia for nearly 2 h and resulted in facilitation of these withdrawal reflexes. Thus, we show for the first time an inactivation of TRPA1 and TRPV1 channels by NSAIDs to channel agonists in behavioral assays.



Disclosures: **M.G. Tsagareli:** None. **I. Nozadze:** None. **N. Tsiklauri:** None. **G. Gurtskaia:** None. **E. Abzianidze:** None.

Poster

150. TrpA1

Location: Hall A

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Program#/Poster#: 150.02/L39

Topic: D.08. Pain

Support: EmmyNoether-Program of the Deutsche Forschungsgemeinschaft (SCHM 2533/ 2-1)

Title: TRPA1-protein complexes: tuning a noxious stimuli detector

Authors: ***L. AVENALI**, P. NARAYANAN, T. ROUWETTE, I. CERVELLINI, M. W. SEREDA, O. ABATE FULAS, J. SONDERMANN, O. JAHN, D. GOMEZ-VARELA, M. SCHMIDT;
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Abstract: The transient receptor potential A1 (TRPA1) channel is essential for vertebrate pain. TRPA1 plays a fundamental role as a primary detector of noxious stimuli of physical and chemical nature, and is critically involved in different pain states. Even though TRPA1 activation modalities have been studied extensively, the network of protein interactions regulating TRPA1 (the so-called TRPA1 interactome) is only poorly understood. Considering the crucial role of TRPA1 in pain signaling, it is mandatory to shed light on the elusive molecular machinery regulating TRPA1 channels in sensory neurons. To this end we established a mass spectrometry-based proteomics approach to identify and characterize proteins interacting with native TRPA1 channels from mouse sensory neurons. In this way we have recently uncovered

the physical association of Annexin A2 (AnxA2) with native TRPA1. Functional studies suggest that AnxA2 limits the availability of TRPA1 channels at the plasma membrane and furthermore regulates TRPA1-dependent nociception in mice. These results demonstrate a role for AnxA2 as an endogenous modulator of TRPA1 activity and define a mechanism capable of controlling TRPA1-mediated nociception in vertebrates. In addition, these findings underscore the idea that TRPA1 surface availability and function are controlled by protein-protein interactions and pave the way for a more thorough investigation of the dynamic changes in TRPA1-associated protein complexes. To accomplish this we used an established inflammatory pain mouse model and identified changes in TRPA1 interactome by state-of-the-art quantitative mass spectrometry. This work revealed TRPA1-protein complexes specifically altered in the context of inflammatory pain. New players in the development or maintenance of inflammatory pain might be uncovered, and their characterization is the focus of our current investigations. We anticipate that this study will contribute to our molecular understanding of the processes which give rise to TRPA1-dependent nociception and open the possibility to develop targeted therapeutics for specific TRPA1-related pain disorders.

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Poster

150. TrpA1

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 150.03/L40

Topic: D.08. Pain

Support: NIH Grant GM067762

Title: Possible contribution of TRPA1 to capsaicin-induced analgesia in deep tissue pain

Authors: *D. SUGIYAMA[1], S. KANG[1], H. GU[1], T. J. BRENNAN[2];
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Abstract: We have previously shown that peripheral nerve block with a selective TRPV1 agonist capsaicin (CAP) significantly inhibited non-evoked guarding pain behavior after deep tissue incision in rats. On the other hand, TRPV1 antagonist had limited analgesic efficacy on guarding pain. This suggests that TRPV1-expressing afferents, rather than the TRPV1 itself, are

crucial for non-evoked pain behavior after deep tissue incision. TRPA1 is mostly co-localized with TRPV1 in primary afferents, and recent studies suggest that TRPA1 may play a role in postoperative pain. Therefore, perineurally applied CAP may exert its analgesic effect, at least in part, via blocking pain signals transmitted by TRPA1 in deep tissue. Our preliminary data indicate that injection of H2O2 into deep tissue induced spontaneous pain behavior through TRPA1. We hypothesized that TRPV1-expressing afferents mediate spontaneous pain behavior evoked by activation of TRPA1 in deep tissue, and that nerve block with will inhibit this pain behavior. H2O2 (100 μM- 5 mM) induces Ca2+ influx via TRPA1 receptors in rat DRG neurons (L3-5) in a concentration-dependent manner. H2O2 elicited Ca2+ transients in 15-25% of DRG neurons, all of which also responded to TRPA1 agonist AITC (100 μM). The H2O2-evoked Ca2+ transients were inhibited by TRPA1 antagonists, HC-030031 (100 μM) and AP-18 (100 μM). We studied the relationship between H2O2- and CAP-responsive DRG neurons: the majority (92%) of H2O2-responsive neurons also responded to CAP (0.5 μM), and 52% of CAP-responsive neurons responded to H2O2. We examined the effect of nerve block with CAP on spontaneous pain behavior induced by injection of H2O2 into deep tissue in rats. Percutaneous sciatic nerve block was performed using the following drugs: (1) 0.05% CAP plus 0.5% bupivacaine (BUP) or (2) 0.5% BUP only (control). H2O2 (100 mM, 0.6 ml) was injected into the gastrocnemius muscle at various time points (1-7 days) following the nerve block, and the total time spent flinching, lifting, and licking was recorded for 60 minutes. Compared to control, CAP+BUP group showed significantly decreased H2O2-induced spontaneous pain behavior throughout the testing period ($p < 0.05$): duration of H2O2-induced pain behavior in CAP+BUP vs. control groups were 69 ± 74 vs. 2212 ± 1119 sec at 1 day after nerve block, and 1060 ± 1241 vs. 2337 ± 213 sec at 7 days after nerve block. Our results suggest that H2O2 causes deep tissue pain via TRPA1 receptors, and that interfering with TRPA1 activation may contribute, at least in part, to the CAP-induced analgesia in deep tissue pain. Blockade of TRPA1 receptors in deep tissue might provide an effective therapy for postoperative pain conditions.

Disclosures: **D. Sugiyama:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Hydra Biosciences. **S. Kang:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Hydra Biosciences. **H. Gu:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Hydra Biosciences. **T.J. Brennan:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Hydra Biosciences.

Poster

150. TrpA1

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 150.04/L41

Topic: D.08. Pain

Support: NIH Grant GM067762

Title: Hydrogen peroxide (H2O2) induces pain via TRPA1 receptors in deep tissue

Authors: ***S. KANG**, D. SIGIYAMA, H. GU, T. J. BRENNAN;
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Abstract: TRPA1 receptors mediate inflammatory and nerve injury pain, making them key targets for pain therapies. TRPA1 may have a role in postoperative pain. While various endogenous chemicals that activate TRPA1 have been identified, the endogenous TRPA1 ligand that mediates postoperative pain is currently unknown. Hydrogen peroxide (H2O2) activates TRPA1 in organs and is generated in wounds to recruit leukocytes and support the wound healing process. Previously, we have shown that incised deep tissue rather than skin has a central role in the genesis of spontaneous activity in pain transmitting pathways and non-evoked guarding behavior after plantar incision in rats. Therefore, we hypothesized that H2O2 exerts its nociceptive effects, particularly spontaneous pain, via TRPA1 in the deep tissue. To evaluate H2O2-induced spontaneous pain behavior, various concentrations (10-100 mM) and volume (0.4-1.0 ml) of H2O2 was injected into the gastrocnemius muscle or subcutaneous (S.C.) tissue overlying the gastrocnemius muscle in SD rats, and the total time spent flinching, lifting, and licking was recorded for 60 minutes. The control group was injected with same volume of synthetic interstitial fluid (SIF) instead of H2O2. Intramuscular (I.M.) injection of H2O2 produced spontaneous pain behavior in a concentration- and volume-dependent manner. I.M. injection of H2O2 (0.6 ml, 100 mM) produced greater spontaneous pain behavior (1675 ± 378 sec), compared to S.C. injection (83 ± 74 sec; $p=0.0045$). I.M. injection of SIF did not produce pain behavior (27 ± 19 sec). Pre-treatment with locally injected TRPA1 antagonists, HC-030031 or AP-18 (0.3 ml, 50mM each), significantly reduced pain behavior after I.M. injection of H2O2 (0.3 ml, 100 mM), ($p < 0.0001$). To further evaluate spontaneous pain behavior induced by H2O2, Conditioned Place Aversion (CPA) test was conducted in three separate groups of animals: (1) I.M. and (2) S.C. injection of H2O2, and (3) I.M. injection of SIF. CPA score was calculated by subtracting time spent in the drug-paired chamber during baseline from time spent in the drug-paired chamber during testing. After conditioning, rats developed aversion to the chamber paired with I.M. injection of H2O2 (0.6 ml, 30 mM) (CPA score 203 ± 133), but not to the chambers paired with either S.C. injection of H2O2 (CPA score 10 ± 108) or I.M. injection of SIF (CPA score 28 ± 99) ($p < 0.05$). Our results strongly indicate that H2O2 induces spontaneous pain when injected into deep tissue, but not into S.C. tissue. The reduction of pain behavior in rats given TRPA1 antagonist HC-030031 and AP-18 prior to I.M. injections of H2O2 suggests that H2O2-induced pain behavior is mediated via TRPA1.

Disclosures: **S. Kang:** None. **D. Sigiyama:** None. **H. Gu:** None. **T.J. Brennan:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Hydra Bioscience.

Poster

150. TrpA1

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 150.05/L42

Topic: D.08. Pain

Title: TRPA1 in lung innervating nodose neurons

Authors: ***M. M. KAELBERER**[1,2], S.-E. JORDT[2];
[1]Pharmachology, Yale Univ., New Haven, CT; [2]Anesthesiol., Duke Univ. Med. Ctr., Durham, NC

Abstract: Life sustaining airways through which we inhale oxygen are also paradoxically, the same avenues whereby harmful irritants enter our bodies. To detect these irritants our lungs are innervated pain sensing neurons, or C-fibers, which are part of a vast network of sensory neurons. These sensory fibers are derived from cell bodies in the nodose ganglia of the vagus nerve. The nodose is a diverse population of nerves that extend not only to the lungs but also the heart, and gut, and controls many vital functions such as respiration, heart rate food intake, and sensory pain. Pain sensing neurons throughout the body contain the noxious heat and mechanical pain transducing channel TRPV1. Of these neurons, a subset also have the wasabi activated channel TRPA1 which is sensitive to many known airborne environmental irritants. Given the diversity of sensory function serviced by the nodose ganglia of the vagus nerve, it is important to isolate specific sub-populations within the nodose to address their different contributions. In this study we focus on the unique identity of pain sensing C-fibers in the lungs, specifically the abundance TRPV1 and TRPA1.

Disclosures: **M.M. Kaelberer:** None. **S. Jordt:** None.

Poster

150. TrpA1

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 150.06/L43

Topic: D.08. Pain

Title: Transient receptor potential cation channel A1 (TRPA1) is a sensory nociceptor in a rodent model of ocular pain

Authors: *A. M. SCHUMACHER[1], M. GADD[2], J. LAO[3], H.-S. CHOI[3], J. WATSON[3], D. TULLY[3], A. PATAPOUTIAN[4], M. PETRASSI[3];
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Abstract: Ocular pain can occur as a result of corrective surgery, injury, or exposure to smoke and noxious, reactive chemicals. Current standard of care largely consists of topical corticosteroids and NSAIDs, with a focus on control of inflammation. Use of topical anesthetics is limited due to inhibition of corneal wound healing, short duration of action, and abuse potential. The identification of additional nociceptive targets and associated therapeutic modalities to treat ocular pain and irritation without inducing ocular anesthesia is therefore of great interest. The non-selective cation channel Transient Receptor Potential A1 (TRPA1) is expressed in sensory neurons and activated by endogenous inflammatory compounds as well as exogenous chemical compositions, such as smoke and tear gas. TRPA1 activation has also been implicated in the release of pro-inflammatory peptides that play a role in pain transmission. In this study we report the expression of TRPA1 protein in both rodent and human eyes, and the robust induction of a TRPA1-dependent blinking response to formalin-induced irritation in the rat eye. This pain response was blocked by topical pre-treatment of the rat eye with a potent, selective antagonist of the TRPA1 channel activity. This analgesic effect was observed up to 30 min following administration of the antagonist. Significantly, we did not observe any concurrent anesthetic effect of TRPA1 antagonism using Cochet-Bonnet esthesiometry. This suggests that TRPA1 plays a role in transmitting chemically-but not mechanically-induced pain in the eye.

Disclosures: **A.M. Schumacher:** A. Employment/Salary (full or part-time):; Genomics Institute of the Novartis Research Foundation. **M. Gadd:** None. **J. Lao:** None. **H. Choi:** None. **J. Watson:** None. **D. Tully:** None. **A. Patapoutian:** None. **M. Petrassi:** None.

Poster

150. TrpA1

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 150.07/L44

Topic: D.08. Pain

Support: NIH R01 NS87988

NIH R01 NS89479

Title: Characterization of toll-like receptors and TRPA1 in human primary sensory neurons

Authors: *T. BERTA, Y. KIM, F. TANG, K. KWAK, R.-R. JI;
Dept. of Anesthesiol., Duke Univ. Med. Ctr., Durham, NC

Abstract: Although significant progress has been made in revealing the molecular and cellular mechanisms of pain and itch in rodents, it has been difficult to translate these basic findings to new therapeutics in humans, in part due to the limitations of animal models. Our recent studies have shown that toll-like receptors (TLR3 and TLR7) are expressed by primary sensory neurons in mouse dorsal root ganglion (DRG) and play an important role in itch and pain sensation in mice. We have also identified functional coupling between TLR7 and TRPA1 in mouse DRG neurons. In particular, we found that microRNA let-7b is an endogenous ligand of TLR7 and activates TRPA1 in mouse DRG neurons via a non-canonical signaling mechanism (Park et al., Neuron, 2014). In this study, we investigated whether human DRG neurons also express functional TLRs using histochemical, molecular, and electrophysiological approaches. We obtained fresh non-diseased human DRG tissues from National Disease Research Interchange (NDRI). Immunohistochemistry revealed the expression of TLR7 in small-sized (<60 micrometers) neurons of human DRGs. Single-cell PCR analysis in dissociated small-sized neurons showed that TLR7 is completely co-localized with TRPA1 in human DRG neurons. Notably, the percentage of TRPA1 expressing human DRG neurons (80%) is much higher than that of mouse DRG neurons (40%). Patch-clamp recordings also confirmed that a very high percentage (~73.3%) of small-sized human DRG neurons responded to the TRPA1 AITC with inward currents. Notably, Let-7b was sufficient to induce inward currents in small-sized human DRG neurons (~62.5%), and these inward currents were completely blocked by the TRPA1 antagonist HC030031. Our results indicate that (1) TLR7 is also present in human DRG neurons and (2) there is functional coupling of TLR7 and TRPA1 in human DRG neurons. Currently, we are investigating if functional TLR3 and TLR4 are also present in human DRG neurons. These studies will validate TLRs in human DRG neurons as novel targets for pain and itch treatment. Future studies will also be designed to test the efficacy of novel TLR and TRPA1 inhibitors in human DRG neurons before these compounds are tested in clinical trials.

Disclosures: **T. Berta:** None. **Y. Kim:** None. **F. Tang:** None. **K. Kwak:** None. **R. Ji:** None.

Poster

150. TrpA1

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 150.08/M1

Topic: D.08. Pain

Support: NIH-NIDCR DE16062

Title: Epigenetic regulation of TRPA1 in trigeminal ganglia under inflammatory muscle pain condition

Authors: *J. ASGAR, K. LEE, Y. ZHANG, M.-K. CHUNG, J. RO;
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Abstract: Inflammation and injury produce rapid and reversible changes in the methylation status of genes and thereby regulate gene transcription, and these changes have been implicated in the development or maintenance of persistent pain. Transient receptor potential cation channel, subfamily A, member 1 (TRPA1) plays an important role in acute and persistent mechanical hyperalgesia in craniofacial muscles. However, the role of epigenetic regulation of TRPA1 gene in persistent muscle pain condition has not been studied. We have shown that Complete Freund's Adjuvant (CFA)-induced inflammation of the masseter muscle leads to the upregulation of TRPA1 expression in TG. Here we provide evidence that TRPA1 gene in TG is under epigenetic regulation and that DNA methylation plays a critical role in the transcriptional regulation of TRPA1. We first showed that the CFA-induced masseter inflammation was associated with a significant decrease in global DNA methylation in TG. Furthermore, CFA-induced masseter inflammation led to the downregulation of DNA methyltransferases (DNMTs) in TG. More specifically, masseter inflammation significantly reduced the methylation of specific CpG sites within the TRPA1 promoter in TG. Using the chromatin immunoprecipitation assay, we obtained the evidence that two DNMTs (1 and 3a) bind to the CpG region of the TRPA1 promoter. Consistent with these findings, the inhibition of DNMTs with 5-Aza-2′-deoxycytidine induced a significant increase in TRPA1 expression in TG cultures. Treatment of TG cultures with reactive oxygen species, such as H2O2, induced a significant increase in TRPA1 expression. We confirmed that TG cultures treated with H2O2 led to significantly less DNMT3a binding to the TRPA1 promoter region, suggesting that oxygen-derived free radicals that are produced during inflammation can alter DNMTs from binding to the TRPA1gene. In summary, this study provides evidence of the epigenetic regulation of TRPA1 in TG under a myositis condition and suggests the role of DNA methylation as a key epigenetic mechanism. The functional role of such epigenetic regulation of TRPA1 in persistent muscle pain conditions is now being investigated.

Disclosures: **J. Asgar:** None. **K. Lee:** None. **Y. Zhang:** None. **M. Chung:** None. **J. Ro:** None.

Poster

150. TrpA1

Location: Hall A

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Program#/Poster#: 150.09/M2

Topic: B.02. Ligand-Gated Ion Channels

Title: Electrophysiological characterization of internal and external ligands on human TRPA1

Authors: A. BRUGGEMANN[1], M. RAPEDIUS[1], T. GOETZE[1], C. HAARMANN[1], I. RINKE[1], M. VOGEL[1], T. STENGEL[1], J. STIEHLER[1], *J. L. COSTANTIN[2], R. HAEDO[2], J. OESTREICH[2], C. BOT[2], G. OKEYO[2], M. GEORGE[1], N. FERTIG[1];
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Abstract: The bottle neck for drug discovery on ion channels are usually electrophysiological assays. Nanion's SyncroPatch® offers a high-throughput gigaseal platform that records from up to 384/768 cells at the same time, and therefore helps fill the gap. TRPA1 is a member of the TRP (Transient Receptor Potential) family of ion channels that is thought to play a predominant role in the sensation of noxious cold and inflammatory pain (1,2). TRPA1 is activated by a range of environmental irritants causing pain, pungent compounds found in food (mustard oil, garlic, cinnamon) or low temperatures (3). Consistent with its proposed function in nociception, TRPA1 has been shown to be primarily expressed in sensory neurons (1). Further, due to the increasing role in physiology and pathophysiology, TRPA1 has also been considered as an important potential therapeutic target in drug discovery for the treatment of pain, respiratory diseases, cancer and immune disorders (4). Preclinical data and data from a recent human genetic study indicate TRPA1 antagonists as a promising new approach for the treatment of acute and chronic pain (5). Indeed, a TRPA1 antagonist has been shown positive results in a proof of concept study for diabetic neuropathic pain (6). Our results show activation of hTRPA1 by Supercinalaldehyde (SCMA) and specific inhibition by A967079. Furthermore, we also show the activation of TRPA1 by intracellular activation using the automated internal perfusion feature. Reliable pharmacology on hTRPA1 expressing HEK cells will be shown, allowing an in-depth biophyiscal characterization of the protein. References: 1. Story G.M., et al. 2003. Cell. 112(6): 819-29.; 2. Bautista D., et al. 2006. Cell. 124: 1269-1282; 3. Bautista D., et al., 2005. Natl. Acad. Sci. U.S.A.,102 (34):12248-52; 4. Clapham, D. 2003. Nature. 426: 517-524; 5. Kremeyer, B., et al. 2010. Neuron. 66: 671-680; 6. Macpherson, M.J., et al. 2007. Nature. 445: 541-45; 7. Chen, J., et al. 2011. Pain. 152(5): 1165-72; 8. Bandell, M., et al. 2004. Neuron. 41 (6): 849-57; 9.

http://www.prnewswire.com/news-releases/glenmarks-trpa1-antagonist-grc-17536-shows-positive-datain-a-proof-of-concept-study-275445961.html

Disclosures: **A. Bruggemann:** A. Employment/Salary (full or part-time):; Nanion Technologies. **M. Rapedius:** A. Employment/Salary (full or part-time):; Nanion Technologies. **T. Goetze:** A. Employment/Salary (full or part-time):; Nanion Technologies. **C. Haarmann:** A. Employment/Salary (full or part-time):; Nanion Technologies. **I. Rinke:** A. Employment/Salary (full or part-time):; Nanion Technologies. **M. Vogel:** A. Employment/Salary (full or part-time):; Nanion Technologies. **T. Stengel:** A. Employment/Salary (full or part-time):; Nanion Technologies. **J. Stiehler:** A. Employment/Salary (full or part-time):; Nanion Technologies. **J.L. Costantin:** A. Employment/Salary (full or part-time):; Nanion Technologies. **R. Haedo:** A. Employment/Salary (full or part-time):; Nanion Technologies. **J. Oestreich:** A. Employment/Salary (full or part-time):; Nanion Technologies. **C. Bot:** A. Employment/Salary (full or part-time):; Nanion Technologies. **G. Okeyo:** A. Employment/Salary (full or part-time):; Nanion Technologies. **M. George:** A. Employment/Salary (full or part-time):; Nanion Technologies. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies.

Poster

150. TrpA1

Location: Hall A

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Program#/Poster#: 150.10/M3

Topic: B.02. Ligand-Gated Ion Channels

Support: Ernst Schering Foundation, Germany

German Academic Exchange Service

Deutsche Schmerzgesellschaft e.V.

Title: Agonist-induced sensitization of the irritant receptor ion channel TRPA1

Authors: ***J. E. MEENTS**[1,2], M. J. M. FISCHER[3,2], P. A. MCNAUGHTON[4,2];
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Abstract: The TRPA1 ion channel is expressed in nociceptive neurons and responds to a wide variety of chemical irritants, such as acrolein in smoke or isothiocyanates in mustard. In this

study we show that TRPA1 is sensitized by repeated agonist application in mouse DRG neurons as well as heterologously expressed. Activation by an agonist is essential, because repeated activation by depolarization did not sensitize the channel. Agonist-induced sensitization is independent of the site of action of the agonist, because covalent and non-covalent agonists were equally effective. Mutating N-terminal cysteines, the target of covalent agonists, did not affect sensitization by non-covalent agonists. Sensitization is unaffected by agents blocking ion channel trafficking or signalling pathways involving ATP, protein kinase A or the formation of lipid rafts, and does not require ion flux through the channel. Examination of the voltage-dependence of TRPA1 activation shows that sensitization is caused by a slowly developing shift in the voltage dependence of TRPA1 towards more negative membrane potentials. This shift reverses very slowly and is partly conserved upon repeated agonist stimulation. Sensitization is therefore intrinsic to the TRPA1 channel. Agonist-induced sensitization may represent a slowly developing gain in function and may play a role in exacerbating the pain caused by prolonged activation of TRPA1.

Disclosures: **J.E. Meents:** None. **M.J.M. Fischer:** None. **P.A. McNaughton:** None.

Poster

151. Pain: Descending Modulation

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Program#/Poster#: 151.01/M4

Topic: D.08. Pain

Support: NINDS NS048602

NINDS NS081707

Title: Divergent PAG neuronal populations drive pain and itch processing

Authors: ***V. K. SAMINENI**[1], J. GRAJALES-REYES[2], B. COPITS[2], D. O'BRIEN[2], M. BRUCHAS[2], R. GEREAU IV[2];
[1]Washington Univ. Pain Ctr. and Dept. of Anesthesiol., Washington Univ., St Louis, MO;
[2]Washington Univ. in St. Louis, St. Louis, MO

Abstract: Chronic itch, like chronic pain, is a major clinical problem. Despite the similarities between itch and pain, the underlying neural circuitry for itch is poorly understood, as is the mechanism by which itch is suppressed by pain. Profound analgesia occurs with stimulation of the periaqueductal gray, however the precise role of the PAG in itch is unknown. We

hypothesized that specific subsets of neurons within the PAG could suppress itch and provide a neural substrate for the inverse interaction between itch and pain. To determine the role of PAG in mediating itch and pain, we activated or inhibited PAG neurons using engineered G-protein coupled receptors (GPCRs) activated exclusively by synthetic, systemically administered small molecules (i.e., DREADD technology). Engineered excitatory (Gq) or inhibitory (Gi) GPCRs were expressed in PAG neurons via adeno-associated viral vectors. We found that chemogenetic activation of non-specific PAG neurons produced a reduction of both itch and pain, whereas inhibition of non-specific PAG neurons resulted in enhanced itch and pain. In contrast, when only the GABAergic neurons in the PAG were targeted using VGAT Cre mice, activation resulted in decreased itch, but increased behaviors. Conversely, inhibition of PAG GABAergic neurons resulted in increased itch, but decreased pain behaviors. When glutamatergic neurons in the PAG were selectively targeted we found that activation led to enhanced itch and decreased pain, while inhibition neurons produced decreased itch and increased pain. We conclude that the PAG bidirectionally modulates itch and pain signaling and is a neural control center for pruritus.

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Poster

151. Pain: Descending Modulation

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Topic: D.08. Pain

Support: NIH Grant NS082020

Title: A possible neural mechanism for photosensitivity in chronic pain

Authors: *M. M. HEINRICHER[1], M. E. MARTENSON[2], N. HAMMACK[2];
[1]Dept Neurol Surgery, Mail Stop L-472, [2]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Abnormal sensitivity to light is a symptom associated with a range of neurological and neuro-ophthalmic disorders, including migraine, various eye conditions, traumatic brain injury, and some chronic pain disorders. Although candidate pathways by which light could interact with nociceptive transmission pathways have been described, these circuits do not adequately explain the general intolerance to light unaccompanied by localized light-induced pain seen in many clinical conditions. The photosensitivity seen in patients with some chronic pain disorders is often part of a more general sensory hypersensitivity. Here we show, in lightly anesthetized

rats, that a subset of pain-modulating neurons in the rostral ventromedial medulla unexpectedly responds to light. ON-cells (known to exert a net facilitating effect on spinal nociceptive processing) and OFF-cells (known to exert a net inhibiting effect on spinal nociceptive processing) were recorded in the rostral ventromedial medulla (RVM). These neurons have historically been defined by changes in activity during noxious somatic stimulation. Approximately half of the pain-facilitating ON-cells sampled, and a similar proportion of pain-inhibiting OFF-cells showed a change in firing with light. Light exposure also resulted in a measurable decrease in the threshold for heat-evoked paw withdrawal. We also investigated three possible relays through which information about ambient light could reach the RVM: the olivary pretectal nucleus, posterior thalamus, and trigeminal pathways. Light-evoked changes in ON- and OFF-cell firing were prevented by inactivation of the olivary pretectal nucleus, but did not require a trigeminal or posterior thalamic relay. These data demonstrate integration of information about ambient light intensity with somatic nociceptive input at the level of single pain-modulating neurons in the brainstem. These data provide a novel mechanism for photosensitivity in chronic pain states. Since activation of RVM pain-facilitating neurons is known to induce an aversive state, our findings suggest that ambient light could modulate sensory processing such that normally innocuous inputs are perceived as aversive or even painful.

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Poster

151. Pain: Descending Modulation

Location: Hall A

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Topic: D.08. Pain

Support: NIH Grant DA16272

Title: Sex differences in glial activation and subsequent cytokine release affect morphine efficacy in the rat

Authors: *H. H. DOYLE[1], A. Z. MURPHY[2];
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Abstract: Morphine continues to be one of the most widely used analgesics for pain management in the US; unfortunately, clinical and animal models show that morphine is a less potent analgesic in females than in males. The midbrain periaqueductal gray (PAG) is a central

locus in pain signaling and opioid action. Recent studies suggest that morphine binds to the innate immune receptor toll-like receptor 4 (TLR4) on glia, resulting in increased activation of microglia and astrocytes, increased cytokine release, and paradoxical opposition of morphine analgesia. Here we test the hypothesis that males and females have dissimilar patterns of glial activation in the brain, specifically within the PAG, that may contribute to observed sex differences in morphine analgesia. To test this hypothesis, lipopolysaccharide (LPS), an immunoreactive component of gram negative bacteria and a known TLR4 agonist, was administered either peripherally, (i.p., 1mg/10ml/kg) or centrally (bilateral intra-PAG, 5ug/.5ul/side) in male and female rats, and body temperature, glial activation and morphine efficacy were determined. Peripheral LPS did not sex-specifically affect febrile responses, however, significantly more activated glia were observed within the PAG of LPS-treated females compared with LPS-treated males. Similar effects on glial activation were observed following intra-PAG administration of LPS. Current experiments are testing the hypothesis that sex differences in glial activation result in disproportionate release of pro-inflammatory molecules in females. mRNA levels of IL-1β, IL-6, IL-9 and TNFα, as well as TLR4, will be quantified for the PAG of male and female rats given LPS or morphine. Together, these results suggest that microglial activation in the PAG of males and females is innately different, and contribute to the dimorphic effects of morphine.

Disclosures: H.H. Doyle: None. **A.Z. Murphy:** None.

Poster

151. Pain: Descending Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 151.04/M7

Topic: D.08. Pain

Support: DA034975

Title: Investigating the role of the anterior cingulate cortex in opioid-sensitive descending pain modulatory pathways

Authors: *L. GOMTSIAN[1], E. NAVRATILOVA[1], A. OKUN[1], C. QU[1], X. YUE[1], D. LU[1], D. ROBLES[1], F. PORRECA[1], J. OYARZO[2];
[1]Pharmacol. Dept., Univ. of Arizona, Tucson, AZ; [2]Mayo Clin., Scottsdale, AZ

Abstract: Pain is a multidimensional phenomenon composed of sensory and affective features. Opioids such as morphine are used for treatment of moderate to severe pain and have been

suggested to act primarily by alleviating the aversive aspects of pain. However, they have many harmful side effects. There is an urgent medical need to elucidate the mechanisms and opioid-sensitive circuits by which pain and pain relief is modulated. Cortical regions may contribute to descending pathways modulating pain through projections to the peri-aqueductal gray (PAG) and the rostral ventromedial medulla (RVM). We explore the effect of morphine administration at different areas of the anterior cingulate cortex (ACC) and its effect on mechanical hypersensitivity in a rodent model of neuropathic pain. Adult, male Sprague-Dawley rats received nerve injury by ligation of the L5/L6 spinal nerves (SNL injury). Evoked tactile allodynia was evaluated following microinjection of morphine into the ACC and RVM. Rats with SNL developed tactile hypersensitivity that was not reversed by morphine injection into various regions of the ACC. In contrast, morphine injection in the RVM reversed evoked thresholds in SNL animals, as shown previously. We have demonstrated that sites in the ACC can modulate the affective aspects of neuropathic pain without altering the sensory component. The primary goal of this research is to understand the nature of the neuroanatomical contributions of the ACC and its role in descending pain modulatory pathways. Further studies exploring other sites within the ACC may ultimately lead to a better understanding of opioid-sensitive circuitry.

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Poster

151. Pain: Descending Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: MRC G0901269

Title: What the young brain tells the spinal cord: the changing developmental profile of top down serotonergic modulation of dorsal horn sensory circuitry

Authors: F. SCHWALLER, *M. FITZGERALD;
Univ. Col. London, London, United Kingdom

Abstract: The development of postnatal sensory circuits is shaped by central modulation of sensory inputs. Experimental evidence in rodents has shown that adult descending modulation of dorsal horn activity is both inhibitory and facilitatory, whereas in the first weeks of life,

descending facilitation is dominant. In adults, serotonergic neurons in the rostroventral medial medulla (RVM) are an important contributor to descending controls. Here we have investigated the contribution of this system, and specifically 5HT3 receptors, to descending facilitation in young animals. Mapping the postnatal maturation of 5-HT terminals in the lumbar spinal dorsal horn using 5-HT transporter (5-HTT) immunoreactivity revealed a marked increase after postnatal day (P) 14, suggesting the arrival of descending serotonergic fibres in the third postnatal week. Ablation of descending serotonergic neurons with intrathecal 5,7-DHT resulted in increased dorsal horn neuron pinch-evoked firing activity at P40, but a strong decrease in brush and pinch-evoked firing activity at P21. Mapping 5-HT3 receptor expression in the dorsal horn revealed an adult-like distribution from postnatal day 7 (P7). Spinal administration of the 5-HT3 receptor antagonist ondansetron did not change neuron activity in naive adults, but dose dependently decreased brush and pinch-evoked firing at P21. Weaker reduction of facilitation of pinch evoked neuron responses was observed following ondansetron application at P10. These findings show that when descending serotonergic terminals first arrive in the lumbar spinal dorsal horn in the third postnatal week, they strongly facilitate both low and high threshold input via dorsal horn 5-HT3 receptors. In adulthood, the profile of descending serotonergic modulation changes. We suggest that this is due to the late maturation of inhibitory interneuronal circuits, recruited via different classes of spinal 5-HT receptors.

Disclosures: **F. Schwaller:** None. **M. Fitzgerald:** None.

Poster

151. Pain: Descending Modulation

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Title: Dose dependent conditioned place aversion following chemogenetic activation of the locus coeruleus

Authors: ***S. HIRSCHBERG**[1], Y. LI[1], A. RANDALL[2], A. PICKERING[1];
[1]Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom; [2]Med. Sch., Univ. of Exeter, Exceter, United Kingdom

Abstract: The Locus coeruleus (LC) is the principle noradrenergic (NA) nucleus in the CNS. Dysfunction of the NAergic system has been associated with pathologies such as major

depression, Alzheimer's disease and neuropathic pain. We have therefore been interested in developing means to produce long term activation of the NA system. We describe here the use of an engineered excitatory receptor-ionophore (PSAM) to manipulate LC activity. We developed a lenti-viral vector (lenti-PRS-EGFP2aPSAM-HA) with catecholaminergic promoter (PRS) to allow expression of a traceable (HAtagged) excitatory ion channel (PSAM-HA) in NAergic LC neurons. Expression was evaluated using immunofluorescence for EGFP and dopamine beta hydroxylase (DBH) after vector delivery into the LC of Wistar rats (P19-21). Cell properties and effects of agonist application were investigated using patch clamp recordings from acute pontine slices and *in vivo* spike recordings with multi-barrelled electrodes. To assay for behavioural effects Wistar rats (250-300g) were transduced with either lenti-PRS-EGFP2aPSAM-HA (N=11) or a control vector lenti-PRS-EGFP (N=7) and tested in a conditioned place preference (CPP) protocol with intraperitoneal (i.p.) dosing of 5mg/Kg and 10mg/Kg PSEM308 (selective agonist). After behavioural testing, transduced animals were dosed with PSEM308, culled 2.5 hours later and pontine tissue processed for c-Fos expression. After LC injection of lenti-PRS-EGFP2aPSAM-HA, fluorescence (285+/-75 cells per LC, N=3) was restricted to DBH-positive neurons and PSAM-HA was only detected in membranes of EGFP-positive cells. Patch recordings showed functional expression of PSAM that was well tolerated by the LC neurons. *In vivo*, four out of ten LC neurons were reversibly excited by local PSEM308 pressure application (1mM) with a dose dependency. Mean increase in baseline firing was 4.5+/-0.74 Hz,. Conditioning with 10mg/Kg PSEM308 caused aversion to the drug paired chamber in PSAM-HA transduced rats (N=8; $P<0.05$, Bonferoni post-test saline vs drug, 20% difference; Interaction: $P=0.052$ $F(1.14)=4.47$ Two way repeated ANOVA) whilst 5mg/Kg (N=11) had no effect. No effect of PSEM308 was seen in control animals at either dose. The percentage of transduced neurons that were double labelled with c-Fos was increased by PSEM308 from 18.4+/-5.9% in EGFP and 29.8+/-9.4% in PSAM-HA transduced animals. Our findings show that chemogenetic LC activation causes a dose-dependent aversive effect. It remains to be determined whether targeting of subsets of LC neurons (such as the pontospinal group) will produce the same aversive behaviour.

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Poster

151. Pain: Descending Modulation

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Support: Canadian Institute of Health Research (CIHR)

Fonds de Recherche du Québec – Santé (FRQS)

Title: Time-frequency EEG analysis of pain modulation by hypnosis, suggestions and distraction

Authors: *B. HOUZÉ[1,2], A. STREFF[1,2], M. PICHÉ[1,3], P. RAINVILLE[1,2];
[1]CRIUGM, Montreal, QC, Canada; [2]Univ. de Montréal, Montréal, QC, Canada; [3]Univ. du Québec à Trois-Rivières, Trois-Rivières, QC, Canada

Abstract: Pain modulation by hypnosis is well documented but the specific brain mechanisms underlying the effects of hypnotic suggestions on acute pain are not fully understood. To investigate this issue, we examined pain-related time-frequency (TF) electroencephalographic responses elicited by noxious electrical stimulation under various modulatory conditions. In 18 subjects (SHSS hypnotisability score 7.9 ±3.4), hypnotic hypoalgesia (HypnoHypo) and hypnotic hyperalgesia (HypnoHyper) were assessed in a hypnotic modulation session. Non-hypnotic suggestions of hypoalgesia (SuggHypo) and distraction hypoalgesia (DT) were assessed in a non-hypnotic session. In both sessions, stimulus intensity was set individually at 120% RIII-reflex threshold, producing moderate pain. Stimuli were administered in 5 successive blocks in the target experimental conditions (2 blocks of 9 stim), alternating with neutral control conditions (3 blocks of 6 stim). After trial-averaging of TF decomposition activity using a Morlet wavelet transform, 3 regions of interest were defined on TF maps: somatosensory-evoked potential (SEP:0-500 ms, 3-7 Hz, ROI1), event-related synchronisation in beta band (ERS β: 0-250 ms, 13-17 Hz, ROI2) and event-related desynchronisation in alpha band (ERD α: 350-1000 ms, 8-12 Hz, ROI3). The means of the 10% highest amplitude values were computed for each ROI and compared between the 3 hypoalgesic conditions, and between the 2 hypnotic pain modulation conditions. All modulatory conditions produced robust pain modulation compared to control blocks ($p<.00005$). HypnoHypo suggestions produced a marginal decrease in frontal ROI1 activity compared to DT ($p=.053$) and a context effect was observed on parietal ROI3 activity, with a more ERD α elicited under hypnosis (HypnoHypo), in both the control and experimental conditions compared to DT ($p=.009$). HypnoHypo and HypnoHyper conditions increased frontal ROI1 activity, indicating a generalized effect of hypnotic suggestions independent of the direction of suggestions. HypnoHypo further decreased activity in frontal ROI2 ($p=.034$) and ROI3 ($p=.014$). Parietal ROI3 activity was also reduced by HypnoHypo suggestions and was increased by HypnoHyper suggestions ($p=.032$). The SHSS-scores was associated with changes in pain ratings in the hypnosis and suggestion conditions ($p<.05$) but not in DT. However, SHSS-scores were not associated with changes in brain activity for any ROI, although a trend was observed for the HypnoHypo condition ($R2 > 0.17$, $p <.09$). Altogether, these results suggest that hypnosis, suggestions and distraction modulate pain through partly distinct brain mechanisms.

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Poster

151. Pain: Descending Modulation

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Support: NIH Grant AG023477

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Medical Research Foundation of Oregon

Title: Extrasynaptic GABAA signaling in the ventrolateral periaqueductal gray differentially affects morphine antinociception in male and female rats during persistent inflammation

Authors: *K. J. TONSFELDT[1], K. L. SUCHLAND[2], S. L. INGRAM[2];
[1]Oregon Hlth. and Sci. Univ., Portland, OR; [2]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The ventrolateral periaqueductal gray (vlPAG) is a key structure in the descending pain modulatory circuit. Activation of the circuit is dependent on removing GABAergic inhibition or disinhibition of vlPAG output neurons. Mu-opioid receptors (MOPrs) reduce GABA-mediated inhibition of vlPAG output neurons to activate the descending pain modulatory system and induce antinociception. Both tonic and phasic GABAA-mediated currents are observed in the vlPAG. Tonic GABAA currents are reduced in vlPAG neurons from female rats pretreated with Complete Freud's Adjuvant (CFA) for 5-7 days to induce chronic inflammatory pain, but similar changes were not observed in male rats (Two-way ANOVA, Effect of Sex; $F(1,31) = 5.681$, $p < 0.05$; Sidak's multiple comparison, * $p < 0.05$). However, mIPSCs are increased in females following CFA treatment (Mann-Whitney $U = 21$, $p = 0.003$). The mRNA and protein levels of the GABAA delta subunit, known to mediate tonic currents in other brain areas, are unchanged in the vlPAG of female pretreated with CFA, but the mRNA expression is reduced compared to males (Main Effect of Sex; $F(1,16) = 16.89$, * $p = 0.008$) . The selective agonist of GABAAδ receptors THIP (gaboxadol) induced similar amplitude currents in naïve and CFA-treated rats (Effect of Concentration, $F(1,23) = 33.87$, $p < 0.0001$). In addition, a positive allosteric modulator of the GABAAδ subunit DS2 increased tonic currents and revealed a contribution of GABAAδ receptors to evoked GABAergic IPSCs (Paired t-test; $t(8) = 2.518$, * $p < 0.05$), indicating that GABAAδ receptors remain on the cell surface after CFA treatment. Finally, we explored the role of the GABAA δ subunit in morphine-induced antinociception. Importantly, opioid inhibition of mIPSC frequency was increased selectively in CFA-treated female vlPAG neurons suggesting that opioid activation of the descending pain circuit is

facilitated during persistent inflammation (Effect of Pretreatment, $F(1,23) = 9.016$, $p = 0.006$; Sidak's multiple comparison, * $p < 0.05$). Indeed, systemic morphine antinociception was enhanced in female rats pretreated with CFA and this effect was reversed with microinjections of DS2 directly into the vlPAG (Effect of Pretreatment, $F(1,23) = 9.016$, $p = 0.006$; Sidak's multiple comparison, * $p < 0.05$). Together, these data suggest that decreased activation of GABAAδ receptors is a compensatory mechanism to enhance descending inhibition of pain in persistent inflammation in females.

Disclosures: K.J. Tonsfeldt: None. **K.L. Suchland:** None. **S.L. Ingram:** None.

Poster

151. Pain: Descending Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 151.09/M12

Topic: D.08. Pain

Support: NIH Grant R01 NR004778

Title: Sex differences in the lateral hypothalamus-induced descending noradrenergic system in nociceptive and hyperalgesic pain types

Authors: B. N. MANTHA[1], M. A. WAGNER[1], T. BANERJEE[1], Y. JEONG[2], *J. E. HOLDEN[1];
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Abstract: The lateral hypothalamus (LH) modifies nociception at the level of the spinal cord dorsal horn. In a model of nociception, LH stimulation produces antinociception when norepinephrine (NE) binds to alpha2-adrenoceptors and pronociception when NE binds to alpha1-adrenoceptors. Stimulating the LH with 500 nmol of carbachol produces antinociception in male and female rats for both nociceptive and hyperalgesic pain types. The purpose of this study was to determine whether LH-induced alpha-adrenergic modulation occurs in hyperalgesic as well as nociceptive pain conditions. Male and female Sprague-Dawley rats were randomly assigned to either nociceptive or hyperalgesic pain groups (n = 12 - 15 rats per group). The hyperalgesic group received chronic constriction injury (CCI) with left sciatic nerve ligation to induce hyperalgesia. Fourteen days later, carbachol (500 nmol in 0.5 µl normal saline [NS]) was microinjected into the LH and paw withdrawal latency (PWL) from a thermal stimulus was measured at 1, 5, and 10 min. At 10 min rats were then given IT infusion of either the alpha1-adrenoceptor antagonist WB4101, the alpha2-adrenoceptor antagonist yohimbine (97 nmol each)

or normal saline for control. PWL was then measured at 1 min and then every 5 min for 45 min. Results from generalized estimating equation (GEE) analysis showed no difference in PWL between pain types. Female rats had a 3.29 sec increase in PWL ($Z = 4.61$, $p < 0.0001$) over controls after blocking alpha1-adrenoceptors with WB4101 and a 2.47 sec decrease from controls after blocking alpha2-adrenoceptors with yohimbine ($Z = -3.52$, $p = 0.0004$). Male rats showed a 2.06 sec increase over controls in PWL after WB4101 ($Z = 2.91$, $p = 0.0036$) and a 2.44 sec decrease from controls after yohimbine ($Z = -3.69$, $p = 0.0002$). The findings of this study suggest that stimulating the LH modulates nociception in part at both alpha1 and alpha2-adrenoceptors, but the extent of modulation depends on sex of the rat.

Disclosures: **B.N. Mantha:** None. **M.A. Wagner:** None. **T. Banerjee:** None. **Y. Jeong:** None. **J.E. Holden:** None.

Poster

151. Pain: Descending Modulation

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Topic: D.08. Pain

Support: North Carolina State University College of Veterinary Medicine Internal Grant

NIH Grant T35 / IBRTP

Title: Relationship of activation of phenotypically-distinct neuronal populations in the extended centromedial amygdala with the expression of acute pain, conditioned fear, and fear-conditioned analgesia

Authors: ***R. K. BUTLER**[1], S. EHLING[2], M. BARBAR[1], J. M. THOMAS[1], M. A. HUGHES[1], A. E. THOMSON[1], S. J. WALL[1], V. ZARIC[1], B. CASE[1], D. KNAZOVICKY[1], M. E. GRUEN[1], W. BÄUMER[2], R. M. RODRIGUIZ[3], V. M. POGORELOV[3], D. K. ARYAL[3], W. C. WETSEL[3], B. D. X. LASCELLES[1];
[1]Clin. Sci., [2]Mol. Biomed. Sci., North Carolina State Univ. Col. of Vet. Med., Raleigh, NC;
[3]Psychiatry and Behavioral Sci., Duke Univ. Med. Ctr., Durham, NC

Abstract: Fear-conditioned analgesia (FCA) is a survival response whereby an organism suppresses nociceptive behaviors in an environment previously paired with an aversive stimulus. FCA is facilitated through activation of the descending inhibitory pain pathway which originates in brain regions including the amygdala. The amygdala can be broadly divided into two

components: the basolateral amygdala complex (BAC) and the extended centromedial amygdala (ECMA). Previously, we investigated the role of phenotypically-distinct neuronal populations in the BAC in the expression of acute pain, conditioned fear, and FCA; in these experiments, we investigated the ECMA. The ECMA is a group of structures within the basal forebrain. The centromedial group is found in the dorsomedial portion of the BAC and consists of the central (CEA), medial, and the amygdaloid part of the bed nucleus of stria terminalis. The ECMA is a mediator of aversive behaviors including the expression of conditioned fear and is involved in the emotional processing of pain. To date, it is unknown how the ECMA mediates or modulates the descending inhibitory pain pathway and FCA. Neurons within the ECMA can be distinguished based on their neuropeptide-containing phenotype. In the CEA, neurons are almost exclusively GABAergic; however, neurons which contain the opioid peptide enkephalin (ENK) do not project outside of the ECMA (interneuronal) whereas neurons which contain corticotropin-releasing factor (CRF) project outside the ECMA. In these experiments, we combined Pavlovian fear-conditioning with the formalin test of persistent pain which resulted in four groups: control (non-fear conditioned, saline), acute pain (non-fear conditioned, formalin), conditioned fear (fear conditioned, saline) and FCA (fear conditioned, formalin). We demonstrate that expression of nociceptive behaviors are significantly reduced in formalin-treated mice which are re-exposed to a contextually-aversive environment compared to formalin-treated, unconditioned mice. Quadruple-immunostaining of serial brain sections throughout the ECMA with 1) ENK, 2) CRF, 3) cFos (a marker of neuronal activation), and 4) NeuN (a non-specific neuronal nuclear marker) revealed an increase in co-localization of cFos with CRF in the capsular part of the CEA in mice expressing FCA compared to pain alone. This suggests that a CEA CRF-containing neuronal population may be important in amygdala-mediated analgesia induced upon exposure to a threatening environment. These data enhance our knowledge of the role of the amygdala in facilitating FCA and they provide a viable target for the development of analgesics.

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Poster

151. Pain: Descending Modulation

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 151.11/M14

Topic: D.08. Pain

Support: ibs-r001-01

Title: Negative role of PLCb4 in descending analgesia gating

Authors: *J. KIM;
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Abstract: Endogenous opioids generate analgesic signals in the periaqueductal gray (PAG). These signals are projected to and relayed by the rostral ventromedial medulla (RVM) to the dorsal horn of the spinal cord (SC), which is called descending analgesia circuit. It is known that PAG GABAnergic neurons in Cav3.1 mice showed impaired opioid-dependent analgesia in the absence of T currents and low-threshold burst spikes. Furthermore, Phospholipase c beta 4(PLCb4) knockout mice with increased bursting in TC neurons showed reduction of visceral pain responses. Howevere, the role of PLCb4 in the control of descending pain pathway is unknown. Here, we found that PLCb4 is highly expressed in NPYnergic neuron in PAG, but not in GAD67-positive neurons or PAG-RVM projecting neurons. Also, PLCb4(-/-) mice displayed enhancement of anti-nociceptive effect by morphine administration and swim stress-induced analgesia(SSIA). Moreover, mice with PAG-specific knockdown of PLCb4 reproduced the phenotype of PLCb4 knockout. These findings suggest that role of PLCb4 signaling in PAG is negative control of opioid-dependent analgesia gating in descending pain pathway.

Disclosures: J. Kim: None.

Poster

151. Pain: Descending Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 151.12/M15

Topic: D.08. Pain

Support: CAPES

Title: Effect of long-lasting voluntary physical activity on noxious stimulus-induced antinociception in mice

Authors: *I. J. BONET, M. O. F. PAGLIUSI, Jr, C. A. PARADA, C. R. SARTORI, C. H. TAMBELI;

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Abstract: Background and aim: It has been previously demonstrated that exercise can modulate pain in humans, which justifies its application as a non-pharmacological clinical intervention in various pathological pain conditions. However, it is not known whether it can affect the activity of the endogenous analgesic circuits. The ascending nociceptive control is a novel spino-striato-rostral ventral medulla pain modulation circuit that produces heterosegmental pain-induced analgesia, i.e., noxious stimulus-induced antinociception (NSIA) mediated by endogenous opioids in the nucleus accumbens. Therefore, the aim of this study was to investigate whether the exercise modulates the activity of NSIA. Methods: Male C57Bl/6JUniB mice (20-28g) were used in this study and all experimental procedures were approved by the Ethics Committee in Animal Research at the UNICAMP. The mechanical nociceptive threshold was quantified by an electronic pressure-meter test (Von Frey). The animals had access to wheel activity for 28 days. At the 28th day, Carrageenan (100µg / paw) was subcutaneously injected (15µL/paw) in the hindpaw 3 hours before behavioural testing. Capsaicin (7.5µg/paw) was locally injected in the subcutaneous forepaw to induce NSIA and the mechanical nociceptive threshold was quantified in the carrageenan injected hindpaw 1, 15, 30, 45 and 60 min after the capsaicin injection.. Two-way ANOVA followed by Tukey test was used for statistical analysis ($P<0.05$). Rats per group = 5 to 6. Results: NSIA significantly reduced carrageenan-induced hyperalgesia. Surprisingly, the exercise reduced 67.22% of NSIA. Conclusion: These data demonstrated that long lasting voluntary exercise reduces the activity an endogenous analgesic circuit.

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Poster

151. Pain: Descending Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: JSPS KAKENHI 26670290

Title: Central neuronal circuit underlying odor-induced analgesia in mice

Authors: S. ISHIKAWA, R. YAMAGUCHI, S. TASHIRO, Y. KANMURA, K. KAJIYA, T. KUWAKI, *H. KASHIWADANI;
Kagoshima Univ., Kagoshima, Japan

Abstract: Recently we found that odor exposure of odorant-X, one of the terpenoids derived from plant extract, had significant analgesic effects in mice. The effects were not observed in anosmic model mice, indicating that the effects were triggered by olfactory input evoked by odorant-X exposure. Furthermore, orexin mutant mice did not show the effects, indicating that orexin neurons in the hypothalamus play a pivotal role for the effects. Because odorant-X exposure did not induce aversive behaviors or elevation of plasma corticosterone, one of the stress markers, odorant-X-induced analgesia is, most probably, different from stress-induced analgesia. To address the central neuronal circuits underlying odorant-X-induced analgesia, we first examined the localization of neuronal activity induced by odorant-X exposure using c-Fos immunohistochemistry. After 1 hour odorant-X exposure, mice were perfused with saline following 4% paraformaldehyde under deep anesthesia. Sections of 40 um thickness were made from the fixed brain samples and were stained with rabbit antiserum prepared against c-Fos. The immunoreactivity was visualized by Alexa 568-conjugated secondary antibody. Though various brain structures were activated by odorant-X exposure, c-Fos expressing neurons were substantially increased in olfactory cortices (anterior olfactory nucleus, anterior piriform cortex, cortical amygdaloid nucleus), association corticies (prelimbic cortex, lateral orbitofrontal cortex, cingulate cortex), amygdala (medial amygdaloid nucleus), septum (lateral septum), thalamus (centromedial thalamic nucleus), hypothalamus (lateral hypothalamus), periaqueductal gray (dorsolateral part, ventrolateral part), and pons (laterodorsal tegmental nucleus). The contribution of each brain structures to odorant-X-induced analgesia has yet to be elucidated, however, we will further discuss the central neuronal circuits underlying the analgesia by comparing the odorant-X-induced c-Fos expression in orexin deficient mice.

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Poster

151. Pain: Descending Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 151.14/M17

Topic: D.08. Pain

Title: The prediction of placebo analgesia : a bayesian approach

Authors: *Y. CHAE[1], W.-M. JUNG[2], Y.-S. LEE[2];
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Abstract: Background: From the perspective of predictive coding, brain is not passively waiting for external stimuli but is actively making inferences based on prior experiences. Bayesian formulation within the predictive coding framework can be applied to the prediction of placebo analgesia, a hypoalgesia induced by experience and expectations of pain. Objectives: The present study established an experimental placebo analgesia using two different levels of precision of prior experiences with cue conditioning paradigms. We applied Bayesian model and predicted the individual placebo responses based on pain behaviors during the conditioning period. Methods: We implemented a medical decision task which was a modified trust game. In the task, participants (n=24) received a high pain (512mN) by PINPRICK and made a decision of choosing a virtual treatment among two given options: taking medicine or being treated by a doctor. The decision to take the medicine was followed by moderate pain (256mN) to patients whereas the decision to be treated by the doctor was followed by moderate (256mN) or mild pain (64mN) by the chance of 50%. Different monetary value was linked to the choice of both medicine and doctor, which also balanced the biased pain intensity. Participants reported relative intensity of the reduced pain to the original pain prior to making the choice. The task was repeated 40 times as a conditioning procedure. After conditioning, same task was repeated 8 times as a test, but instead of moderate or mild pain, high pain was always given. We formulated a Bayesian model by fitting pain ratings during conditioning period given each cue as the prior probability, and by fitting pain ratings to each pain stimuli without prior knowledge about cue as the likelihood. Pain ratings in the test period were predicted by the posterior probability of the fitted Bayesian model using Markov chain Monte Carlo (MCMC) algorithm. Results: Participants reported significantly less pain when they chose doctor or medicine compared to control, in which placebo analgesia were successfully induced. Using Bayesian model, the predicted pain ratings to doctor or medicine cue showed significant correlations to the actual average of pain ratings in the test period. Conclusions: For the prediction of placebo analgesia, we applied the Bayesian formulation within the predictive coding framework utilizing differences in the magnitude and the precision of conditioned experience. The Bayesian model could benefit for the understanding of individual differences of placebo analgesia.

Disclosures: **Y. Chae:** None. **W. Jung:** None. **Y. Lee:** None.

Poster

151. Pain: Descending Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 151.15/M18

Topic: D.08. Pain

Title: Descending pain modulation in irritable bowel syndrome

Authors: *R. J. CHAKIATH[1,2,3], P. SIDDALL[4,3], J. KELLOW[5,3], P. WRIGLEY[1,2,3];
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Abstract: Background. Descending neural modulatory circuits from the brain are able to inhibit or facilitate ascending nociceptive transmission (descending pain modulation, DPM). Changes in DPM profoundly influence pain perception. Neurophysiological measures of DPM have been developed however the clinical utility of these techniques remains to be determined. Recently, it has been suggested that results from these measures may contribute to the development of a person's *pain modulation profile* which range from pro- to anti-nociceptive. **Method.** With this in mind, we undertook a case-control study (age/gender matched) comparing DPM in participants with irritable bowel syndrome (IBS, n=19) and healthy controls (HC, n=19). Using a conditioned pain modulation (CPM) protocol we performed nociceptive spinal cord withdrawal (RIII) reflexes by applying an electrical test stimulus to the sural nerve (ankle) and muscle potentials were recorded (Synergy, Nicolet EDX, USA) from the biceps femoris muscle (upper leg). After baseline measurements, participants placed their left hand in a refrigerated bath (JeioTech RW-3025G, Korea) previously adjusted from 10^{o}C, to achieve a pain intensity 30-70/100. Reflex potential measurements were repeated at 2 and 5 minutes immersion and 2 minutes post immersion. The change in RIII reflex potential (AUC) and pain intensity scores (NRS101) from baseline, during the second minute of testing and post testing were calculated and grouped as reflecting either an inhibitory (change from baseline < 0) or facilitatory effect (change from baseline > 0) or no change (change = 0). Depression, anxiety and stress were also measured prior to testing using the Depression Anxiety and Stress Questionnaire (DASS-21). Independent sample t-tests were used to assess differences in depression, anxiety and stress. **Results.** People with IBS report greater anxiety, stress and depression when compared to HC ($p < 0.05$). There were no significant differences between groups during and post CPM in the proportion of participants with inhibitory or facilitatory effects using change in reflex potential (subjects classified as inhibitory during 5th minute of CPM: IBS = 10/19, HC = 15/19, Facilitatory: IBS = 9/19, HC = 4/19) or pain scores (subjects classified as inhibitory during 5th minute of CPM: IBS = 13/19, HC = 13/19, Facilitatory: IBS = 5/19, HC = 5/19, No change: IBS 1/19, HC = 1/19). **Conclusions.** These results show that while mood is altered in people with IBS, the responses to the DPM protocol in people with IBS were not different to HCs. These initial findings suggest that uniform changes in DPM do not occur in people with IBS which varies from inhibitory to facilitatory.

Disclosures: **R.J. Chakiath:** None. **P. Siddall:** None. **J. Kellow:** None. **P. Wrigley:** None.

Poster

151. Pain: Descending Modulation

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Topic: D.08. Pain

Support: NIH, Grant R01 NR004778

Title: The effect of cobalt chloride on lateral hypothalamic-induced pain modulation in male and female hyperalgesic rats

Authors: ***M. A. WAGNER**, J. E. HOLDEN;
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Abstract: We recently reported that tonically active alpha1-adrenoceptors contributed to centrally mediated hyperalgesia in the chronic constriction injury (CCI) model of neuropathic pain. The lateral hypothalamus (LH) is part of a descending system that modulates nociception in the spinal cord dorsal horn via a connection with the A7 cell group, which provides norepinephrine (NE) to the spinal cord dorsal horn in rat. To verify whether the source of tonically released NE is the A7 catecholamine cell group, we microinjected cobalt chloride (CoCl; a reversible blocker of synaptic transmission) into the A7 cell group and blocked antinociception induced by intrathecal (IT) application of the alpha-1 adrenoceptor antagonist, WB4101, in both male and female hyperalgesic rats. To determine the role of the LH in tonic activation of alpha1-adrenoceptors, we tested the effect of microinjecting CoCl into the LH on WB4101-induced antinociception. Male and female Sprague-Dawley rats (5-6 per group) received left sciatic nerve ligation via CCI surgery to model hyperalgesia. Fourteen days later, rats received IT infusion of either WB4101 (97 nmol) or normal saline for control. Left paw withdrawal latency (PWL) from a thermal stimulus was measured at 1, 3 and 5 min. After the 5 min measurement, CoCl (100 mM/0.5 μl) was microinjected into the left LH and PWL was measured every 5 min for 45 min. Male rats demonstrated significant antinociception for left PWL following IT administration of WB4101 (9.93 ± 0.91 vs. 5.35 ± 0.83 sec, WB4101 vs. saline, respectively) that was blocked by administration of CoCl in the LH. PWL returned to baseline levels within 10 min as compared to control rats. This was not the case for female rats. Left PWL in female rats receiving WB4101 remained significantly different ($p < 0.001$) from those receiving saline control after microinjection of CoCl in the LH throughout the 45 min of

testing. These findings are suggestive of bilateral hypothalamic innervation of the A7 cell group in females compared to males, and a difference in descending modulation based on sex.

Disclosures: M.A. Wagner: None. **J.E. Holden:** None.

Poster

151. Pain: Descending Modulation

Location: Hall A

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Topic: D.08. Pain

Support: NIH R01 DA23576

NIH F31 NS 073250

Title: Dissecting the mechanism of substance P release in the rat rostral ventromedial medulla during inflammatory injury

Authors: *M. V. HAMITY[1], U. MADUKA[2], D. HAMMOND[1];
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Abstract: It is accepted that substance P (SP) acts in the rostral ventromedial medulla (RVM) to facilitate pain under conditions of peripheral inflammatory nociception induced by ipl injection of complete Freund's adjuvant (CFA). The mechanism may entail an enhanced release of SP that is dependent on presynaptic N-methyl D-aspartate receptors (NMDARs). We therefore examined the release of SP from prisms of RVM tissue obtained from saline or CFA-treated rats. The RVM was dissected from the brainstem of rats that received an ipl injection of CFA or saline in one hindpaw four days earlier. The tissue was chopped into prisms that were incubated at 37 °C for one hr and superfused with artificial cerebrospinal fluid. Releasates were collected and analyzed for SP by enzyme immunoassay. Basal release of SP did not differ between saline and CFA-treated rats (0.007 ± 0.0007 and 0.007 ± 0.0005 pg/ml per mg protein, respectively). Release evoked by 50 mM K^+ also did not differ between saline (0.028 ± 0.003) and CFA-(0.033 ± 0.006) treated rats. As hypothesized, the addition of NMDA to the perfusate facilitated K^+-evoked SP release in both saline- (0.068 ± 0.007) and CFA- (0.072 ± 0.008) treated rats. However, contrary to expectations, the facilitation of SP release by NMDAR activation was not enhanced in CFA-treated rats. Substance P measured in releasates represents overflow from the synaptic cleft. Because peripheral inflammatory injury increases the expression of NK1R in the RVM, we considered the possibility that the upregulation of NK1R in CFA-treated rats served to

effectively internalize and sequester SP internally - making it less available for measurement. To test this, we repeated these experiments in the presence of aprepitant, a NK1R antagonist. The addition of 30 μM aprepitant significantly increased the amount of K^+-evoked release of SP in both treatment groups (0.047 ± 0.004 and 0.053 ± 0.005 pg/ml per mg protein) , but again the release of SP was not significantly greater in CFA-treated rats. Overall, our data support the hypothesis that presynaptic NMDAR can facilitate SP release in the RVM, but do not support the hypothesis that this is the mechanism by which excitatory inputs to pain facilitatory neurons in the RVM are facilitated. However, the finding that aprepitant significantly increased the amount of SP in releasates suggests that binding to and subsequent internalization with the NK1R is significant mechanism to remove SP from the synapse and extracellular environment. Supported by R01 DA23576 and F31 NS 073250

Disclosures: M.V. Hamity: None. **U. Maduka:** None. **D. Hammond:** None.

Poster

151. Pain: Descending Modulation

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Topic: D.08. Pain

Support: NIDA Grant DA07255

Title: Nicotinic receptor excitation of descending pain modulatory pathways

Authors: *I. C. UMANA[1], B. A. MILLER[2], V. T. WAN[3], C. A. DANIELE[4], D. S. MCGEHEE[4];
[1]Univ. of Chicago MSTP, Chicago, IL; [2]PREP Program, [4]Anesthesia and Critical Care, [3]Univ. of Chicago, Chicago, IL

Abstract: The α7 subtype of the nicotinic acetylcholine receptor (nAChR) may be an alternative target for analgesic drug development, as α7 selective agonists and positive allosteric modulators produce antinociception without evidence of adverse side effects. Interestingly, icv administration of α7 agonists decreases nociceptive behavior in chronic pain models, suggesting supraspinal sites of action of these drugs. A strong candidate site of action is the periaqueductal gray (PAG), which is part of the descending pain control pathway. The PAG integrates inputs from higher centers and sends projections to the rostral ventromedial medulla (RVM), which in turn projects to the spinal cord to modulate incoming nociceptive signaling. We recently found functional α7 nAChR expression in 63% of recorded vlPAG-RVM projection neurons. In

addition, somatic μ-opioid receptor (MOR) expression was rare in neurons containing nAChRs, suggesting segregation of these two receptor classes. Therefore, our working model is that activity of vlPAG α7-expressing (α7+) neurons suppresses ascending nociception and activity of MOR-expressing vlPAG neurons facilitates nociceptive signaling. We explored the modulation of excitatory and inhibitory drive to vlPAG-RVM projection neurons by presynaptic nAChRs. RVM-projecting vlPAG neurons were identified by *in vivo* injection of a fluorescent dye into the RVM. In neurons lacking somatic nAChRs (α7-), nicotine enhanced spontaneous inhibitory postsynaptic current (sIPSC) frequency (12/19 neurons), whereas in α7+ neurons, sIPSC frequency increase was less prevalent (7/24 neurons, Fisher's test $p<0.05$). Nicotine-induced enhancement in sIPSC frequency also occurred in the presence of methyllycaconitine, an α7 selective antagonist, suggesting contribution of non-α7 nAChRs. Finally, nicotine bath application increased miniature EPSC frequency in α7+ and α7- neurons. Thus, nicotine enhances excitatory, but not inhibitory drive to α7+ neurons that we propose are pain-inhibiting. Together, our data suggest that presynaptic non-α7 and somatic α7 nAChRs contribute to excitation of putative pain-inhibiting neurons in the vlPAG, which could be a supraspinal mechanism for α7-mediated analgesia. We used the formalin test to assay acute and chronic antinociceptive potential of vlPAG α7 nAChRs. Administration of the selective α7 agonist, PHA-543613, into the vlPAG substantially reduced Phase II nociceptive responses. This antinociception was completely blocked by intra-vlPAG administration of α-bungarotoxin, an α7 selective antagonist. Our findings demonstrate vlPAG α7 nAChR is a promising target for analgesic drug development.

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Poster

151. Pain: Descending Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 151.19/M22

Topic: D.08. Pain

Support: R37-GM48085

Title: Anatomical plasticity in spinal terminals and functional release of oxytocin after peripheral nerve injury

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Abstract: Speed of recovery from pain following nerve injury varies across individuals, with some individuals having pain for many years. Recovery from pain involves many mechanisms, including increased inhibition or decreased sensitization. This study focuses on oxytocin (OT) induced inhibition after injury. Spinal OT fibers originating in the paraventricular nucleus of the hypothalamus terminate in the superficial laminae of the spinal dorsal horn (SDH) where OT excites inhibitory interneurons that modulate primary afferent activity to produce antinociception. Preliminary work suggests that the density of OT fibers in the SDH increases after sub-acute pain states, but whether this occurs after nerve injury has not be investigated. To test this, we performed partial L5 spinal nerve ligation (pSNL), and quantified OT immunoreactivity (IR) in spinal regions ipsilateral (IPSI) and contralateral (CONTRA) to nerve injury. We randomly assigned male, Sprague-Dawley rats at 7W age to pSNL surgery or not and quantitatively assessed spinal cord IR in animals at 9W and 19W (n=8/group). OT fiber density was greater in the IPSI lumbar SDH in the pSNL compared to normal groups ($p=0.012$), but not in the CONTRA SDH ($p=0.343$). This effect was regionally constrained to the lumbar region of the spinal cord. These data suggest an increased capacity for OT release in the lumbar spinal level, where afferents injured with pSNL terminate. We further hypothesized that since spinal OT IR is increased after injury, OT release might also be increased after injury, either tonically or when evoked by noxious stimulation. We performed spinal microdialysis in the IPSI lumbar SDH of male rats in the same 4 groups as the OT IR study above (n=8/group). Baseline OT concentration did not differ among groups, and peripheral noxious stimulation did not evoke spinal release of OT in any group ($p>0.05$). These data suggest that OT anatomical plasticity occurs after injury, but there is no significant increase in spontaneous or evoked OT release in the paradigm tested. Supported by R37-GM48085 from the NIH.

Disclosures: **A.L. Severino:** None. **C.A. Aschenbrenner:** None. **J.C. Eisenach:** A. Employment/Salary (full or part-time):; Anesthesiology.

Poster

151. Pain: Descending Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 151.20/M23

Topic: D.08. Pain

Title: Nabilone (CB1-agonist) reverses fibromyalgia (FM) symptomology. Therapeutic role of endocannabinoid system

Authors: *J. I. ROMERO[1], D. ROMERO-CANO[2];
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Abstract: FM, a complex, diffused syndromatic bodily disarray and peripheral-central multi-domain nervous system disorder, cannot be narrowed down to a simple pathology. Multiples mechanisms for FM phenotype complex symptomology have been proposed: hyper ascending peripheral pain transmission, increased excitatory neurotransmitters, inflammatory cytokines in cerebral spinal fluid, dysfunctional descending modulation, central sensitization, oxidative stress, distinct anatomical cerebral alterations for pain and affective symptoms and early life stress or prolonged severe stress affects brain modulatory circuitry of pain and emotional in genetically susceptible individuals. Years of proposed drug guidelines to modulate different neurotransmitters system have not accomplished satisfactory clinical results. Endocannabinoid signaling system (ECBS) has recently been considered as a therapeutic alternative for FM, since it subserves a multiplicity of cellular and behavioral functions. FM broad symptomology seems to fit possible ECB dysfunctional disarray. Hypothesis: Nabilone, CB1-synthetic agonist, a therapeutic medical alternative will revert ECB hypo-dysfunctional state in FM patients, improving quality of life by increasing sleep time, reducing awakenings, pain intensity and anxiety. Methodology: 30 female patients ranging from 8 to 40 years of confirmed diagnosis by rheumatologist or internist were accepted under consent agreement to participate in a Nabilone open trail, for a period of 10 weeks, each patient paid for her own treatment. Rheumatologic and psychiatric treatments had already been suspended. Following scale were used to evaluate patients pre-trans and post: Fibromyalgia Impact Questionnaire (FIQ), Modified Athens scale to assess sleep and fragmented sleep pattern, VAS pain scale and Hamilton anxiety scale. Results: No-parametric statistical analysis have shown significant change ($P<0.05$) in all four main symptoms. Nabilone alone restores sleep by reducing FSP, quality of profound sleep, reduced pain intensity and anxiety, Sumary: Nabilone activated ECB signaling system reverting disarray of bodily, central and autonomous nervous system. However, ECB system alone does not bring about the cure, but does allow for control of symtoms and an improvement in que quality of life of each patient within 10 weeks and has continue to do so for over a year.

Disclosures: J.I. Romero: None. **D. Romero-Cano:** None.

Poster

151. Pain: Descending Modulation

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Topic: D.08. Pain

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Title: Parabrachial input to pain-modulating circuit in acute and chronic pain

Authors: *Q. CHEN, M. HEINRICHER;
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Abstract: An important factor in both normal and clinically significant pain is the intrinsic pain-modulating system that regulates nociceptive processing via projections from the brainstem to the dorsal horn. The output of this modulating system, the rostral ventromedial medulla (RVM), can facilitate or suppress nociceptive transmission at the dorsal horn by the respective action of two physiologically and pharmacologically distinct classes of neurons, “ON-cells” and “OFF-cells”. Both classes are known to respond to noxious inputs: ON-cells are activated, leading to a “burst” of activity associated with behavioral responses to noxious stimulation, whilst OFF-cell firing is suppressed, producing a “pause” in any ongoing activity. However, the pathway through which noxious inputs drive changes in RVM activity has not yet been defined. Anatomically, the RVM receives direct afferents from the parabrachial complex (PB), which is the major target of supraspinal projections from the superficial dorsal horn. The spinoparabrachial pathway is known to be crucial in behavioral hypersensitivity in chronic pain. The aim of this study was to test the hypothesis that noxious information is relayed to the pain-modulating neurons of the RVM via the PB in acute and persistent pain. Single-cell recording studies were performed in lightly anesthetized rats. ON-cells and OFF-cells were recorded in the RVM before and after block of the PB using local microinjection of muscimol. Spontaneous activity and responses to von Frey probes applied to the paw were recorded. The reflex-related ON-cell burst and OFF-cell pause were attenuated by inactivation of the PB. Spontaneous firing of both cell classes was also altered. Optogenetic manipulation of PB terminals in RVM demonstrated that this relay is at least in part direct. In animals subjected to persistent inflammation (CFA), PB block also attenuated ON- and OFF-cell responses to noxious stimuli. However, PB block did not eliminate the sensitization to innocuous stimuli. These data show that a substantial component of the relevant nociceptive drive to RVM pain-modulating neurons is relayed through the parabrachial complex. The differential effect of PB block in a persistent inflammatory pain state implies altered or additional input to the RVM in persistent pain. While the parabrachial nucleus is well known as an important relay for ascending nociceptive information, the connection with the RVM allows the spinoparabrachial pathway to access descending control systems as well.

Disclosures: Q. Chen: None. **M. Heinricher:** None.

Poster

151. Pain: Descending Modulation

Location: Hall A

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Program#/Poster#: 151.22/M25

Topic: D.08. Pain

Support: MRC

Title: Periaqueductal grey EP3 receptor activity facilitates spinal nociception in arthritic secondary hyperalgesia

Authors: *R. DRAKE[1], L. J. LEITH[1], B. M. LUMB[1], L. F. DONALDSON[2];
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Abstract: The midbrain periaqueductal grey (PAG), together with downstream nuclei within the medulla and brain stem, can affect pain experience by regulating the spinal processing of A- and/or C-nociceptor inputs and subsequent transmission of nociceptive information to the brain. The balance of descending inhibitory and/or facilitatory control of spinal nociceptive processing plays a major role in shaping the pain phenotype following tissue injury. For instance, sensitisation to noxious stimuli develops within undamaged tissue adjacent, and also distant to, the damaged site (secondary hyperalgesia) and spinal neuronal pools that serve this secondary domain are dominated by descending facilitatory controls that amplify spinal inputs from unsensitised peripheral nociceptors. Cyclooxygenase enzyme activity and/or prostaglandin E 2 signalling within the ventrolateral (vl) PAG is known to be pro-nociceptive in naïve and acutely inflamed animals but its role in more persistent inflammation or, specifically, its contribution to secondary hyperalgesia remains unknown. We have used a combined pharmacological and electrophysiological approach to determine the effects of prostaglandin EP3 receptor activity within the vlPAG on the spinal processing of A- and C-nociceptor inputs in naïve and arthritic rats, and its contribution to secondary hyperalgesia in arthritic animals. In naïve male Wistar rats blockade of COX enzyme activity or EP3 receptor activity increased thermal withdrawal thresholds in the awake animal. Additionally, in anaesthetised naïve animals, activation or inhibition of vlPAG EP3 receptors affected C-, but not A-, nociceptor evoked reflex withdrawal thresholds. Arthritic rats (Freund's complete adjuvant model) developed a mechanical and thermal secondary hyperalgesia of the hind-paw that was associated with a sensitisation of withdrawal thresholds to A-, but not C-, nociceptor activation. Notably, in arthritic animals the influence of EP3 receptor activity within the vlPAG extended to the spinal processing of A-nociceptor inputs and PAG EP3 receptor blockade increased withdrawal thresholds to A- and C-

nociceptor activation in the area of secondary hyperalgesia. Unexpectedly, vlPAG EP3 receptor blockade increase the firing threshold of spinal dorsal horn neurones to C-, but not A-, nociceptor activation in the area of secondary hyperalgesia. We conclude that in the naïve animal EP3 receptor activity in the vlPAG facilitates the spinal nociceptive processing of C-nociceptor inputs alone but affects the spinal processing of both A- and C-nociceptor inputs from an area of secondary hyperalgesia in the arthritic animal.

Disclosures: **R. Drake:** None. **L.J. Leith:** None. **B.M. Lumb:** None. **L.F. Donaldson:** None.

Poster

152. Mechanisms of Neuropathic Pain I

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RVO67985823

GAUK138215

Title: Modulation of TRPV1 activity in spinal cord neurons presynaptic endings by paclitaxel is mediated by TLR4 activation

Authors: P. ADAMEK[1,2], P. MROZKOVA[1,2], *J. PALECEK[3];
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Abstract: Paclitaxel is widely used in the clinical practice as chemotherapeutic, but the treatment is frequently accompanied with development of persistent painful peripheral neuropathy. In our study we tested hypothesis that these adverse effect could be at least partialy mediated due to activation of Toll Like (TLR4) and Transient Receptor Vanilloid 1 (TRPV1)

receptors at the spinal cord level. Whole-cell patch clamp recordings of miniature (mEPSC), spontaneous (sEPSC) and dorsal root stimulation evoked (eEPSC) excitatory postsynaptic currents were made from superficial dorsal horn neurons (Voltage-Clamp configuration at -70 mV) in acute spinal cord slices (300 μM) prepared from adult male mice C57BL/6 weighting 25 to 30 g and from male Wistar rats 21 days old. All the recordings were made in the presence of strychnine (5 μM) and bicuculline (10 μM), TTX (0.5 μM) application was used for mEPSC detection. Our data demonstrated that acute application of low concentration paclitaxel (50 nM) induced significant increase in the frequency of mEPSC. This effect was prevented by the TRPV1 antagonist SB366791 (10 μM) pretreatment. However, the paclitaxel application did not influence frequency or amplitude of the sEPSC and eEPSC. These results indicate direct modulation of presynaptic endings by paclitaxel. In other set of experiments we tested the effect of paclitaxel application on repeated capsaicin evoked response in superficial dorsal horn neurons recorded as mEPSC frequency. Under control conditions the mEPSC frequency of the second response to capsaicin (0.2 μM) was significantly smaller when compared with the first one (32,6 %). Acute application of paclitaxel before the second capsaicin application prevented the decrease of mEPSC frequency and was not different from the first response. This effect was prevented by coapplication of the paclitaxel with TLR4 antagonist LPS-RS (2 μg/ml). Our results demonstrate that functional interaction between TLR4 and TRPV1 receptors may play an important role in modulation of nociceptive synaptic transmission in spinal cord dorsal horn after paclitaxel treatment. Understanding these mechanisms is needed to improve treatment of the chemotherapy-induced neuropathic pain.

Disclosures: **P. Adamek:** None. **P. Mrozkova:** None. **J. Palecek:** None.

Poster

152. Mechanisms of Neuropathic Pain I

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RVO67985823

GAUK 1566314

Title: The role of proinflammatory cytokines and TRPV1 receptors in paclitaxel induced peripheral neuropathy

Authors: *N. KALYNOVSKA[1,2], M. DIALLO[1], J. PALECEK[1];
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Abstract: Chemotherapy-induced peripheral neuropathy often represents a dose-limiting negative side effect for the use of paclitaxel in clinical practice. However, the underlying mechanism of the disease remains still poorly understood. Accumulating evidence from animal models of peripheral neuropathy has implicated a number of peripheral and central proinflammatory processes to play an important role in the development of neuropathic pain. In our experiments we have focused on the function of TNFalpha, CCL2 and TRPV1 receptors in paclitaxel induced neuropathy. The model of peripheral neuropathy was established in adult male Wistar rats by i.p. injection of paclitaxel solution (5 x 2 mg/kg) on five alternate days. On days 10 and 21 after the first injection, lumbar dorsal root ganglia (DRGs) and spinal cord dorsal horn (SCDH) tissues were collected and further used for Western blot and RT PCR experiments. The acute effect of paclitaxel application was tested on spinal cord slices from 21 days old rats under *in vitro* conditions. Paclitaxel treatment significantly increased protein expression of TRPV1 receptors, glial cell marker GFAP, protein levels of TNFalpha and CCL2 in lumbar DRGs at day 10 and with exception of TNFalpha also at 21 days after the first injection of paclitaxel. In the spinal cord dorsal horn, paclitaxel induced TRPV1 overexpression only at day 10, but not at day 21. GFAP, TNFalpha and CCL2 increased levels were detected on day 21, but not on day 10. mRNA levels of TRPV1 and proinflammatory markers did not change significantly during both tested periods (days 10 and 21). We have used *in vitro* spinal cord slices to study molecular mechanisms of paclitaxel-induced cellular changes within the central nervous system. In the first experiments, c-Fos expression was evaluated in dorsal horn neurons after incubation with 100 nM paclitaxel for 60 min. The paclitaxel application induced a significant increase in c-Fos expression within the superficial area of spinal cord dorsal horn. This increased expression of c-Fos protein was significantly attenuated by preincubation of lumbar spinal cord slices with TRPV1 antagonists (SB 366791 and AMG 9810). Our data indicate an important role of TRPV1 receptors in the cellular mechanisms of paclitaxel-induced neuropathy and possible modulation of their function by proinflammatory cytokines.

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Poster

152. Mechanisms of Neuropathic Pain I

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Topic: D.08. Pain

Support: NS046606

H.E.B. Professorship

Title: Modulation of TRPV1 activity in DRG neurons by paclitaxel is mediated by TLR4 activation

Authors: *P. M. DOUGHERTY[1], H. ZHANG[2], C. E. TATSUI[3], L. D. RHINES[3], Q. LI[3], A. K. KOSTURAKIS[4], H. ZHANG[3], R. M. CASSIDY[2], J. P. CATA[3], K. SAPIRE[3], D. S. HARRISON[5], R. M. KENNAMER-CHAPMAN[2], A. B. JAWAD[2], A. GHETTI[6], J. YAN[3], Y. LI[3];
[1]Pain Med., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [2]The Univ. of Texas Hlth. Sci. Ctr. Houston, Houston, TX; [3]The Univ. of Texas M.D. Anderson Cancer Ctr., Houston, TX; [4]The Univ. of Texas Hlth. Sci. Ctr. San Antonio, Houston, TX; [5]Duke Univ. Sch. of Med., Durham, NC; [6]Anabios, Inc., San Diego, CA

Abstract: Peripheral neuropathy is dose-limiting in cancer chemotherapy with paclitaxel and can often induce persistent pain and discomfort in cancer survivors. The hypothesis tested was that chemotherapeutic agents produce these adverse effects at least in part by sensitizing transient receptor potential vanilloid subtype 1 (TRPV1) through Toll-like receptor 4 (TLR4) signaling. The data show that paclitaxel-induced behavioral hypersensitivity to mechanical stimuli is prevented and reversed by spinal administration of a TRPV1 antagonist (AMG9810). TLR4 is expressed in TRPV1-positive rat and human dorsal root ganglion (DRG) neurons. TRPV1 expression is increased in DRG neurons in paclitaxel-treated rats. Perfusion of paclitaxel or the archetypal TLR4 agonist lipopolysaccharide (LPS) directly activated rat DRG neurons and produces acute sensitization of TRPV1 via a TLR4-mediated mechanism. All these effects were prevented by co-administration with LPS-RS (TLR4 antagonist). Paclitaxel and LPS sensitize TRPV1 in HEK293 cells stably expressing TLR4 and transiently expressing TRPV1. Finally, paclitaxel directly activates and sensitizes TRPV1 responses in dissociated human DRG neurons. In summary, TLR4 was activated by paclitaxel and led to sensitization of TRPV1. This mechanism could contribute to paclitaxel-induced acute pain and chronic painful neuropathy.

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Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

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Topic: D.08. Pain

Title: Increase of mast cells through TRPV1-expressing primary afferents are involved in oxaliplatin-induced mechanical allodynia in mice

Authors: *T. ANDOH, A. SAKAMOTO, Y. KURAISHI;
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Abstract: The chemotherapeutic agent oxaliplatin induces peripheral neuropathy, as a dose-limited side effect. However, the mechanisms of oxaliplatin-induced peripheral neuropathy are still unclear. In this study, we demonstrated that cutaneous mast cell was involved in oxaliplatin-induced mechanical allodynia in mice. Male C57BL/6NCr mice were used, except for one series of experiments in which male mast-cell deficient mice (WBB6F1 W/Wv) and the normal littermates (WBB6F1+/+) were used. In addition, neonatal capsaicin-treated C57BL/6NCr mice were also used. A single intraperitoneal injection of oxaliplatin (3 mg/kg) or vehicle (5% glucose) was given in above mice. Mechanical allodynia was evaluated using a von Frey filament (0.69 mN). A single intraperitoneal injection of oxaliplatin elicited mechanical allodynia, which peaked on day 10 after the injection. Mast cell deficiency inhibited significantly and completely oxaliplatin-induced mechanical allodynia. Oxaliplatin-induced mechanical allodynia was significantly inhibited by serine protease inhibitor camostat mesilate and proteinase-activated receptor 2 antagonist FSSLY-NH2, but not H1 histamine receptor antagonist terfenadine. In the plantar skin of mice treated with oxaliplatin, the number of total mast cells was significantly increased. In neonatal capsaicin-treated adult mice, an intraperitoneal injection of oxaliplatin did not induce mechanical allodynia and increase the number of cutaneous mast cells. Neonatal capsaicin-treatment results in the deletion of TRPV1 expressing peripheral sensory neurons. Thus, it is suggested that TRPV1-expressing primary afferents are involved in the increase of cutaneous mast cells. In addition, serine proteases from mast cells may play an important role in oxaliplatin-induced mechanical allodynia.

Disclosures: **T. Andoh:** None. **A. Sakamoto:** None. **Y. Kuraishi:** None.

Poster

152. Mechanisms of Neuropathic Pain I

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Topic: D.08. Pain

Support: CB-2012/179294

Title: The transcription factor Sp1 regulates the expression of the Ca^{2+} channel α2δ-1 auxiliary subunit in neuropathic pain

Authors: ***K. GOMEZ**[1], M. PEREZ-RODRIGEZ[1], P. BARRAGAN-IGLESIAS[1], V. GRANADOS-SOTO[1,2], R. GONZALEZ-RAMIREZ[2,3], R. FELIX[3];
[1]Farmacobiologia, Cinvestav, Sede Sur, Ciudad DE Mexico, Mexico; [2]Biología Mol. e Histocompatibilidad, Hosp. Gen. Dr. Manuel Gea Gonzalez, Ciudad DE Mexico, Mexico; [3]Biología Celular, Cinvestav, Ciudad De Mexico, Mexico

Abstract: High voltage-activated calcium (CaV) channels are a family of transmembrane proteins that form Ca2+-selective pores. These channels are oligomeric complexes of a CaVα1 ion-conducting subunit and CaVβ and CaVα2δ auxiliary subunits that open by depolarization of the plasma membrane, being essential regulators of the intracellular Ca2+ concentration. At the transcriptional level, the expression of the α2δ-1 subunit is regulated by the Sp1 transcription factor. The main role of this auxiliary subunit is to promote the trafficking of functional CaV channels to the plasma membrane and to modify the channel kinetics and its voltage-dependent properties. During peripheral nerve injury, an upregulation of the CaV α2δ-1 auxiliary subunit occurs in the dorsal root ganglia (DRG) ipsilateral to the nerve injury, which has been correlated with allodynia. In the present report, in order to investigate the possible involvement of the transcription factor Sp1 as a molecular determinant of the exacerbated expression of CaVα2δ-1 auxiliary subunit we examined the expression of α2δ-1 and Sp1 in the DRG using the L5/L6 spinal nerve ligation model in rats with or without intrathecal administration of mithramycin-A, a Sp1 inhibitor, and its correlation with tactile allodynia. Our data indicate that tight ligation of the L5/L6 spinal nerve results in a time-dependent α2δ-1 and Sp1 upregulation in the DRG that correlates with tactile allodynia. Mithramycin-A treatment of spinal nerve ligated animals, at doses that alleviate allodynia, markedly decreased the levels of Sp1 and prevented the elevation of α2δ-1 expression that normally occurs in neuropathic pain, in a dose-dependent manner. Thus, it is reasonable to hypothesize that Ca2+ currents may be increased in DRG neurons during

neuropathic pain development, and that this effect may be explained, at least in part, by an elevation of α2δ-1 expression as a result of an overexpression of Sp1 in sensory neurons. Kimberly Gomez, Marian Jesabel Perez-Rodriguez and Paulino Barragan-Iglesias are Conacyt fellows. Ricardo González-Ramírez was supported by a post-doctoral fellowship from Conacyt (CB-2012/179294). Vinicio Granados-Soto and Ricardo Felix were supported by a Conacyt grant (CB-2012/179294).

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Poster

152. Mechanisms of Neuropathic Pain I

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Topic: D.08. Pain

Title: Molecular mechanisms for the upregulation of $Ca_v3.2$ T-type calcium channels in the dorsal root ganglion of rats with spinal nerve injury-induced neuropathy: involvement of Egr-1 and USP5

Authors: *S. TOMITA, F. SEKIGUCHI, M. TSUBOTA, A. KAWABATA;
Kinki Univ. Sch. Pharm., Higashi-Osaka, Japan

Abstract: We have reported that the upregulation of $Ca_v3.2$ T-type calcium channels in the primary sensory nerves contributes to the development of neuropathic pain in rats with L5 spinal nerve cutting (L5SNC). It has been reported that expression of $Ca_v3.2$ is transcriptionally promoted by early growth response 1 (Egr-1) and suppressed by RE-1 silencing transcription factor (REST), and that $Ca_v3.2$ protein is protected by ubiquitine specific peptidase 5 (USP5), a deubiquitinating enzyme, from proteasomal degradation. We thus determined protein levels of Egr-1, REST and USP5, in parallel with $Ca_v3.2$, in the dorsal root ganglia (DRG) after L5SNC in the rats, and analyzed their possible involvement in the upregulation of $Ca_v3.2$ and development of neuropathic pain. After L5SNC, mechanical nociceptive threshold in the ipsilateral hindpaw, as assessed by the paw pressure test and von Frey test, significantly decreased on day 6 (early phase) and reached a bottom level on days 9-14 (persistent phase). The hyperalgesia/allodynia induced by L5SNC was suppressed by administration of RQ-00311651, a T-type calcium channel blocker. Protein levels of Egr-1 and $Ca_v3.2$ significantly increased in L4, but not L5 or L6, DRG on days 6 and 14 after L5SNC, although REST expression remained constant before and after L5SNC. USP5 was upregulated on day 14, but not day 6, in L4 DRG, while it was

upregulated on days 6 and 14 in L5 DRG. Knockdown of Egr-1 in the early phase with the antisense method inhibited the neuropathic hyperalgesia and upregulation of $Ca_v3.2$ in L4 DRG on days 8-9 after L5SNC. Knockdown of USP5 in the persistent phase abolished the neuropathic hyperalgesia and upregulation of $Ca_v3.2$ in L4 DRG on day 14 after L5SNC. These data suggest that, in the L4 DRG of rats after L5SNC, Egr-1 induces $Ca_v3.2$ upregulation and contributes to the development of neuropathy in the early phase, and USP5 plays a critical role in the maintenance of the upregulated $Ca_v3.2$ and neuropathic pain in the persistent phase.

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Poster

152. Mechanisms of Neuropathic Pain I

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Title: Complement 3a receptor mediates dorsal horn Ca^{2+} signaling evoked by the VGF peptide TLQP-21 in the setting of neuropathic pain

Authors: *S. DOOLEN[1], K. F. KITTO[2], C. A. FAIRBANKS[2,3,4], L. VULCHANOVA[2], B. K. TAYLOR[1];
[1]Dept. of Physiol., Univ. of Kentucky, Lexington, KY; [2]Dept. of Neurosci., [3]Dept. of Pharmaceutics, [4]Dept. of Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: VGF (non-acronymic) is granin-like neuropeptide precursor whose expression is upregulated in sensory and spinal cord neurons after peripheral nerve injury. Intrathecal administration of the VGF-derived peptide TLQP-21 produces hyperalgesia, while TLQP-21 immunoneutralization reduces behavioral signs of neuropathic pain. Recently, the complement 3a receptor (C3aR1) was identified as a receptor for TLQP-21. We have shown that TLQP-21 induced thermal hyperalgesia is dose-dependently reversed by co-administration with a C3aR1 antagonist, SB209157. Here, we tested the hypothesis that TLQP-21 contributes to neuropathic pain through the activation of C3aR1. We determined whether TLQP-21/C3aR1 activation increases Ca^{2+} signaling in dorsal horn, and whether these effects are potentiated by nerve injury.

Spared nerve injury was performed in adult mice, and mechanical hypersensitivity was confirmed 14 d later. Transverse lumbar slices (450 um; L3/L4) were bulk loaded with 10 uM fura-2 AM for ratiometric Ca^{2+} imaging, labeled with the astrocyte marker SR-101, and evaluated for TLQP-21-evoked Ca^{2+} signaling. Superfusion with TLQP-21 evoked Ca^{2+} signals in SR-101-negative lamina II profiles (presumably cells) from both sham and injured mice, suggesting that the TLQP-21 induced Ca^{2+} responses were evoked in either neurons or microglia, but not astrocytes. The majority (86.4%) of TLQP-21-evoked Ca^{2+} signals did not respond to glutamate, suggesting a non-neuronal (likely microglial) origin. Nerve injury significantly increased peak Ca^{2+} signals evoked by either 0.1 or 1.0 μM TLQP-21. This was significantly decreased in the presence of SB290157 (1 μM). These data support the hypothesis that TLQP-21 activation of C3aR1 in dorsal horn of spinal cord contributes to spinal neuroplasticity under conditions of neuropathic pain.

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Poster

152. Mechanisms of Neuropathic Pain I

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Topic: C.13. Sensory Disorders

Support: NIH Grant R44NS086343-01

Title: AFA019 differentially inhibits T-type Ca^{2+} currents over Na+ and K+ currents in DRG neurons and mitigates neuropathic pain in mice

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Abstract: Overactivation of the T-type Cav3 Ca2+ channel (T-channel) is involved in development of seizures and neuropathic pain. Current pharmacotherapeutics do not treat neuropathic pain well. To discover a potent and selective T-channel inhibitor for the treatment of neuropathic pain, we took a rational drug design approach. A new series of 1,3,4-oxadiazole compounds was synthesized and evaluated. The versatility of the synthetic strategy was using two different substituents at C2 and C5 of the oxadiazole scaffold to generate new molecules. This involved coupling reactions of various acid halides with 5-substituted-2-amino-1,3,4-oxadiazoles, which were made from the ring closing sequence of acid chlorides with

thiosemicarbazide followed by 1,3-dibromo-5,5-dimethylhydantoin. AFA019 represents one of these chemicals producing potent inhibition of T-currents *in vitro* and analgesic efficacy against neuropathic pain in mice. Using voltage-clamp recordings of acutely isolated dorsal root ganglion (DRG) neurons from adult mice, we observed that AFA019 (1 μM) inhibited T-Ca2+ current by 50 ± 9.7% (n = 8). At the same concentration, AFA019 caused 16.5 ± 8.9% (n = 7) inhibition of voltage-gated Na+ currents, and 18.9 ± 11.8% (n = 4) inhibition of early transient K+ currents and 24.1 ± 14.6% (n = 4) inhibition of the delayed steady K+ currents. The effect on the T- currents was concentration-dependent with an IC50 of 0.93 μM. In contrast, the inhibitory effect of AFA019 on Na+ and K+ currents appears to be less concentration-dependent. At 30 μM the inhibition of AFA19 was around 20~30% on both Na+ and K+ currents. The selectivity against T-current over high-voltage-activated Ca2+ currents is under investigation. For comparison, we tested Z944 which is in clinical trials for treatment of neuropathic pain. Z944 inhibited T-currents in DRG neurons with an IC50 of 0.2 μM. However, it also potently inhibited Na+ currents with an IC50 of 0.51 μM. At 1 μM, Z944 also caused 45% inhibition of K+ currents. Administration of AFA019 (0.3 - 30 mg/kg, i.p. n = 8) significantly mitigated neuropathic pain in both spared nerve injury- and paclitaxel (Taxol, 4 mg/kg, i.p.)-induced chronic pain models in mice in a dose-dependent manner. AFA019 (30 mg/kg, i.p.) also significantly reduced seizures and fatality induced by either pentylenetetrazole (40 mg/kg, i.p.) or maximal electric shocks in mice. These results demonstrate that AFA019 could be a potentially effective therapeutic to treat both neuropathic pain and epilepsy.

Disclosures: **B. Zou:** None. **C. Pascual:** None. **C. Lieu:** None. **M.J. Gunaratna:** None. **M. Zhang:** None. **D.H. Hua:** None. **S.X. Xie:** None.

Poster

152. Mechanisms of Neuropathic Pain I

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Topic: D.08. Pain

Support: MEXT KAKENHI 24791558

NEXT KAKENHI 30597084

NEXT KAKENHI 25462303

Title: Interferon-gamma activates NMDA receptors in the dorsal horn of spinal cord

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Abstract: A pro-inflammatory cytokine, interferon-gamma (IFNγ), is upregulated in the dorsal horn after peripheral nerve injury, and it has been implicated as one cause of neuropathic pain. It was reported that intrathecal injections of IFNγ induce pain related behaviors in rats and mice, whereas IFNγ receptors knock-out mice does not respond. However, little is known about the roles of IFNγ in the spinal cord. To elucidate how the excitatory synaptic transmissions mediated by IFNγ in the spinal cord, we investigated the effects of IFNγ on synaptic transmission by using whole-cell patch-clamp recordings from substantia gelatinosa (SG) neurons. At first we investigated the action of IFNγ on AMPA (α-amino-3-hydroxy-5-methyl-4-Isoxazole-4-propionic acid) receptor-mediated currents in the voltage-clamp mode -70 mV. Bath application of IFNγ did not affected AMPA-induced current. While, NMDA (N-methyl-D-asparate)-receptor-mediated currents were significantly increased by the application of IFNγ in the voltage-clamp mode -50 mV. This IFNγ-induced facilitatory actions of NMDA current was inhibited by IFNγ receptor selective antagonist. In addition, minocycline, an inhibitor of microglia activation, also blocked the facilitatory effect of IFNγ on NMDA-induced currents. These results suggest that IFNγ binds own receptor and enhances the activity of NMDA but not AMPA receptors in the dorsal horn of spinal cord. It is also considered that this mechanism includes the microglia-neuron crosstalk. The activation of NMDA receptors in SG neurons by IFNγ may contribute the persistent neuropathic pain.

Disclosures: M. Sonekatsu: None. **W. Taniguchi:** None. **M. Yamanaka:** None. **N. Nishio:** None. **S. Tsutsui:** None. **H. Nishi:** None. **H. Hashizume:** None. **T. Nakatsuka:** None. **M. Yoshida:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

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Program#/Poster#: 152.10/M35

Topic: D.08. Pain

Support: CB-2012/179294

Title: Role of anion exchanger 3 in different models of chronic pain

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Abstract: Primary sensory neurons keep a high intracellular chloride concentration ([Cl−] in). In addition, these neurons show a depolarizing GABAA receptor conductance not only during the development but also in its mature state. The polarity and magnitude of these currents is determined primarily by [Cl-]i. In normal conditions This mechanism contributes to the development of primary afferent depolarization (PAD); therefore in the presynaptic inhibition. However, after tissue or nerve injury this phenomenon instead of inhibitory becomes excitatory, contributing to pain states. Thus, chloride plays an important role in pain processing. It is known that the anion exchanger 3 (AE3) is expressed in dorsal root ganglia (DRG) and also contributes to maintain [Cl-]i in the primary neurons. It has been shown that AE3 inhibitors produce acute antinociception in the formalin test while formalin injection increases AE3 expression. However, the role of the AE3 in chronic pain conditions is unknown. The purpose of this study was to determine the participation of AE3 in two different models of chronic pain. Neuropathic pain was induced by spared nerve injury (SNI) whereas chronic inflammatory pain was induced by intraplantar injection of complete Freund's adjuvant (CFA). Tactile allodynia and thermal hyperalgesia of the left hind paw (ipsilateral) and right hind paw (contralateral) were assessed at 0, 3, 7, 14 and 21 days after ligation or administration of CFA. Western blotting was used to determine AE3 protein expression in DRG. Axotomy and ligation of tibial and common peroneal nerves and intraplantar injection of CFA induced tactile allodynia and thermal hyperalgesia, which were maintained for 21 days. The AE3 protein expression was modulated differentially and time-dependent manner in DRG after SNI or CFA injection. An enhancement of AE3 protein expression in L4 and L5 DRG was observed at 7 and 14 days after SNI. On the other hand, AE3 protein expression in L4 DRG increased 14 days after CFA injection, while the increment in L5 and L6 occurred 7 days after injection. Inthrathecal pre-treatment (-10 min) with the chloride-bicarbonate anion exchanger inhibitor, 4,4´-diisothiocyanatostilbene-2,2-disulfonic acid (DIDS, 50 µg/10µl) partially prevented SNI- and CFA-induced nociception. These results suggest that chronic pain upregulates AE3 expression in DRG. Data also suggest that AE3 participates in the development and maintenance of chronic pain. This work was partially supported by a Conacyt grant (CB-2012/179294 to VG-S). MJP-R and PB-I are Conacyt fellows.

Disclosures: M.J. Pérez Rodríguez: None. **K. Gomez:** None. **P. Barragan-Iglesias:** None. **V. Granados-Soto:** None.

Poster

152. Mechanisms of Neuropathic Pain I

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Topic: D.08. Pain

Support: Deutsche Forschungsgemeinschaft (DFG) within the framework of the Excellence Initiative (EXC 307)

Deutscher Akademischer Austauschdienst (DAAD) to Flavia Frattini

Title: The role of SorLA, a sorting protein-related receptor, in neuropathic pain development

Authors: *F. FRATTINI[1,2], J. HU[1];
[1]Ctr. For Integrative Neurosci., Tuebingen, Germany; [2]Grad. Sch. of Neural & Behavioural Sciences/International Max Planck Res. School, Eberhard Karls Univ. Tübingen, Tuebingen, Germany

Abstract: Neuropathic pain is an intractable and frequent condition characterized by exaggerated response to painful stimuli (hyperalgesia) or normally innocuous stimuli (allodynia), and spontaneous pain. One of the mechanisms involved in the development of neuropathic pain is the loss of inhibition mediated by γ-aminobutyric acid (GABA) in the spinal cord after peripheral nerve injury. Altered intracellular chloride (Cl-) concentration regulated by brain-derived neurotrophic factor (BDNF) upon potassium chloride co-transporter 2 (KCC2) and sodium-potassium-chloride co-transporter 1 (NKCC1) plays an important role to promote neuropathic pain since it affects GABAA receptor reversal potential, thus leading to loss of inhibition or even a switch to excitation. But how BDNF regulates KCC2/NKCC1 remains unclear. SorLA (sorting protein-related receptor with A-type repeats), an intracellular trafficking receptor which is associated with Alzheimer disease, has been shown to regulate the phosphorylation of NKCC2 (the renal analog of NKCC transporters) in kidney, influencing Cl- intake and supporting the maintenance of renal function. Interestingly, BDNF has been reported to regulate SorLA expression in neurons. We therefore hypothesize that SorLA may be involved in the development of neuropathic pain while acting downstream of BDNF signaling pathway. We first detected a prominent expression of SorLA in the spinal cord and dorsal root ganglion neurons, indicating that this protein could have a function in somatosensory system. Furthermore, after sciatic nerve injury, animals lacking SorLA did not develop allodynia, the major cause of complaints among neuropathic pain patients. Similar observation was made after mimicking nerve injury pain through BDNF intrathecal injection, confirming that BDNF regulates pain behavior through SorLA. Taken together, our findings suggest that SorLA influences pain perception induced by mechanical stimulus after nerve injury and support the hypothesis that it acts downstream in the BDNF pain pathway to promote neuropathic pain. Further experiments will address whether SorLA influences KCC2/NKCC1 regulation in neuropathic pain. This study may unravel an innovative role for SorLA as a mediator between

BDNF and chloride regulation in neurons after injury and may have relevant clinical implications for patients suffering from neuropathic pain.

Disclosures: **F. Frattini:** None. **J. Hu:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.12/M37

Topic: D.08. Pain

Title: Nerve injury promotes loss of nociceptive neuron-specific Gαi-interacting protein (Ginip) expression in neuropathic pain rat

Authors: *H. YU, Z. LIU, F. WANG, G. FISCHER, Q. H. HOGAN;
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Abstract: Gαi-interacting protein (Ginip) is a signal transduction molecule that expresses specifically in dorsal root ganglion (DRG) nociceptive neurons and plays a role in modulating metabotropic GABAb receptor (GBR)-mediated antinociception via interactions with Gαi. Genetic deletion of *Ginip* leads to impaired responsiveness to GBR agonist-mediated analgesia in mouse. However it has not been defined whether nerve injury changes Ginip expression. Here we showed that Ginip expresses in ~40% of total lumbar DRG neurons in normal adult rats and its immunoreactivity (IR) is distributed in ~80% of IB4+ (nonpeptidergic) and ~30% of CGRP+ (peptidergic) neurons. The Ginip+ central axonal fibers terminate in lamina II of spinal dorsal horn (DH). Ginip is expressed in DRG neurons immunopositive for GBR1, GBR2, Gαi(s), and Gαo; and its IR is also extensively colabeled with multiple nociceptive neuronal markers, including $Ca_V2.2$α1b, $Ca_V3.2$α1b, $Na_V1.7$, TRPV1, and Trek2. Importantly, peripheral nerve injury by L5 spinal nerve ligation (L5-SNL) significantly decreases the proportion of Ginip-positive neurons from 40±8.4% to 1.8±1.2% ($p<0.01$) and the total Ginip protein to 1.3%±0.04% of its basal level ($p<0.01$, n = 6 animals in each group, mean± SD) in the ipsilateral L5 DRGs. In contrast, no significant change in Ginip was found in adjacent non-injured L4 DRGs. These data support that Ginip is a marker predominant for nonpeptidergic small nociceptive neurons, and also point that nerve injury triggers loss of Ginip expression. Results also indicate that the signal transduction roles of Ginip may be diverse as it colabeled with various subgroups of nociceptive neurons. Future studies may investigate details of the signaling mechanism engaged by Ginip, as well as the pathophysiological significance of lost expression of Ginip in neuropathic pain.

Disclosures: **H. Yu:** None. **Z. Liu:** None. **F. Wang:** None. **G. Fischer:** None. **Q.H. Hogan:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: NHMRC program grant APP569927

Title: α9-nAChR: Novel analgesic target or crucial for mental health?

Authors: ***S. MOHAMMADI**, M. J. CHRISTIE;
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Abstract: The α9-subunit of nicotinic acetylcholine receptors (α9-nAChR) has recently been identified as a novel analgesic target. However, the evidence for the involvement of this receptor in pain has been indirect and confounded. Peptides from marine cone snail venom, known as conotoxins, are highly selective for mammalian receptors and ion channels. These conotoxins are proving to be incredibly useful pharmacological tools for dissecting the specific roles of cellular proteins in the mammalian system. A number of conotoxins that are highly selective for α9-nAChRs have been identified and these have been shown to be effective analgesics in animal models of chronic pain. From these analgesic conotoxins, it has been inferred that the α9-nAChR is a valid target for analgesics. However, evidence has emerged that α9-nAChR-inhibition is not necessary or sufficient for the broad analgesic effects of these conotoxins, nor is it their primary mechanism of analgesia. Furthermore, α9-nAChRs are necessary for only one (mechanical hyperalgesia) of the many pain modalities that are affected by chronic pain. Behavioural testing of mice with germline α9-nAChR-deletion revealed a vital role of the α9-nAChR in coping mechanisms during acute and chronic stress conditions. This highlights, for the first time, potential side effect liabilities of α9-nAChR-inhibiting analgesics. α9-nAChR knockout (KO) mice demonstrated both behavioural and physiological aberrations in their stress-responses, and demonstrated a susceptibility to stress-induced anxiety and depression-like behaviour compared to their wildtype (WT) counterparts. Behaviourally, KO mice exhibited reduced stress-induced arousal in the forced swim test (FST) and the elevated plus maze (EPM), increased stress-induced anxiety-like behaviour on the EPM, and increased stress-induced depression-like behaviour in a sucrose preference test, compared with WT mice. Physiologically, KO mice exhibited dysregulation of the stress-hormone response, with muted corticosterone responses to

an acute stressor, but exaggerated corticosterone responses after persistent stress. Inhibition of the α9-nAChR is unlikely to offer broad analgesic relief to pain sufferers, and the present results suggest that such inhibition would cause detrimental side effects manifesting as dysregulation of the stress response.

Disclosures: S. Mohammadi: None. **M.J. Christie:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.14/M39

Topic: D.08. Pain

Title: Involvement of anoctamin-1 and bestrophin-1 in the development and maintenance of neuropathic pain

Authors: *J. B. PINEDA FARIAS[1], P. BARRAGÁN-IGLESIAS[1], H. I. ROCHA-GONZÁLEZ[2,3], R. DELGADO-LEZAMA[4], J. G. RAYA-TAFOLLA[4], E. LOEZA-ALCOCER[4], F. PÉREZ-SEVERIANO[5], V. GRANADOS-SOTO[1];
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Abstract: Calcium-activated chloride channels (CaCCs) activation produces a chloride efflux in primary sensory neurons that might facilitate their depolarization contributing to action potentials generation. Previous studies have suggested the participation of bestrophin-1 and anoctamin-1, members of the CaCCs family, in inflammatory pain models. However, their role in neuropathic pain is unclear. The aim of this investigation was to study the involvement of anoctamin-1 and bestrophin-1 in rats submitted to the L5/L6 spinal nerve ligation model. Intrathecal administration of non-selective CaCCs inhibitors 9-anthracenecarboxylic acid (9-AC, 10-300 microg), 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB, 30-300 microg) and niflumic acid (NFA, 10-300 microg), but not vehicle, reduced in a dose-dependent manner established tactile allodynia. Moreover, intrathecal administration of selective CaCCs inhibitors (T16Ainh-A01 and CaCCinh-A01, 0.01-10 microg) diminished in a dose-dependent fashion tactile allodynia and thermal hyperalgesia. Both anoctamin-1 and bestrophin-1 mRNA and

protein were expressed at the dorsal spinal cord and dorsal root ganglia of naïve, sham and neuropathic rats. L5/L6 spinal nerve ligation increased mRNA and protein expression of anoctamin-1, but not bestrophin-1, at the dorsal spinal cord and dorsal root ganglia from day 1 to day 14 after nerve ligation. In addition, repeated administration of CaCCs inhibitors (T16Ainh-A01, CaCCinh-A01 and NFA) or antibodies anti-bestrophin-1 or anti-anoctamin-1 prevented spinal nerve ligation-induced rise in anoctamin-1 mRNA and protein expression. Although bestrophin-1 was not upregulated by spinal nerve ligation, we found that bestrophin-1 is expressed in non-peptidergic neurons at outer laminas of the dorsal horn. Spinal nerve ligation increased the compound action potential of C fibers while administration of selective CaCCs inhibitors (T16Ainh-A01 and CaCCinh-A01) attenuated such increase. Our results indicate that CaCCs are present in sites related to the nociceptive processing. Also, our data imply that CaCCs participate in the maintenance of neuropathic pain and therefore selective inhibitors may function as analgesics in nerve injury pain states. This work was partially supported by a Conacyt grant CB-2012179294 (VG-S). JBP-F and PB-I are Conacyt fellows.

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Poster

152. Mechanisms of Neuropathic Pain I

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Topic: D.08. Pain

Support: Korea Health technology R & D Project, Ministry of Health & Welfare, Republic of Korea (A120254)

Title: Involvement of ROS-dependent PI3-kinase activation in leptin-induced enhancement of NMDA receptor-mediated tactile hyperalgesia in neuropathic rats

Authors: J. B. JUN[1,2], S. J. JUNG[1], H. A. KIM[1], *J. W. LEEM[1,2];
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Abstract: Recent studies have shown that leptin (an adipocytokine) played an important role in influencing neuropathic pain. It was revealed that leptin enhanced NMDA-induced spinal neuronal excitation. We have previously displayed that upregulation of phosphatidylinositol

(3,4,5)-triphosphate (PIP3) levels via reactive oxygen species (ROS) in the dorsal horn was involved in tactile hyperalgesia seen in neuropathic rats. In the present study, we investigated whether leptin aggravated NMDA receptor-mediated neuropathic pain behavior and, if so, whether this leptin-induced effect was mediated through the ROS-PI3 kinase pathway. Tactile hyperalgesia of the hind paw, evaluated by measuring paw withdrawal threshold upon the application of von Frey hairs, was induced using naive rats either by lumbar 5 spinal nerve ligation (L5 SNL) or by intrathecal (i.t.) administration of leptin or glutamate. The L5 SNL-induced tactile hyperalgesia was attenuated by i.t. administration of leptin antagonist, NMDA antagonist MK-801, ROS scavenger alpha-phenyl-N-tert-butyl nitron (PBN), or PI3-kinase inhibitor LY294002. When intrathecally administered in naive rats, both leptin and glutamate induced tactile hyperalgesia. Leptin and glutamate administered together induced more severe tactile hyperalgesia than glutamate alone. Leptin-induced tactile hyperalgesia was attenuated by MK801. Both leptin-induced and glutamate-induced tactile hyperalgesia were attenuated by either ROS scavenger PBN or LY294002. The results suggested that leptin enhanced NMDA receptor-mediated tactile hyperalgesia, via the ROS-PI3K pathway, in the neuropathic state.

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Poster

152. Mechanisms of Neuropathic Pain I

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Support: NIH Grant DA033390

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Rita Allen Foundation

Title: Dorsal root ganglion transcriptome analysis following peripheral nerve injury in mice

Authors: ***S. WU**[1], B. M. LUTZ[2], L. LIANG[3], K. MO[3], Y.-J. CHANG[4], P. DU[4], A. Y. BEKKER[3], Y.-X. TAO[3];
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Newark, NJ; [4]High Performance and Res. Computing, Office of Information Technology, New Jersey Med. School, Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Peripheral nerve injury leads to the changes in some gene expression in primary sensory neurons of dorsal root ganglia (DRG). These changes are believed to involve in neuropathic pain genesis. However, the alternation of whole transcriptomes in DRG following nerve injury is still unclear. Next generation RNA-seq is a highly sensitive method of analyzing differential expression of not only mRNAs but also non-coding RNAs and splice variants. The present study chose the eliminate mRNA poly-A selection with strand-specific and higher depth next generation sequencing to analyze whole transcriptomes in DRG following spinal nerve ligation (SNL). Our results showed that more than 50 million (M) mapped sequences in pairs with strand information were yielded in each group (51.87 M-56.12 M in sham vs. 51.08 M-57.99 M in SNL). Six days after SNL, expression levels of 11,164 out of total 38,925 identified genes in the injured DRG were significantly changed, including 3,278 transcriptional isoforms. The largest transcriptional changes were observed in protein-coding genes (91.5%) followed by non-coding RNAs. Within 944 differentially expressed non-coding RNAs, the markedly observed changes were seen in long interspersed non-coding RNAs followed by antisense RNAs, processed transcripts, and pseudogenes. We observed a notable proportion of reads aligning to intronic regions in both groups (44.0% in sham vs. 49.6% in SNL). Using quantitative real-time PCR, we validated that the levels of DNMT3a mRNA, DNMT1 mRNA and Gm26883 RNA (a non-coding RNA) were significantly up-regulated and that the amounts of Kcna2 mRNA, Oprm1 mRNA and 4732491K20Rik RNA (another non-coding RNA) were down-regulated following SNL. Our findings suggested that next generation RNA-seq can be used as a promising approach to analyze the changes of whole transcriptomes in DRG following nerve injury and to possibly identify new targets for prevention and treatment of neuropathic pain.

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Poster

152. Mechanisms of Neuropathic Pain I

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Title: Spinal cord injury activates astrocyte sigma-1 receptors leading to astrocyte activation, increases in connexin 43 expression and the development of bilateral below-level mechanical allodynia in mice

Authors: *J.-H. LEE[1,2], S.-R. CHOI[2], D.-H. ROH[3], S.-Y. YOON[4], S.-G. KWON[2], H.-S. CHOI[2], J.-Y. MOON[2], S.-Y. KANG[5], H.-J. HAN[2], H.-W. KIM[6], A. J. BEITZ[7], S.-B. OH[4]; [1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Dept. of Vet. Physiol., BK21 PLUS Program for Creative Vet. Sci. Res., Res. Inst. for Vet. Sci. and Coll. of Vet. Med., Seoul Nat'l Univ., Seoul, Korea, Republic of; [3]Dept. of Maxillofacal Tissue Regeneration and Res. Ctr. for Tooth and Periodontal Tissue Regeneration, Sch. of Dentistry, Kyung Hee Univ., Seoul, Korea, Republic of; [4]Pain Cognitive Function Res. Center, Dept. of Brain and Cognitive Sci., Coll. of Nat. Sci., Dent. Res. Inst. and Dept. of Neurobiol. and Physiol., Sch. of Dentistry, Seoul Nat'l Univ., Seoul, Korea, Republic of; [5]Acupuncture, Moxibustion & Meridian Res. Group, Med. Res. Division, Korea Inst. of Oriental Med., Daejeon, Korea, Republic of; [6]Dept. of Physiol., Inst. of Brain Res., Chungnam Nat'l Univ. Med. Sch., Daejeon, Korea, Republic of; [7]Dept. of Vet. and Biomed. Sci., Coll. of Vet. Med., Univ. of Minnesota, St. Paul, MN

Abstract: We have recently demonstrated that following thoracic spinal cord injury (SCI) astrocyte activation spreads via gap junctions to the lumbar spinal cord and this rapid intercellular communication is critical to the induction of bilateral below-level neuropathic pain. The present study determined whether sigma-1 receptors (Sig-1Rs) are involved in astrocyte activation and whether they modulate the expression of the astrocyte-specific gap junction protein connexin 43 (Cx43) ultimately leading to SCI-induced below-level chronic neuropathic pain. SCI was performed by transverse hemisection of the right thoracic spinal cord between the $T_{11\text{-}12}$ vertebral segments in mice. Below-level mechanical allodynia and thermal hyperalgesia were evaluated bilaterally in the hindpaws of SCI mice. Immunohistochemistry and western blotting were performed to determine potential changes in connexin, glial fibrillary acidic protein (GFAP), and Sig-1R expression in the spinal cord. SCI significantly increased the expression of Sig-1Rs in astrocytes, but not in neurons or microglia, in the dorsal horn of lumbar enlargement spinal segments located below the level of injury and this increase peaked at the same time as SCI-induced mechanical allodynia. In addition, there was a bilateral, significant increase in the expression of GFAP and the astrocyte gap junction protein, connexin 43 (Cx43) in the spinal cord dorsal horn, which was significantly reduced by repeated injection of the Sig-1R antagonist, BD1047 in SCI mice. Conversely there were no changes in oligodendrocyte (Cx32) or neuronal (Cx36) gap junction protein expression after SCI. Administration of BD1047, the non-selective gap junction blocker (carbenoxolone), or the selective Cx43 blocker (Cx43 mimetic peptide ^{43}Gap26) significantly reduced SCI-induced bilateral below-level mechanical allodynia. Collectively these findings demonstrate that SCI-induced Sig-1R stimulation activates astrocytes and increases the expression of the astrocyte gap junction protein Cx43 in the lumbar regions of the spinal cord dorsal horn. This ultimately contributes to the induction of the bilateral below-

level chronic mechanical allodynia in SCI mice and raises the possibility of using Sig-1R antagonists to treat spinal injury-induced neuropathy.

Disclosures: **J. Lee:** 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.; a grant (2014R1A2A2A01007695) from National Research Foundation (NRF) funded by the Korean Government (MSIP), Republic of Korea. **S. Choi:** None. **D. Roh:** None. **S. Yoon:** None. **S. Kwon:** None. **H. Choi:** None. **J. Moon:** None. **S. Kang:** None. **H. Han:** None. **H. Kim:** None. **A.J. Beitz:** None. **S. Oh:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.18/M43

Topic: D.08. Pain

Support: NIH Grant NS079855

NIH Grant NS090689-01

Farber Family Foundation Grant

Title: Phosphorylation of Ser-481 stabilizes the inactivated state of the Kv3.4 channel: hinting at a mechanism of SCI-induced neuropathic pain

Authors: *B. M. ZEMEL, M. COVARRUBIAS;
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Abstract: The voltage-gated K+ channel Kv3.4 undergoes protein kinase C (PKC)-dependent modulation of both fast inactivation and functional expression in dorsal root ganglion (DRG) nociceptors. Although the mechanism underlying elimination of fast inactivation upon phosphorylation of four N-terminal serines is well understood, the basis of the PKC-dependent downregulation of the Kv3.4 current is unknown. To tackle this problem, we are investigating: 1) the phosphorylation site(s) responsible for the downregulation; 2) the kinase(s) and phosphatase(s) involved; and 3) the mechanism of the downregulation. Here, we report that persistent PKC activation coupled with calcineurin (PP2B) inhibition induces downregulation of the Kv3.4 current in CHO cells and DRG neurons. This downregulation results from a negative shift in the steady-state inactivation curve, which indicates a relative stabilization of the

channel's inactivated state. Mutational analysis revealed that Ser-481 plays a major role in this modulation. Whereas S481A nearly eliminates the downregulation by PKC activation and PP2B inhibition, S481D mimics the negative shift of the steady-state inactivation curve in heteromeric channels. These results are mechanistically tantalizing because S481 is located at the channel's activation gate. Additionally, immunostaining and coimmunoprecipitations showed that PKC-ε and Kv3.4 co-localize and interact, suggesting that this PKC isoform might be responsible for the phosphorylation-dependent modulation of the Kv3.4 channel in DRG. Further supporting this possibility, PKC-ε is robustly expressed in DRG neurons. We propose that the Kv3.4 channel, PKC-ε and possibly PP2B coexist in a signaling complex expressed in DRG neurons. There, the Kv3.4 channel undergoes PKC/PP2B-dependent modulation to eliminate fast N-terminus-dependent inactivation (due to phosphorylation of four N-terminal serines) and enhance a novel inactivation mechanism (due to phosphorylation of Ser-481). Additional results suggest that these mechanisms could be implicated in peripheral pain sensitization induced by spinal cord injury (SCI).

Disclosures: B.M. Zemel: None. **M. Covarrubias:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.19/M44

Topic: D.08. Pain

Support: NIH grant NS046606

Title: Upregulation of T-type calcium channels activated by toll like receptor 4 in primary sensory neurons in paclitaxel induced peripheral pain

Authors: *Y. LI[1], R. M. CASSIDY[2], A. K. KOSTURAKIS[3], D. S. HARRISON[4], P. M. DOUGHERTY[1];
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Abstract: Paclitaxel induced peripheral neuropathy is a common dose-limiting side effect in cancer chemotherapy and often persists in cancer survivors. Paclitaxel increases excitability of peripheral sensory neurons and produces these adverse effects at least in part by activation of low voltage activated calcium channels (T-type, Cav3.2) via toll-like receptor 4 (TLR4)

signaling. The data show that the percent of cells showing spontaneous action potentials and the mean frequencies of action potentials are significantly increased at day 7 and day 14 in the paclitaxel treatment group compared to vehicle controls. Analysis of action potential parameters indicated that the enhanced excitability was observed in small size DRG neurons in paclitaxel treated animals. Cav3.2 expression is increased in DRG and spinal cord in paclitaxel-treated rats, is localized to CGRP-positive, IB4-positive small DRG neurons and GFAP-positive spinal cord cells. Cav3.2 was also co-localized with TLR4 in DRG neurons and spinal cord astrocytes. Perfusion of LPS (TLR4 agonist) directly activated DRG neurons, while pretreatment with ML218 (specific T-type calcium channel blocker) significantly blocked this. Paclitaxel-induced behavioral hypersensitivity to mechanical stimuli is prevented but not reversed by spinal administration of ML218. In summary, paclitaxel treatment led to sensitization of Cav3.2 and blockade of TLR4 prevented this. This mechanism could contribute to paclitaxel-induced acute pain and chronic painful neuropathy.

Disclosures: Y. Li: None. **R.M. Cassidy:** None. **A.K. Kosturakis:** None. **D.S. Harrison:** None. **P.M. Dougherty:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.20/M45

Topic: D.08. Pain

Title: Roles of Acid-Sensing Ion Channels in neuropathic pain induced by experimental autoimmune encephalomyelitis

Authors: *I.-C. WANG[1,2], C.-H. LEE[2], S.-H. LIN[2], C.-Y. CHUNG[2], F. LIAO[2], C.-C. CHEN[2,3]; [1]Dept. of Life Science, Natl. Taiwan Univ., Taipei, Taiwan; [2]Inst. of BioMedical Sciences, Academia Sinica, Taipei, Taiwan; [3]Taiwan Mouse Clinic, Natl. Comprehensive Mouse Phenotyping and Drug Testing Center, Academia Sinica, Taipei 115, Taiwan, Taipei, Taiwan

Abstract: Neuropathic pain is one of the major symptoms in Multiple Sclerosis, but the underlying neurological mechanisms is still under debate. Since acidosis has been reported in multiple sclerosis, the Acid-sensing ion channels, which are involved in acid-induced neuropathic pain, may participate in the pain pathway. Therefore, we hypothesize that ASICs may play a role in pain development in MS. We utilize experimental autoimmune encephalomyelitis (EAE), a well-established MS rodent model, to evaluate clinical deficits, mechanical response and pathological alterations in four different ASIC subtypes knockout mice.

Both wild-type and knockout mice showed clinical deficits and mechanical hypersensitivity in the recovery phase of EAE. Also, pathological studies demonstrated that dorsal root ganglion (DRG) neurons are injured after EAE induction by co-staining with neuron injury marker, activating transcription factor 3 (ATF3). Further characterization of injured neurons showed that myelinated (neurofilament 200-positive) and non-peptidergic (isolectin B4-positive) neurons were the principal populations and that peptidergic neurons were excluded (calcitonin gene-related peptide-positive and substance P-positive neurons). These results suggested that peripheral nerve might participate in the nociceptive hypersensitivity in EAE. Moreover, analgesic drug application indicated that EAE-induced neuropathic pain is modulated by ASIC3 mediated anti-nociceptive pathway.

Disclosures: I. Wang: None. **C. Lee:** None. **S. Lin:** None. **C. Chung:** None. **F. Liao:** None. **C. Chen:** None.

Poster

152. Mechanisms of Neuropathic Pain I

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Topic: D.08. Pain

Support: National Research Foundation of Korea grant 2012M3A9B6055414

Hugel Inc.

Title: Anti-nociceptive effects of botulinum toxin type A on trigeminal neuropathic pain in rats

Authors: *M. KIM[1], H. KIM[1], J. SON[1], J. JU[1], K. YANG[1], M. LEE[2], M. PARK[3], J. PARK[4], D. AHN[1];
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Abstract: We investigated the anti-nociceptive effects of botulinum toxin type A (BoNT-A) in a rat model of trigeminal neuropathic pain produced by inferior alveolar nerve injury. Experiments were carried out in male Sprague-Dawley rats. The left mandibular second molar was extracted under anesthesia, followed by a miniature dental implant placement to induce injury to the inferior alveolar nerve. Mechanical allodynia was monitored after subcutaneous injection of 0.3, 1 or 3 U/kg BoNT-A into the vibrissa pad on postoperative day (POD) 3, 7 or 12. Subcutaneous

injections of 1 or 3 U/kg BoNT-A on POD 3 significantly attenuated mechanical allodynia although the 0.3 U/kg BoNT-A did not affect the air-puff threshold. A single injection of 3 U/kg BoNT-A produced prolonged anti-allodynic effects over the entire experimental period. Double treatments with 1 U/kg BoNT-A produced prolonged anti-allodynic effects compared with single treatments. Besides, treatment with BoNT-A on POD 7 and 12, when pain had already been established, also produced prolonged anti-allodynic effects. Treatment with BoNT-A did not affect up-regulated the number of activating transcription factor 3 (ATF3)-positive cells in a rat with inferior alveolar nerve injury. Although nerve injury increased the level of Nav1.6, 1.7, 1.8 in the trigeminal ganglion on POD 3, subcutaneous injection of BoNT-A produced down-regulated only Nav1.7 expression. These results suggest that subcutaneous injection of BoNT-A produced prolonged anti-nociception and this anti-nociception is mediated by the regulation of Nav1.7. In summary, our current results suggest that BoNT-A injections can be used to treat the trigeminal neuropathic pain associated with dental implant surgery. Although our results are encouraging in this regard, however, their general applicability is limited by the use of an animal model. Further clinical human studies involving large cohorts are needed to properly estimate the clinical efficacy of BoNT-A and its potential as new treatment for neuropathic pain.

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Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.22/M47

Topic: D.08. Pain

Support: NIH R01 DA036165 01

Title: HIV-Associated pain and peripheral sensory neuropathy: the role of Wnt5a signaling

Authors: ***S. YUAN**, W. RU, M. DE CABO JAUME, S.-J. TANG;
Neurosci. and Cell Biol., Univ. of Texas Med. Br., Galveston, TX

Abstract: Peripheral sensory neuropathy is a major neurological complication in HIV infected patients. Pathological pain in HIV patients is intimately associated with the development of neuropathy. To study the mechanism of HIV-Associated sensory neuropathy (SN), we used a HIV-1 gp120 mouse model generated by intrathecal injection (i.t.) of gp120. Our recent work showed that the model with three i.t. gp120 injections (i.t. at 1, 3, 5 day, each time 100ng

gp120Bal) developed behavioral, cellular and molecular abnormalities that resembled the pathologies in the HIV patients who had pathological pain, including SN. At day 7 after the first i.t. injection, the density of PGP 9.5+ intraspinosum nerve fiber (ISNF; fibers located in the inner layer of the epidermis) began to decrease (control: 34.84 fibers/mm, model: 21.1 fibers/mm), although it was not statistically significant. At this time point, the PGP 9.5+ intragranulosum nerve fibers (IGNF, c-fibers reaching the out layer of the epidermis) were significantly reduced (control: 18.16 fibers/mm, model: 8.20 fibers/mm, $p<0.05$). At the end of the 3rd week, PGP 9.5+ ISNF density in the gp120 model further dropped to 9.53 fibers/mm, comparing with the 34.84 fibers/mm in the control ($p<0.0001$). Since our recent studies suggested a critical role of Wnt5a-JNK signaling pathway in HIV-Assocaited pain, we wanted to determine the potential contribution of Wnt5a signaling to gp120-induced SN. Repeated administration (i.t.) of a specific Wnt5a agonist foxy-5 (10μg/i.t., 3 times, every other day in the first week and collected tissue at the end of 3 week) caused the severe allodynia and epidermis denervation (ISNF: 10.96 fibers/mm). The effect of Foxy-5 was significantly attenuated by the administration of the JNK inhibitor, SP600125. To further test the role of endogenous Wnt5a, we generated condition knockout (CKO) of Wnt5a in neurons where Wnt5a was predominantly expressed. We found that the Wnt5a CKO diminished gp120-induced denervation in the epidermis. These dada collectively indicate the important role of Wnt5a signaling in gp120-caused SN.

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Poster

152. Mechanisms of Neuropathic Pain I

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Topic: D.08. Pain

Support: Cure 2013

Rita Allen Foundation 2012

Title: MeCP2 regulates gene expression in dorsal root ganglia after peripheral nerve injury

Authors: ***M. T. MANNERS**[1], A. ERTEL[2], Y. TIAN[1], K. MCCORMICK[2], Z. ZHOU[3], S. AJIT[1];
[1]Pharmacology/Physiology, Drexel Univ. Col. of Med., Philadelphia, PA; [2]Dept. of Cancer Biol., Thomas Jefferson Univ., Philadelphia, PA; [3]Dept. of Genet., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Decreased pain perception is commonly reported in children with Rett syndrome (RTT). RTT is caused by mutations in methyl-CpG-binding protein 2 (MECP2), suggesting that normal MeCP2 function is important in modulating pain. Our objective is to investigate genes regulated by MeCP2 in the dorsal root ganglia (DRG), as well as microRNA (miRNA)-mediated regulation of MeCP2 in neuropathic pain. Our previous study showed that miRNAs predicted to target Mecp2 are downregulated in the DRG from a rodent model of neuropathic pain. We confirmed miRNA-mediated regulation of MeCP2 *in vitro* and MeCP2 upregulation in the DRG following nerve injury. MeCP2 regulates brain derived neurotrophic factor (BDNF) and repression of MeCP2 by miRNAs resulted in a concomitant decrease in Bdnf. Mecp2-null and MeCP2 T158A knockin mice have decreased Bdnf in DRG and MeCP2 T158A mice exhibited reduced mechanical sensitivity. Diminished pain sensitivity resulting from mutations in Mecp2 could be mediated by downregulation of Bdnf. Increased MeCP2 expression and binding can play a critical role in inducing gene expression changes in a pain state. We performed ChIP-seq using DRG from a spared nerve injury (SNI) model of neuropathic pain and a subset of loci with enriched MeCP2 binding including miR-126 and Ptger1 were further investigated. miR-126 expression decreased in SNI model while its target, Vegfa and Dnmt1 were upregulated. These data suggests that increased MeCP2 binding induced by neuropathic pain can lead to the repression of this miRNA. Conversely, prostaglandin E receptor 1 (Ptger1), a gene implicated in mediating pain, was increased in the SNI model and decreased in Mecp2-null mice, indicating MeCP2 may activate expression of Ptger1. Our studies using rodent models of pain and RTT can lead to a better understanding of the mechanistic basis of pain and thus to innovative approaches for its treatment.

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Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.24/N1

Topic: D.08. Pain

Support: Korea University Grant K1220201

Title: Effects of NR2B mediated signaling on neuropathic pain in a rat model of peripheral nerve injury

Authors: *Y. KIM[1], J. KIM[2], Y. YOON[1];
[1]Korea Univ., Col. Med., Seoul, Korea, Republic of; [2]Korea Univ., Col. Hlth. Sci., Seoul, Korea, Republic of

Abstract: N-methyl-D-aspartate receptor 2B (NR2B), one of the NMDAR subunits, is restrictively located in the sensory region of the central nervous system, especially, in the superficial lamina of the spinal cord. Besides, that has various phosphorylation sites in the c-terminal and interacts with postsynaptic density protein-95 (PSD-95), which is a scaffold protein located in the postsynaptic density. Thus, these signaling may be important to the mechanism of neuropathic pain. Therefore, this study was performed to find out the roles of NR2B mediated signaling pathway in the development and/or maintenance of neuropathic pain after peripheral nerve injury. Left side of the L5 spinal nerve was tightly ligated with 6/0 silk under isoflurane anesthesia in the male Sprague-Dawley (Crl: CD, 180-200 gram) rats. Quadrants of the L4 and L5 spinal cord were obtained from normal and SNL rats. The temporal expression of NR2B, NR2B Tyr1472, NR2B Ser1303, Ca++/calmodulin dependent complex II (CaMKII), Thr286 CaMKII, protein kinase C (PKC) and PSD-95 were quantified after SNL. In addition, RO 25-6981, autocamtide-2 related inhibitory peptide (AIP) or chelerythrine (CHE) were intrathecally injected in the early phase (1-4 days after SNL) and in the late phase (7-21 days after SNL) after nerve injury. The paw withdrawal threshold was assessed with series of von Frey filaments before and after drug injection. Co-immunoprecipitation (co-IP) was performed to analyze the interaction between Ser1303 and CaMKII, Ser1303 and PKC, and Ser1303 and PSD-95. NR2B and Thr286 CaMKII expression were more increased at the L5 spinal cord in the early phase than in the late phase after SNL. On the contrary to this, PKC expression was increased in the late phase. Phosphorylation of NR2B Ser1303 was increased from the early phase until the late phase. Moreover, Ro 25-6981 and AIP elevated paw withdrawal threshold more efficiently in the early phase than in the late phase, whereas CHE elevated that in the late phase. Interaction between Ser1303 and CaMKII was increased in the early phase, and interaction between Ser1303 and PKC was existed in the late phase. Interaction between Ser1303 and PSD-95 was gradually increased over time after injury. Above all, AIP and Ro 25-6981 reduced the interaction between Ser1303 and PSD-95, but CHE did not. Thus, our results suggested that NR2B mediated signal transduction pathway may be a possible connector from the developmental phase to the maintenance phase of neuropathic pain after peripheral nerve injury.

Disclosures: **Y. Kim:** None. **J. Kim:** None. **Y. Yoon:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.25/N2

Topic: D.08. Pain

Support: Eir - Research & Business Park

Title: Assessing properties of small cutaneous nerve fibers

Authors: *C. D. MORCH[1], K. HENNINGS[3], S. FRAHM[2], L. PETRINI[2], M. B. JENSEN[2], L. ARENDT-NIELSEN[2], O. K. ANDERSEN[2];
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Abstract: Introduction: Peripheral neuropathy is a common pathology in e.g. diabetes and after chemotherapy. The integrity and membrane properties of large peripheral nerve fibers can be assessed by nerve conduction studies and the threshold tracking. Today, diagnostic methods for assessing changes in small fiber membrane properties are inadequate. Here we present a novel method for assessing the membrane properties of small and large cutaneous nerve fibers using perception threshold tracking. Methods: 20 healthy human subjects (9 females, mean age: 29.4 years) participated in the study (ethical approval: N-20120046). Small cutaneous nerve fibers were activated by a circular array of 16 small area electrodes (diameter = 0.2mm) and large cutaneous nerve fibers were activated by a surface electrode (20 x 15 mm). Perception thresholds were assessed by an adaptive staircase method. The strength duration time constant and rheobase were estimated by fitting the perception thresholds to rectangular constant current electrical pulses of 50µs, 100µs, 200µs, 400µs, 800µs, 2ms, 8ms, and 16ms to Weiss' law. The threshold electrotonus was assessed as the perception threshold reduction by depolarizing conditioning pulse at 10ms, 20ms, 40ms, and 80ms and hyperpolarizing conditioning pulse at 30ms and 80ms with an intensity of 20% of the perception threshold of a 1ms rectangular pulse. Results: The average time constant of the small fibers (1060µs ± 690µs) was significantly longer than the time constant of the large fibers (580µs ± 160µs; $p = 0.01$, paired t-test). The rheobase of the small fibers (0.070mA ± 0.041mA) was significantly lower than the rheobase of the large fibers (0.43mA ± 0.10mA; $p < 0.001$, paired t-test). The threshold electrotonus protocol showed no statistical difference between small and large fibers threshold reduction for depolarizing pulses and 30ms hyperpolarizing pulses. For the 80ms hyperpolarizing pulse, the threshold increase was higher for the small (61.2% ± 11.2%) than the large (27.1% ± 2.3%) cutaneous nerve fibers (RM ANOVA and Bonferroni, $p = 0.006$). Conclusion: This novel perception threshold tracking technique enables assessment of the membrane properties of small and large cutaneous nerve fibers based on automatic detection of perception thresholds. Different membrane properties observed may relate to different diameters, myelination, and distribution of ion channels. The method provides information about small fiber membrane properties and may be used for diagnosis of thin fiber neuropathy.

Disclosures: **C.D. Morch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The method is patented by AAU. **K. Hennings:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The method is patented by AAU. **S. Frahm:** None. **L. Petrini:** None. **M.B. Jensen:** None. **L. Arendt-Nielsen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The method is patented by AAU. **O.K. Andersen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The method is patented by AAU.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.26/N3

Topic: D.08. Pain

Support: Harrison Award

Harold Carron Endowment

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Inje Research and Scholarship Foundation

Title: GABAA α2 subunit in dorsal root ganglia plays an important role in the development and maintenance of neuropathic pain post-crush injury of the sciatic nerve in female rats

Authors: *A. L. OBRADOVIC[1], J. SCARPA, Jr.[2], H. P. OSURU[3], J. L. WEAVER[4], J.-Y. PARK[6], S. PATHIRATHNA[3], A. PETERKIN[3], Y. LIM[7], M. JAGODIC[3], S. M. TODOROVIC[5], V. JEVTOVIC-TODOROVIC[5];
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Abstract: Objectives: The pathophysiology of neuropathic pain (NPP) after peripheral nerve injury remains unknown and it likely results from repetitive high frequency bursts of peripheral afferent activity, leading to long-lasting changes in synaptic plasticity in the spinal dorsal horn (DH). Although treatments that promote GABA activity in the DH provide partial relief of neuropathic symptoms, their beneficial properties are usually complicated by numerous side effects. We examined how *in vivo* silencing of the $GABA_A$ α_2 gene in dorsal root ganglion (DRG), known as a main peripheral sensory relay formation, controls development of NPP in rats. **Methods:** After crush injury to the right sciatic nerve of female rats, the α_2 $GABA_A$ antisense and mismatch oligodeoxynucleotides or NO-711 (a GABA uptake inhibitor) were applied to the L5 DRG. *In vivo* assessment of thermal and mechanical hyperalgesia was conducted prior to the injury and ensuing 10 days. *In vitro* quantification of α_2 $GABA_A$ protein and electrophysiology studies of $GABA_A$ currents were performed on acutely dissociated L5 DRG neurons at relevant time-points. **Results:** NPP post-crush injury of a sciatic nerve in adult female rats coincides with significant down-regulation of the α_2 subunit expression in the ipsilateral DRG (about 30%); this effect was not observed in sham operated animals. Selective down-regulation of α_2 expression by antisense oligodeoxynucleotides in DRGs significantly worsens mechanical and thermal hypersensitivity in crush-injured animals and also, causes development of profound hypersensitivity in sham animals. Conversely, up- regulation of endogenous GABA *via* blockade of its uptake by NO-711 in DRG alleviates NPP. We also found that prolonged NPP phenotype correlate with significant α_2 subunit protein down-regulation even at post-operative day (POD) 10, which suggests strong association between GABA function in DRG cells and the NPP phenotype. **Conclusions:** We demonstrate that peripheral nerve injury resulting in NPP is accompanied by a significant down-regulation of $GABA_A$ α_2 subunit in the ipsilateral DRG neurons. Further down-regulation of this subunit results in worsening of pain, while selective inhibition of GABA reuptake at DRG level alleviates thermal hyperalgesia. Also, we showed that at the later days of NPP progression (POD 10) pain phenotype is still associated with significant down-regulation of α_2 $GABA_A$ subunit.

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Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.27/N4

Topic: D.08. Pain

Support: NIH grant 1P20GM103643

Title: Role of CD4 in spinal cord chemokine responses in a murine model of neuropathic pain

Authors: *L. CAO[1], J. T. MALON[2];
[2]Biomed. Sci., [1]Univ. New England, Biddeford, ME

Abstract: Previously, using a murine model of neuropathic pain, spinal nerve L5 transection (L5Tx), we showed that there is a transient infiltration of CD4+ T lymphocytes into the lumbar spinal cord post-L5Tx and these cells are predominantly type I helper T cells that can promote pro-inflammatory responses within the spinal cord. Behavioral tests indicated that CD4+ T lymphocytes contribute to the maintenance phase of L5Tx-induced mechanical hypersensitivity. It is well-known that chemokines (such as CCL2) plays important role in the development of neuropathic pain. To further understand the role of CD4+ T lymphocytes in peripheral nerve injury-induced spinal cord chemokine response, we determined the levels of various chemokines in the lumbar spinal cord in both CD4 knockout (KO) and wild type mice post-L5Tx. A total of eight chemokines CXCL1 (KC), CCL1 (TCA-3), CCL2 (MCP-1), CCL3 (MIP-1 alpha), CCL5 (RANTES), CCL11 (Eotaxin), CCL17 (TARC), and CCL22 (MDC) were examined. CD4 KO mice displayed blunt responses in L5Tx-induced CCL2 and CCL5. This is consistent with our previous observation that significantly less spinal cord infiltrating cells were detected in CD4 KO mice post-L5Tx. Further, L5Tx-induced upregulation of phosphorylated p38 MAPK was not observed in CD4 KO mice, suggesting a role of CD4+ T lymphocytes in activation of p38 pathway. Altogether, our data indicate a contributing role of CD4+ T lymphocytes in L5Tx-induced chemokine responses and possibly upstream p38 MAPK pathways.

Disclosures: L. Cao: None. **J.T. Malon:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.28/N5

Topic: D.08. Pain

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Univesité de Strasbourg

Title: VIP deficient mice exhibit reversible alterations in molecular and epigenetic determinants of cold and mechanical nociception

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Abstract: The vasoactive intestinal (neuro)peptide (VIP) is involved in a variety of functions including nervous system development and regeneration. Several studies have reported opposite effects of exogenous VIP on nociception depending on administration site. Here, we investigated the role of endogenous VIP in nociception using VIP knockout (VIP KO) mice. Compared to wild type (WT) and heterozygous (Hz) adults, VIP KO mice exhibited robust cold and tactile hyperalgesia whilst their sensitivity to heat remained unaffected, similar to human neuropathic symptoms. In VIP KO mice, *in vivo* electrophysiology revealed that the C-fiber activation of spinal neurons was triggered by non-noxious mechanical and electrical stimulations compared to WT. Intraperitoneal injection of VIP to deficient mice restored normal C-fiber thresholds and abrogated the pain symptoms for 3 days. This rescue is transduced by VIP receptor type 1 (VPAC1) that initiates rapid changes in gene expression regulation. We then screened for gene candidates classically altered under pain conditions. We showed that expression of specific thermo- and mechano-induced receptors as well as pain-related secreted factors were altered in VIP KO mice but restored following VIP treatment. Gene expression was associated with epigenetic changes including DNA methylation. These results highlight a novel role for VIP in nociceptive sensitivity in basal and pathological conditions. Its analgesic properties in neuropathic pain states warrants further evaluation in a translational context.

Disclosures: T.L. Maduna: None. **P. Juif:** None. **N.P. Uppari:** None. **N. Petit Demouliere:** None. **A. Lacaud:** None. **P. Poisbeau:** None. **V. Lelievre:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 152.29/N6

Topic: D.08. Pain

Support: US Fulbright Foundation

Title: Effects of alpha7 nicotinic acetylcholine receptor positive allosteric modulator on microglia BDNF expression following LPS-induced neuropathic pain in mice

Authors: *M. ABBAS, S. RAHMAN;
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Abstract: Evidence suggests that neuro-immune activation plays a critical role in the pathophysiology and maintenance of neuropathic pain phenotypes involving central nervous system alpha7 nicotinic acetylcholine receptors (nAChRs). Here, we examined the effects of 3a,4,5,9b-Tetrahydro-4-(1-naphthalenyl)-3H-cyclopentan[c]quinoline-8-sulfonamide (TQS), an alpha7 nAChR positive allosteric modulator on microglia brain derived neurotropic factor (BDNF) expression following lipopolysaccharide (LPS)-induced neuropathic pain models in mice. Pretreatment of TQS (1 or 4 mg/kg, i.p.) reduced LPS-induced tactile allodynia and hyperalgesia. Furthermore, pretreatment of TQS (4 mg/kg) reduced (~60% of control, $p<0.05$) LPS-induced increased microglia BDNF expression in hippocampus. Taken together, these results suggest that TQS decreases LPS-induced neuropathic pain by modulating hippocampal microglia BDNF level. Therefore, microglia alpha7 nAChR positive allosteric modulator could be a potential drug candidate for neuropathic pain.

Disclosures: M. Abbas: None. **S. Rahman:** None.

Poster

152. Mechanisms of Neuropathic Pain I

Location: Hall A

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Program#/Poster#: 152.30/N7

Topic: D.08. Pain

Support: NNSFC 31130066

Title: PAP-I activates spinal microglia and induces nociceptive response in rats

Authors: *X. ZHANG, J. LI, H. LIU;
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Abstract: Objective Pancreatitis-associated proteins (PAPs) are a family of secretory proteins and their function is not studied in the sensory system. Previous studies revealed that in the rat chronic pain models with inflammation or nerve injury, PAP-I was dramatically upregulated in distinct types of dorsal root ganglion (DRG) neurons. These interesting findings suggest that PAP-I may contribute to the development of chronic pain. Methods The dorsal roots and sciatic nerves of anesthetized SD rats were ligated after surgical exposure. The nerve segments including the ligation points were dissected at 24 h for further analysis. P14 rat spinal cord was

isolated and disaggregated for cell culture. After 10-14 days, the flasks were gently shaken for microglia to detach and to be harvested from culture medium. We used Neuro Probe BW200L chambers for chemotaxis assay. Primary spinal microglia were seeded and cultured for 7 h before analysis. P3 SD rats were injected i.p. with AAV2/8. Behavior tests were done two months later. Results (1) PAP-I was upregulated and transported by distinct types of rat DRG neurons in the CFA-inflammation model and nerve injury model. (2) Rat recombinant PAP-I served as a chemoattractant and stimulating factor for primary spinal microglia *in vitro*. (3) PAP-I overexpression in DRG neurons induced a slight change of resting membrane potential but a significant increase in neuronal excitability. (4) PAP-I overexpression (intrathecal/intraplantar injection of recombinant PAP-I-myc-His; AAV expressing PAP-I-2A-EGFP) induced stronger responses to nociceptive stimulus. Conclusion PAP-I has a pro-nociceptive effect *in vitro* and *in vivo*. The PAP-I-induced attraction and activation of spinal microglia may play an important role in pathological pain.

Disclosures: X. Zhang: None. **J. Li:** None. **H. Liu:** None.

Poster

153. Diabetic Neuropathy

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 153.01/N8

Topic: D.08. Pain

Support: KU Integrative Medicine

Title: Dietary vitamin B6 differentially modulates cutaneous sensitivity, peripheral sensory axon morphology and spinal cord neurotransmitter content in diabetic and non-diabetic mice

Authors: *S. E. TAGUE[1], M. K. WINTER[1], N. ŞANLı[4], C. E. LUNTE[5], K. E. MCCARSON[2], P. G. SMITH[3];
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Abstract: Vitamin B6 is a coenzyme that is vital for sensory nerves. Due to shifts in vitamin B6 metabolism, many diabetics become deficient in the active form of this coenzyme, pyridoxal 5'-phosphate. Deficiencies in vitamin B6 lead to paraesthesias, reductions in axon density, and decreased conduction velocity. Peripheral sensory neuropathy is also commonly associated with diabetes mellitus. Some diabetic patients experience a loss of sensation, while others have

painful symptoms, yet both may exhibit similar anatomical degeneration of epidermal nerves in the distal extremities. Vitamin B6 has been shown to have anti-nociceptive properties in diabetics; however, the mechanisms are not well defined. In this study diabetes was induced by injection of streptzotocin (STZ) in fasted A/J mice on two consecutive days (85mg/kg and 65mg/kg), while non-diabetic mice were injected with sodium citrate buffer vehicle. Mice were fed vitamin B6 deficient (0.1mg/kg pyridoxine HCL) or replete (35mg/kg pyridoxine HCL) diets. By the end of 5.5 weeks, vitamin B6 deficient nondiabetic mice exhibited reduced non-peptidergic GFRα2-immunoreactive epidermal innervation, which may account for the observed cutaneous mechanical insensitivity after 3 weeks. In contrast to non-diabetics, diabetic mice on a pyridoxine-deficient diet developed cutaneous mechanical hypersensitivity along with cutaneous neuropathy. The vitamin B6 replete diet was able to abrogate the diabetes induced hypersensitivity; however, dietary pyridoxine did not affect the loss of epidermal nerves in the footpad due to diabetes, suggesting that the positive effect of increased dietary vitamin B6 on pain-related behavior was due to central mechanisms. Spinal cord amino acid neurotransmitters were analyzed by HPLC and found to be influenced by both diabetes and dietary vitamin B6. Diabetic mice showed elevated spinal cord levels of glutamic acid, glycine, and GABA, but not taurine. Vitamin B6 deficiency decreased GABA levels in the spinal cord. In diabetic mice, vitamin B6 deficiency resulted in a lower ratio of the inhibitory neurotransmitter GABA compared to the excitatory neurotransmitter glutamic acid, which may diminish attenuation of afferent nociceptive signaling in diabetic neuropathy. In conclusion, vitamin B6 deficiency leads to insensate distal sensory neuropathy in non-diabetic animals, but in diabetics vitamin B6 deficiency increases painful symptoms of diabetic neuropathy, possibly by reducing the content of the inhibitory amino acid neurotransmitter GABA.

Disclosures: **S.E. Tague:** None. **M.K. Winter:** None. **N. Şanlı:** None. **C.E. Lunte:** None. **K.E. McCarson:** None. **P.G. Smith:** None.

Poster

153. Diabetic Neuropathy

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 153.02/N9

Topic: D.08. Pain

Support: Fapesp Grant 2011/23764-0

Title: Dorsal root ganglia proteome profile of early painful diabetic neuropathy in a rat model of type-i diabetes

Authors: *C. A. PARADA[1], M. C. P. ATHIE[2], A. S. VIEIRA[3], J. M. TEIXEIRA[2], E. V. DIAS[2], C. H. TAMBELI[2];
[1]State Univ. of Campinas - Unicamp, Campinas, Brazil; [2]Structural and Functional Biol., [3]Med. Genet. Dept., State Univ. of Campinas, Campinas, Brazil

Abstract: Diabetic Peripheral Neuropathy (DPN) manifests in 50-60% of types I and II diabetic patients and is the major cause of limb amputation. Although electrophysiological and morphological aspects are well described, little is known about its development and progression, undermining effective therapies. Hyperglycemia and insulin signaling impairment are considered the triggering events of oxidative stress production observed in the dying nerves. Several hypotheses try to explain the phenomenon, but until now there are still many gaps in the pathogenesis and the plastic changes it generates. Significant advances in label-free quantitative proteomics by mass-spectrometer coupled to high-performance-liquid-chromatography have been made, allowing a robust and sensitive methodology for identification and quantitation of proteins that could help understand the molecular events observed in early DPN. In this study we show that proteomic changes in L4 and L5's Dorsal Root Ganglia (DRG) already take place after only two weeks of DPN in a rat model for type I diabetes (n=4). We used an LTQ-Velos Orbitrap mass spectrometer for DRG peptides injection, Mascot Search Engine and Scaffold 4 v. 4.4.3 software for protein identification and quantification. 1410 proteins were identified, 55 were differentially expressed between diabetic and control groups by T-test's $p<0,05$. Molecular function analysis grouped proteins involved in cellular transport (6), cell metabolism (9), mitochondrial activity (10), proliferation/cell growth (11), regeneration/degeneration (11) and antioxidant activity (7). Of those, only 3 remained significant after applying Hochberg-Benjamini Correction for multiple tests (1 down - Gstk1; and 2 up-regulated in diabetic group - Sirt2 and Ppp2r4). Gstk1 is a Glutathione S-Transferase protein member present in peroxisomes playing a role as an antioxidant. Sirt2 - Sirtuin 2 is a deacetylase commonly related to other neurodegenerative diseases, such as Alzheimer, believed to positively modulate myelination, microtubule stability but also to be activated by oxidative stress, triggering an autophagic cascade. Finally. Ppp2r4 or Protein phosphatase 2A is also related to neurodegenerative diseases and is a known tau protein phosphatase. This protein profile may reveal that an oxidative stress due to increase in metabolism is ongoing in DRG two weeks after mechanical sensitivity onset, altering protein expression to pathways that compensate stress and maintain cell homeostasis. A better comprehension of proteomic profile might help elucidate the first cellular alterations and how diabetic neuropathy develops and evolve.

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Poster

153. Diabetic Neuropathy

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 153.03/N10

Topic: D.08. Pain

Title: Nociceptin/orphanin FQ receptor antagonist JTC-801 inhibits spinal nitric oxide production and neuropathic pain in diabetic mice

Authors: *K. OHNO, H. SAKAMOTO, T. YASUNAGA, E. OKUDA-ASHITAKA;
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Abstract: Diabetic neuropathy is the most common chronic complication of diabetes mellitus. The pathological mechanisms of diabetic neuropathy include peripheral nerve degeneration and neurological damage. Although it is well known that diabetic neuropathy affects peripheral nerves, the mechanisms of this disease in the central nervous system remain poorly understood. In the present study, we examined the role of spinal nitric oxide (NO) in diabetic mice and the effect of gabapencin and nociceptin/orphanin FQ receptor antagonist JTC-801 on diabetic neuropathic pain. Diabetes was induced in mice by an intraperitoneal injection of streptozotocin (STZ). The STZ-injected mice showed significant loss of body weight and developed mechanical allodynia at 1-3weeks measured by von Frey filaments. NO synthase (NOS) activity in mouse spinal cord was assayed by NADPH-diaphorase histochemistry using beta-NADPH and nitroblue tetrazolium. NADPH-diaphorase activity was significantly increased in the dorsal spinal cord of the STZ-injected mice at 3 weeks, although the expression of NOS mRNAs, inducible NOS (iNOS) and neuronal NOS (nNOS), were not changed by STZ injection. Furthermore, oral administration of gabapentin (30 μg/g), a widely used analgesic drug for neuropathic pain, inhibited the mechanical allodynia as well as the increase of NADPH-diaphorase activity in the spinal cords of the STZ-injected mice. In addition, the increased spinal NADPH-diaphorase activity of the STZ-injected mice was inhibited by the exposure of JTC-801 (100 nM) to spinal cord slice. Oral administration of JTC-801 (10 μg/g) significantly decreased in the mechanical allodynia and NADPH-diaphorase activity in the STZ-injected mice. These results indicate that increased spinal NOS activity, assayed by NADPH-diaphorase histochemistry, contributes to diabetic mechanical allodynia. Furthermore, nociception/orphanin FQ receptor antagonist may provide a novel effective treatment for diabetic neuropathic pain.

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Poster

153. Diabetic Neuropathy

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Topic: D.08. Pain

Support: NSFC81271241

China Ministry of Education 20130001110013

Peking University 20120310

Title: Activation of ephrinB-EphB receptor signaling in rat spinal cord contributes to diabetic neuropathic pain

Authors: *X. T. DENG[1,2], M.-Z. WU[1,2], P.-C. MA[1,2], H. MA[1,2], B. ZHENG[3], P. ZHAO[3], X.-J. SONG[1,2,3,4];
[1]Neurosci. Res. Institute, Peking Univ., Beijing, China; [2]Ctr. for Pain Med., Peking Univ. Hlth. Sci. Ctr., Beijing, China; [3]Ctr. for Clin. Res. and Translational Med., Dongfang Hosp., Lianyungang, China; [4]Parker Reserach Inst., Parker Univ., Dallas, TX

Abstract: EphrinB-EphB receptor signal, which is critical cues in regulating cell shape, adhesion/repulsion, migration and positioning during developmental processes of the nervous systems, plays important roles in the development and maintenance of neuropathic pain after certain forms of severe stress such as peripheral nerve injury, bone cancer and morphine withdrawal. Chronic pain accompanied with diabetic neuropathy is a typical form of neuropathic pain in certain patients with diabetes. Diabetic neuropathic pain (DNP) possesses a major clinical challenge and its underlying mechanisms remain elusive. We hypothesized that DNP might share the similar neural mechanisms with other forms of neuropathic pain, thus the ephrinB-EphB receptor signal might play an important role in DNP. Here, we report that activation of ephrinB-EphB receptor signaling in the spinal cord contributes to DNP in adult Sprague-Dawley rats. DNP was induced by intraperitoneal injection of streptozotocin (STZ, 70 mg/kg body weight), which induced long-lasting mechanical allodynia in the animals in addition to the increased level of blood sugar. Western blotting analysis showed that phosphorylation of EphB1 receptor as well as the proinflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α in the spinal cord were significantly increased in STZ-induced DNP. Intrathecal administration of a blocking reagent for EphB1 receptor, EphB1-Fc (5 μg), greatly suppressed STZ-induced increased expression of TNF-α and IL-1β as well as activation of astrocytes in spinal dorsal horn. A single dose of EphB1-Fc produced a transient inhibition of mechanical allodynia starting within 2 h and lasted for about 8 h; repetitive administration of EPhB1-Fc (5 μg, daily for 3 consecutive days) produced a stable, long-lasting analgesic effect in DNP rats. Our results demonstrate that activation of EphB1 receptor in the spinal cord is important for the maintenance

of DNP at least partly through glial cell activation and proinflammatory cytokine pathways; blocking EphB1 activation can greatly reduce DNP syndromes. This study suggests that EphB receptor may be a potential target for treating chronic pain after diabetic neuropathy.

Disclosures: **X.T. Deng:** None. **M. Wu:** None. **P. Ma:** None. **H. Ma:** None. **B. Zheng:** None. **P. Zhao:** None. **X. Song:** None.

Poster

153. Diabetic Neuropathy

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 153.05/N12

Topic: C.13. Sensory Disorders

Support: NIH Grant R01EY021165

Title: Development of diabetes-induced acidosis in the rat retina

Authors: A. V. DMITRIEV, D. HENDERSON, *R. A. LINSENMEIER;
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Abstract: VEGF is known to be regulated by acidosis in some tissues, but the role of pH in controlling VEGF in the adult retina is unknown. We hypothesized that the retina of diabetics would be unusually acidic due to increased glycolytic metabolism. Acute hyperglycemia markedly acidified the normal cat retina and in a small number of diabetic cats that were available for study, dysregulation of retinal pH accompanied capillary dropout, and acidosis was found in an animal with earlier background retinopathy. Acidosis, or responses to acidosis, may affect retinal functions in addition to VEGF regulation, and could be responsible for neural dysfunction independently. The purpose of this study was to determine the extent of acidosis at the early stages of diabetes in the rat retina. Double-barreled H^+-selective microelectrodes were used to measure profiles of H^+ across the central retina of dark-adapted intact Long-Evans rats. Profiles were measured in adult diabetic rats 1 to 6 months after intraperitoneal injection of streptozotocin and also in age-matched normal rats. In diabetics, blood glucose was at least 300 mg/dl during measurements made weekly prior to the H^+ recordings. The intraretinal electroretinogram (ERG) was evaluated to determine the retinal depth of the electrode and the condition of the retina. Respiration was adjusted to maintain normal arterial blood gas values for pH, PCO_2 and PO_2 in all animals. In both control and diabetic animals, the most acidic part of the retina was the outer nuclear layer. However, the retina of rats tested at very early stages of diabetes (1-1.5 month) was significantly more acidic than the retina of age-matched controls.

The increased acidity was observed in all layers of the retina, but the change in the outer retina was larger. H^+ profiles were more variable in diabetics than in controls, both within an animal and across animals. A substantial part of this variation was correlated with the degree of hyperglycemia. Interestingly, the acidity of diabetic rat retina was diminished at later stages (2 – 6 months) and sometimes diabetic retinae were even less acidic than in controls. Retinal acidosis begins to develop at an early stage of diabetes (1 month) in rats. The elevation of $[H^+]$ in the retina is expected because of increased glucose in the blood. The shape of H^+-profiles, which are generally uniform in healthy rat retinas, are more variable in diabetics, hinting at a disruption of retinal H^+ regulation due to a progressive abnormality in the retinal circulation and/or cellular pH regulatory processes.

Disclosures: **A.V. Dmitriev:** None. **D. Henderson:** None. **R.A. Linsenmeier:** None.

Poster

153. Diabetic Neuropathy

Location: Hall A

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Topic: D.08. Pain

Support: Conacyt Grant CB-2012/179294

Title: Neonatal streptozotocin-induced Type 2 diabetes mellitus: a model to study peripheral neuropathy

Authors: *P. BARRAGAN-IGLESIAS[1], V. OIDOR-CHAN[1], J. PINEDA-FARIAS[1], E. HONG[1], A. SANCHEZ-MENDOZA[2], V. GRANADOS-SOTO[1];
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Abstract: The aim of this study was to characterize the neonatal streptozotocin-induced type 2 diabetes mellitus (n-STZ) as a model of peripheral neuropathy. Three-day-old male Wistar rats were injected with STZ (70 mg/kg, i.p.). Three weeks after n-STZ, rats were weaned and separated in individual cages to perform further behavioral and molecular experiments. A time course of weight, blood glucose, oral glucose tolerance test (OGTT), and insulin secretion was performed at 4, 8, 12 and 16 weeks after n-STZ. At the same time course, we evaluated the pancreatic beta cells damage and the impact in the number of Langerhans islets. Moreover, we also evaluated allodynia and molecular changes in sciatic and L4-L6 spinal nerves by using

western blot and immunofluorescence. n-STZ produced signs of type 2 diabetes including hyperglycemia; glucose intolerance and low secretion of insulin from 4 weeks post n-STZ. In addition, n-STZ also reduced the number of Langerhans islets and pancreatic beta cells. A partial recovery in the number of Langerhans islets and insulin secretion was observed only at 8 weeks post n-STZ. As sign of nerve damage, we observed a time-dependent overexpression of activating transcription factor 3 (ATF3) in L4-L6 dorsal root ganglions (DRGs); satellite glial cells surrounding DRGs, and sciatic nerve. Moreover, we observed overexpression of glial fibrillary acidic protein (GFAP) in satellite glial cells. Interestingly, these changes correlated with the presence of allodynia. The analgesic drugs tested at week 16 showed the following antiallodynic profile: Gabapentin (10-100 mg/kg, p.o.) > morphine (1-3 mg/kg, s.c.) > diclofenac (1-3 mg/kg, p.o.). On the other hand, the AMP-activated protein kinase (*AMPK*) *activator* metformin (200 mg/kg, p.o. during 15 days, starting at week 14) produced an antiallodynic efect in rats subjected to n-STZ. Results demonstrate that n-STZ produces signs of type 2 diabetes and peripheral neuropathy. This model could be used to study diabetic peripheral neuropathy in rats. PB-I, VHO-C and JBP-F are Conacyt fellows.

Disclosures: **P. Barragan-Iglesias:** None. **V. Oidor-Chan:** None. **J. Pineda-Farias:** None. **E. Hong:** None. **A. Sanchez-Mendoza:** None. **V. Granados-Soto:** None.

Poster

153. Diabetic Neuropathy

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 153.07/N14

Topic: D.08. Pain

Support: K08NS079482

Title: Chemogenic silencing of Nav 1.8 nociceptors reverses neuropathic pain and hyper-excitability in Painful Diabetic Neuropathy (PDN)

Authors: *B. BHATTACHARYYA[1], N. JAYARAJ[1], D. REN[2], A. BELMADANI[2], R. J. MILLER[2], D. M. MENICHELLA[1];
[1]Dept. of Neurol., [2]Dept. of Pharmacol., Northwestern Univ., Chicago, IL

Abstract: Painful Diabetic Neuropathy (PDN) is a debilitating affliction present in 26% of diabetic patients with substantial impact on their quality of life. Despite this significant prevalence and impact the electrophysiological mechanisms underlying PDN are not well understood. Neuropathic pain is caused by sustained excitability in sensory neurons that reduces

the pain threshold. Extensive characterization of DRG neurons has revealed molecularly defined sub-populations of sensory neurons (Chiu and Woolf 2014, Usoskin et al., 2015), including Nav 1.8 nociceptors. The specific role of Nav1.8 nociceptors in PDN is unknown. Nav1.8::Cre, Ai9 (td tomato reporter) or Nav1.8::Cre, RC::PDi mice were generated to conditionally express hM4D DREADD receptors in this neuronal subset. Mice were given regular (RD) or high fat diet (HFD) for ten weeks, and then tested to determine glucose intolerance. Immunohistochemical staining was performed to confirm DREADD receptors expression in Nav 1.8 nociceptors. Mice were analyzed by pain behavioral tests and electrophysiological studies. We first studied the properties of these nociceptors in normal (RD) and diabetic (HFD) mice. Action potentials evoked from the Nav1.8 expressing neuronal population (Nav 1.8 cre/tomato) by using depolarizing current injections from a set holding membrane potentials (-50 mV) indicated that the subthreshold and threshold level for generating APs are lower in HFD DRGs vs RD DRGs (subthresold HFD 35 pA±5 and RD is 55 pA±8; n=5; threshold for AP in HFD is 80pA±10 and RD is 123± 12, n=5) indicative of diabetes induced hyperexcitability in these animals. We next generated mice in which hM4D receptors (Designer Receptor Activated by a Designer Drug, DREADD) were expressed exclusively in these DRG neurons. We confirmed that DREADD receptors are expressed in Nav 1.8 nociceptors in both IB4 positive and negative subtypes. Intraperitoneal injection of the drug clozapine-N-Oxide (CNO) signiifcantly reduced allodynia in the HFD model of type II diabetes. Furthermore, electrophysiological current clamp studies show that activation of DREADD receptors with CNO (2.5 mM) blocked action potential generation(n=4) in Nav 1.8 DREADD animals. On the contrary, CNO did not show any effect on Aps Nav1.8::Cre, Ai9 (tomato) nociceptors due to the absence of DREADD receptors in both HFD and RD DRGs. We demonstrated that diabetic mice display hyperexcitabilty in their Nav 1.8 nociceptors. Chemogenetic silencing of Nav 1.8 nociceptors reverses neuropathic pain and hyper-excitability in PDN, suggesting a critical role of Nav 1.8 nociceptive neurons hyperexcitability in the pathogenesis of neuropathic pain in diabetes.

Disclosures: **B. Bhattacharyya:** None. **N. Jayaraj:** None. **D. Ren:** None. **A. Belmadani:** None. **R.J. Miller:** None. **D.M. Menichella:** None.

Poster

153. Diabetic Neuropathy

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Topic: C.13. Sensory Disorders

Support: Dykstra Foundation

RO1 NS075084

RO1 AG037506

RO1 NS075156

RO1 DK097519

Title: Tadalafil promotes recovery from peripheral neuropathy in type ii diabetic mice

Authors: *L. JIA[1], M. CHOPP[1,2], A. SZALAD[1], X. LU[1], Z. G. ZHANG[1], L. WANG[1];
[1]Neurol. Res., Henry Ford Hosp., Detroit, MI; [2]Dept. of Physics, Oakland Univ., Rochester, MI

Abstract: Background: We previously demonstrated that treatment of diabetic peripheral neuropathy with a potent phosphodiesterase 5 (PDE5) inhibitor, sildenafil, improved functional outcome in diabetic db/db mice. To further examine the effect of PDE5 inhibition on diabetic peripheral neuropathy, we investigated the effect of another potent PDE5 inhibitor, tadalafil, on diabetic peripheral neuropathy. Tadalafil is pharmacokinetically distinct from sildenafil and has a longer half-life than sildenafil. Methods and Results: Diabetic db/db mice at age 20 weeks (n=10) were treated with tadalafil (10 mg/kg/ every other day, p.o.) for 8 consecutive weeks, and db/db mice (n=10) at the same age treated with saline were used as a control group. We found that the tadalafil treatment significantly ($p<0.05$) increased motor and sensory conduction velocity by 22 % and 23% ($p<0.05$), respectively, compared to the saline treatment. Tadalafil markedly ($p<0.05$) improved sensory function by reducing the thermal latency measured by the plantar test (7±0.5 m/s vs. 11±1.2 m/s in saline) and by the tail flick test (5±0.3 m/s vs. 6±0.3 m/s in saline). Tadalafil treatment also significantly ($p<0.05$) increased local blood flow in the sciatic nerve (93±5 vs. 58±6% in saline) and increased the density of FITC-dextran perfused vessels (18±2 vs.11±1% in saline). Immunohistological analysis showed tadalafil substantially increased intra-epidermal nerve fibers density (16±1 vs.11±1/mm in saline, $p<0.05$) compared to saline treatment. To investigate the molecular mechanisms that mediate therapeutic effect of tadalafil on peripheral neuropathy, the effect of tadalafil on expression of nerve growth factor (NGF) was examined by Western blot. Tadalafil treatment significantly reversed the diabetic reduced NGF protein level (1.09±0.1 vs.0.7±0.2 in saline, $p<0.05$) in diabetic sciatic nerve. The tadalafil treatment did not significantly alter blood glucose levels, Ac1, triglyceride, and animal body weight compared to the saline treatment. Conclusion: Tadalafil improves regional blood flow in the sciatic nerve, which likely contributes to improved peripheral nerve function, and the amelioration of diabetic peripheral neuropathy.

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Poster

153. Diabetic Neuropathy

Location: Hall A

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Topic: D.08. Pain

Support: NIH Grant K08NS079482

Title: Selective chemokine receptor CXCR4 deletion in Nav 1.8 nociceptive neurons reverses neuropathic pain and small fiber neuropathy in diabetes

Authors: *N. D. JAYARAJ[1], B. J. BHATTACHARYYA[1], D. REN[2], A. BELMADANI[2], R. MILLER[2], D. M. MENICHELLA[1];
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Abstract: Painful Diabetic Neuropathy (PDN) is a debilitating affliction present in 26% of diabetic patients with substantial impact on their quality of life. Despite this significant prevalence and impact, current therapies for PDN are only partially effective. Moreover, the electrophysiological mechanisms underlying PDN are not well understood. Neuropathic pain is caused by sustained excitability in sensory neurons that reduces the pain threshold, so that pain is produced in the absence of appropriate stimuli. Sensory neurons display sustained and enhanced excitability in response to different molecules including chemokines. In particular, by pharmacological blockade with a CXCR4 antagonist, we have recently demonstrated the role of CXCR4/SDF-1 signaling in PDN (Menichella et. al. 2014). Nav1.8 cre::CXCR4 f/f and Nav 1.8 Cre::Ai9 /CXCR4 f/f were generated to conditionally knockout CXCR4 chemokine receptors in Nav 1.8 nociceptors. Mice were given regular or high fat diet (HFD) for ten weeks, and then tested to determine glucose intolerance. *In situ* hybridization was performed to confirm selective ablation of CXCR4 receptors in Nav 1.8 nociceptors. Mice were then analyzed by pain behavioral tests, electrophysiological studies and *in vitro* and *in vivo* calcium studies. We demonstrated that selective chemokine receptor CXCR4 deletion in Nav 1.8 nociceptive neurons reverses neuropathic pain in the HFD induced model of type II diabetes. Furthermore, electrophysiological studies on Nav 1.8 cre::CXCR4 f/f heterozygous HFD induced-diabetic DRG culture demonstrated that application of SDF-1 (20nM) not only depolarized DRG neurons (RMP; d-33.2±0.84 mV n=3) but also increased firing frequencies in response to current injection. On the contrary, SDF-1 application does not display any effects on Nav 1.8 cre::CXCR4 f/f homozygous HFD induced-diabetic DRG culture. Additionally, *in vitro* and *in vivo* calcium image studies revealed increased calcium influx in response to chemokine SDF-1 in Nav 1.8 nocicpetors in diabetes. Finally, we observed significant improvement of skin innervation in diabetic mice when chemokine receptor CXCR4 was selectively deleted in Nav

1.8 nociceptors. These observations establish CXCR4 chemokine signaling as a new candidate responsible for hyperexcitability and calcium influx in a distinct subpopulation of DRG neurons and will add to our understanding of how changes in the excitability and calcium influx of sensory neurons contribute to the progression of small fiber neuropathy in PDN, which is a critical barrier to progression for effective treatment of this currently intractable and widespread affliction.

Disclosures: N.D. Jayaraj: None. **B.J. Bhattacharyya:** None. **D. Ren:** None. **A. Belmadani:** None. **R. Miller:** None. **D.M. Menichella:** None.

Poster

153. Diabetic Neuropathy

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 153.10/N17

Topic: D.08. Pain

Title: Possible involvement of opioid receptors in the antihyperalgesic and antiallodynic effect of celecoxib in diabetic rats

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Abstract: In this study we show the antihyperalgesic and antiallodynic effect of celecoxib in diabetic rats and the possible involvement of opioid receptors in the mechanism of action of celecoxib in these rats. We induced experimental diabetes using streptozotocin. To produce hyperalgesia in non-diabetic and diabetic rats, formalin (0.5%) was injected in the right hindpaw. von Frey filaments were used in order to determine the 50% withdrawal threshold in diabetic rats. Celecoxib (0.3-30 mg/kg) administered orally reduced formalin-induced nociceptive behavior during phase 2. Systemic administration of naltrexone (3 mg/kg) as pre-treatment (-10 min), prevented celecoxib-induced antihyperalgesia in formalin-treated diabetic rats. Additionally, naltrexone as well as the δ and κ opioid receptor antagonists naltrindole (3 mg/kg) and 5'-guanidino naltrindole (1 mg/kg), respectively, fully prevented celecoxib-induced antihyperalgesia (10 mg/kg) in formalin-treated non-diabetic and diabetic rats. Furthermore,

celecoxib (0.3-30 mg/kg) exhibited an antiallodynic effect in diabetic rats. Pre-treatment with naltrexone (3 mg/kg) also prevented the antiallodynic effect of celecoxib (0.3, 3 and 10 mg/kg). Interestingly, this dose of naltrexone only partially prevented the antiallodynic effect of celecoxib 30 mg/kg. Naltrexone and naltrindole (3 mg/kg), but not 5'-guanidino naltrindole (1 mg/kg), fully prevented the antiallodynic effect of celecoxib in diabetic rats. Our data suggest that celecoxib produces an antihyperalgesic and antiallodynic effect indiabetic rats. These effects may result from activation of μ, δ and κ opioid receptors for antinociception and μ and δ for antiallodynia. Thus, celecoxib could be useful to treat neuropathic pain in diabetic patients.

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Poster

153. Diabetic Neuropathy

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 153.11/N18

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Role of macrophage migration inhibitory factor in diabetic peripheral neuropathy

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Abstract: Diabetic peripheral neuropathy (DPN) is a common complication of diabetes mellitus which accompanied with distressful sensory symptoms, initially in the feet. With its dual proinflammatory and metabolic effects, macrophage migration inhibitory factor (MIF) has been recently suggested to be a logical target in the field of diabetes. In this study, we attempted to investigate the peripheral changes that elicit pain symptom in DPN rat model, and then we assessed the expression of MIF to the footpad skin. Also, we determined whether this MIF expression is correlated with the expressions of intraepidermal nerve fiber (IENF) and glyoxalseI (GLO1). Experimental DPN was made in male SD rats streptozotocin (STZ) injection. Body weights (BW) and blood glucose (BG) levels were checked. To compare the pain symptom between DPN and sham group, we weekly evaluated mechanical threshold and serially analyzed footpad thickness for 24 weeks. At the last day, all the footpads were excised and prepared for the following procedures; quantitative RT-PCR, western blot, and immunohistochemistry of MIF, IENF and GLO1 . As compared to sham group, DPN group significantly increased BG levels within one week after STZ injection, after BW decreased progressively, and occurred

hyperglycemia until the 24th week. Likewise, the DPN group also developed significantly lower mechanical threshold as early as 7-8 weeks after the induction of diabetes, at which time the footpad thickness became reduced. On the immunohistochemical staining, the DPN group markedly diminished the expressions of IENF and GLO1, while increased MIF on the footpad skin lesions. Similarly, mRNA and protein levels of IENF and GLO1 were significantly down-regulated as the levels of MIF up-regulated on the lesions of DPN group. As hyperglycemic state was continuously maintained until the 24th weeks, the differences between two groups grew prominent. These results demonstrated that MIF may play a role in the pathogenesis of DPN. To our knowledge, this is the first to investigate MIF expression on the DPN-like footpad skin lesions and its potential role in the correlation with IENF and GLO1. Taken together, it is suggested that MIF can be a therapeutic target to control DPN in footpad skin.

Disclosures: S. Noh: None. **K. Yoon:** None.

Poster

154. Pain Imaging and Perception

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 154.01/N19

Topic: D.08. Pain

Support: Wings For Life

Promobila foundation

Spaulding Gordon foundation

Title: PET-MRI of microglia and neuropathic pain after spinal cord injury

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Abstract: Neuropathic pain is one of the most challenging medical problems following a spinal cord injury. It is personally devastating to a degree that it can exceed the impact of other SCI consequences. Neuropathic pain is also difficult to treat successfully, and it significantly impacts rehabilitative efforts, mood and life satisfaction. Animal studies indicate that microglia, the resident macrophages of the CNS, are overexpressed in SCI-induced neuropathic pain. A first step in translating these findings to clinical applications is to establish the role of microglia in human neuropathic pain after SCI. This study presents data on microglial expression in the human brain in SCI with and without neuropathic pain investigated using simultaneous PET-

MRI and the radioligand 11C-PBR28, a highly selective microglial marker. Preliminary analysis suggests: i) Microglial expression may be elevated in the anterior cingulate (most evident in severe neuropathic pain). ii) Resting state networks (independent component analysis) are more clearly defined in the healthy control subjects that in patients, suggesting potential disruption of both "default" and motor networks in SCI patients, potentially co-localized with neuroinflamamtion. These findings suggest that active microglia, as imaged by 11C-PBR28 PET, may be a key component in neuropathic pain in SCI. Microglia may be an attractive pharmacological target for novel analgesics, and the PET-MR imaging methods developed here may provide new objective endpoints for monitoring treatment success.

Disclosures: C. Linnman: None.

Poster

154. Pain Imaging and Perception

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Topic: C.13. Sensory Disorders

Support: NIH NINDS R01NS0750182

NIH NINDS K24NS064050

Title: The migraine brain in transition: girls versus boys

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Abstract: Migraine is about twice as common in females as in males. Whereas pre-pubertal boys and girls have the same prevalence, with puberty, the prevalence in girls becomes around 3 times greater. However, the underlying mechanisms remain poorly understood. Using magnetic resonance imaging (MRI) we evaluated 28 children with migraine (14 girls and 14 boys) and 28 sex- and age-matched healthy controls to determine differences in brain structure and function

between: (a) girls and boys with migraine, and (b) girls and boys with migraine during early- and midpuberty (ie., 10-11 years vs 14-16 years) compared to matched healthy controls. Cortical thickness preprocessing and analysis steps were performed using FreeSurfer. Cortical thickness analysis was performed using a mask that included the primary sensory cortex (S1), primary motor cortex (M1), insula, Precuneus (PCu), dorsolateral prefrontal cortex (dlPFC), frontal pole (FP), and temporal pole (TP). Results were corrected for multiple comparisons based on Monte Carlo permutations with 5,000 iterations using AlphaSim. Subcortical gray matter (GM) volume analysis was performed with voxel-based morphometry (VBM) using FSL-VBM. VBM was performed using a mask that included thalamus, caudate, putamen, pallidum, hippocampus, amygdala, nucleus accumbens, periaqueductal gray (PAG), and hypothalamus. Results were corrected for multiple comparisons using Monte Carlo simulations with an image-wide threshold of $p < 0.01$. Finally, seed-based resting-state functional connectivity (rsFC) was performed using FEAT in FSL. A data-driven approach was used to perform rsFC. Thus, we performed rsFC from the left and right amygdala, as well as from the right PCu. Results were corrected for multiple comparisons using cluster-correction with $z > 2.3$ and $P < 0.05$. Compared to boys, girls had more GM in the S1, SMA, PCu, basal ganglia, and amygdala, as well as greater PCu functional resting state connectivity to the thalamus, amygdala and basal ganglia, and greater amygdala functional resting state connectivity to the thalamus, cingulate, and SMA. Moreover, in migraine girls, midpuberty was related to more GM in the S1, amygdala, and caudate compared to midpuberty migraine boys and matched healthy controls. This is the first study showing sex and developmental differences in pediatric migraineurs in brain regions associated with sensory, motor, and affective functions, providing insight into the neural mechanisms underlying distinct migraine sex-phenotypes. Acknowledgements: This work was supported by NIH (NINDS R01NS0750182 grant and NINDS K24NS064050) to DB. We thank N Maleki for her help with imaging the patients.

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Poster

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Topic: D.08. Pain

Support: NIH/NCCAM 5R01AT007176

Department of Neural and Pain Sciences, University of Maryland School of Dentistry

NIH/NCRR1KL2RR025006-01

Title: Changes in pain-related brain activity following a mindfulness meditation intervention in chronic migraine are associated with reduced anxiety

Authors: *V. A. MATHUR[1,2], S. A. B. BURROWES[1], M. L. KEASER[1], S. A. KHAN[1], M. GOYAL[3], D. A. SEMINOWICZ[1];
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Abstract: Patients with chronic migraine experience frequent and ongoing periods of severe and disabling headache. Migraine is also associated with altered brain response to pain. Prior research suggests that brief meditation can acutely reduce migraine pain and improve related health outcomes among patients, as well as modulate laboratory pain-related brain response among healthy controls. The aim of the current study was to determine the effect of meditation on pain-related brain networks in chronic migraine, and the relationship between brain changes and improved clinical outcomes. Patients were scanned using fMRI before and after a one-time 10-day intensive Vipassana meditation retreat, as well as at 6- and 12-month follow up visits. At each visit, patients completed two fMRI runs (Siemens 3T Tim-Trio, TR=2.5s, resolution = 1.8x1.8x4mm) where they received heat stimuli (three intensity levels, two painful) on the left forearm within a block design. Patients also completed self-report assessments of average past month migraine pain intensity, pain catastrophizing, and mood states. Twenty patients with chronic migraine (16f, 41.8 ± 2.7 y/o) participated in the study, though not all participants completed all time points. Linear mixed models were used to examine changes in brain response and clinical outcomes after the meditation intervention (two time points) and lasting effects at follow up visits (omnibus model, all four time points). A significant reduction was found in migraine pain intensity (p=.05) and anxious mood (p=.006) after the intervention. These effects persisted in the omnibus models (migraine pain p=.01, anxiety p=.05). Omnibus models also revealed a marginal overall reduction in pain catastrophizing (p=.07). No changes were found in other mood states, including depression. To assess pain-related brain responses, parameter estimates were extracted from five clusters of interest defined based on response to heat pain at BL (p_{FWE}<.05). Omnibus models revealed significant reduction in pain-related activation in the right anterior insula (INS, p=.05), and marginal reductions in the right posterior INS (p=.06) and thalamus (p=.06). Pain-related reductions in these three regions was significantly correlated with reductions in anxious mood state (RaINS p<.001, RpINS p=.01, thalamus p=.007), but not with migraine pain intensity or pain catastrophizing. Our findings suggest that a one-time intensive meditation intervention may have long lasting effects on migraine pain, anxiety, and pain-related

brain networks. These results also suggest that meditation's effects on pain may be mediated by reductions in anxiety.

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Poster

154. Pain Imaging and Perception

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Topic: D.08. Pain

Support: 1R21DE023964-01A1

Title: Hypothalamic functional connectivity in ongoing pain in healthy subjects and spontaneous pain in burning mouth syndrome patients

Authors: *J. S. PAYANO SOSA[1], T. J. MEEKER[2], S. A. KHAN[3], T. F. MEILLER[4], D. SEMINOWICZ[2];
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Abstract: Burning mouth syndrome (BMS) is a chronic pain syndrome characterized by burning in the superficial oral mucosa. About 12-18% percent of postmenopausal women experience a type of BMS. While the mechanisms of BMS are unclear, several lines of evidence implicate dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. Because the hypothalamus is known to link the endocrine and nervous systems and is involved in thermoregulation and circadian rhythm, we examined hypothalamus connectivity as a potential target for understanding BMS symptoms. We investigated resting-state fMRI functional connectivity in a homogeneous sample of 9 female (age range 40-61) peri- or post-menopausal BMS Type I patients and 9 matched healthy control subjects. Patients underwent two scanning sessions on the same day: in the morning when the burning is typically absent or minimal, and in the afternoon when the burning is usually most intense. To control for changes in functional connectivity that could be attributed to ongoing pain not specific to BMS, we included 14 healthy male and female controls (age range 23 to 61), with resting-state scans during pain-free and ongoing pain conditions. Ongoing pain was induced by applying capsaicin cream on the leg and placing a warm thermode on the site of application during the entire time of the scan. We used Conn Toolbox and SPM12 to assess seed-to-voxel connectivity with four seed regions, bilateral medial

(MH) and lateral (LH) hypothalamus. Here we focus on the connectivity of the right MH. We hypothesized that compared to healthy controls, BMS patients have increased connectivity of the MH to regions involved in thermoregulation, pain processing, and emotional circuitry. Patients had increased functional connectivity between MH and the stria terminalis, ventral striatum, midbrain, anterior (aMCC) and posterior (pMCC) midcingulate cortex, posterior insula, and orbitofrontal cortex in the afternoon scans when compared to healthy controls. The increased connectivity in the majority of these regions was explained by the presence of ongoing pain in BMS patients (i.e. determined by BMS morning vs. afternoon). In the ongoing pain healthy control group, increased aMCC overlapping with that in the BMS analyses was related to ongoing pain. We therefore showed abnormal connectivity of the MH in BMS, which was mostly explained by the presence of ongoing pain. The MH-aMCC connectivity in particular appears to be related to ongoing pain, even in the absence of a disease state.

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Poster

154. Pain Imaging and Perception

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Topic: D.08. Pain

Support: NIH Grant P30 NR014129

Title: Brush allodynia susceptibility is related to baseline heat pain sensitivity, regional blood flow differences, and sensitization-induced heat allodynia and mechanical hyperalgesia

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Abstract: Introduction: Brush allodynia (BA) often, but variably occurs in many chronic neuropathic pain conditions. It is not known what factors determine allodynic development in any given individual, or to what extent BA is related to hyperalgesia expression. The goal of this study was to determine 1) whether baseline pain sensitivity can predict BA susceptibility, and 2)

the extent to which a sensitizing provocation engages separate mechanisms for hyperalgesia and allodynia. Methods: 29 healthy participants (15f) between 20-43 years (27.2±7.1) were evaluated before and after exposure to a sensitizing capsaicin-heat pain model (C-HP). The model consisted of topical application of 10% capsaicin cream, accompanied by a heat stimulus for 35 minutes provoking a moderate level of pain. Sensory testing included Heat Pain Thresholds (HPT), and Mechanical Pain Ratings (MPR) of sharp probes (512mN, 256mN, 128mN, 64mN). Further we assessed BA at multiple time points after C-HP exposure. Following these sensory test sessions, a subset of subjects (N=15) participated in a replicate arterial spin label (ASL) session. Results: Half of the participants (15) developed BA within 1 hr of capsaicin-heat exposure. The BA group had a significantly lower HPT both pre- (X^2=-2.532, p=0.011) and post C-HP exposure (Z=-2.706, p=0.007); both groups had significantly reduced HPT post C-HP exposure. Additionally, mechanically evoked hyperalgesia (based on MPR) was significantly greater for the BA group post C-HP exposure, at all forces tested: 512mN (Z=-2.010, p=0.044), 256mN (Z=-2.468, p=0.014), 128mN (Z=-2.706, p=0.012), 64mN (Z=-2.171, p=0.030). This group difference in MPR was seen at all time points tested, but the HPT difference was only seen at the first post C-HP time point. The ASL results revealed that the allodynic group showed greater CBF in anterior mid-cingulate cortex (aMCC) than the non-allodynic group post C-HP. This CBF result was not related to group differences in the pain intensity evoked by C-HP exposure. Discussion: In this preliminary analysis we show that sensitization-induced brush allodynia is related to greater baseline heat pain sensitivity. Further, BA is associated with greater heat allodynia and mechanical hyperalgesia, although the time courses of these two phenomena are distinctly different, implying separate underlying mechanisms at work. Finally, the group difference in CBF suggests a particular role for aMCC in the development of BA.

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Poster

154. Pain Imaging and Perception

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: NIH/NCCAM 5R01AT007176

Title: Superior colliculus: A role in migraine?

Authors: *D. SEMINOWICZ[1], H. JOHANI[1], S. YOO[1], R. MASRI[1], R. L. QUITON[2]; [1]Dept of Neural & Pain Sci., Univ. of Maryland, Baltimore, Baltimore, MD; [2]Univ. of Maryland, Baltimore County, Catonsville, MD

Abstract: The superior colliculus (SC) may play an important role in the pathophysiology of migraine headaches, and abnormal processing in SC may explain key features of migraine attacks such as photophobia, phonophobia and cutaneous allodynia. The intermediate and deep layers of the SC contain neurons that integrate multiple modalities of inputs (visual, auditory, and somatosensory) and project to brain areas that are abnormally active in migraineurs including PAG, medial thalamus, and PFC. Approximately 70% of inputs to deep and intermediate SC neurons arise from trigeminal afferents, many of which are activated by noxious stimuli. We aimed to show evidence of SC involvement in migraine headache onset and maintenance in humans and rodent models. We examined pain-related activity in the SC and controlled for activity in the neighboring periaqueductal gray (PAG) in 17 patients with chronic migraine and 20 matched healthy controls. Pain was evoked with a thermal stimulus on the left forearm at one of two painful heat levels with an innocuous warm stimulus as control. We also tested for abnormal functional connectivity between SC and brain structures involved in pain processing and sensory function. In rodent studies, we tested if noxious stimulation of meningeal afferents is associated with the expression of the immediate early gene, c-Fos (a marker of neuronal activation), in the SC. Heat pain consistently activated SC in patients, but not controls. PAG activity was elevated in patients compared to controls, but this was true even during nonpainful stimulation. Thus, patients had consistently higher SC activity compared to controls when controlling for PAG activity, especially on the left side. During resting state, patients had increased SC connectivity to primary sensory cortices and areas involved in nociceptive processing. In rats, noxious chemical stimulation of meningeal afferents resulted in a significant expression of c-Fos in the SC compared to controls. These results support our prediction of a role for SC in migraine. Ongoing work will test this role more specifically by comparing interictal and migraine attack states in a within-patient design and using rodent fMRI studies.

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Poster

154. Pain Imaging and Perception

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Topic: D.08. Pain

Support: FAPESP Brazil 2014/20983-1

Title: Does anti-NGF reverse symptoms of chronic neuropathic pain?

Authors: ***J. T. SILVA**[1,2], B. EVANGELISTA[1], D. SEMINOWICZ[2], M. CHACUR[1];
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Abstract: Introduction: Nerve growth factor (NGF) has been widely studied by the scientific community for its pro-nociceptive role in chronic pain conditions, and it is characterized as a chemical mediator responsible for the induction and maintenance of these pathologies. Chronic neuropathic pain is characterized by spontaneous burning pain accompanied by allodynia and hyperalgesia. Anti-NGF drugs have been used to reduce these symptoms in cancer pain, irritable bowel pain and osteoarthritis in both animals and human models. However, in chronic neuropathic pain its multiple actions are not fully understood.Methods: Male Wistar rats (200-220g, 2 months old) underwent induction of neuropathic pain by chronic constriction injury of the sciatic nerve (CCI). Control groups included sham-operated animals (Sham), which underwent the same incision, but without nerve ligation, and Naive animals, which underwent no surgical procedures. We performed Western blot to detect NGF in the sensory ganglia (DRG L4-6) of CCI, Sham and Naive animals. In addition, anti-NGF was injected (1 and 3ug, ipl.) in the hindpaw 14 days after surgery and a dose-response curve was performed. Sensory testing included mechanical nociceptive thresholds, thermal hyperalgesia and cold allodynia.Results: We observed an increase in NGF synthesis in the CCI group compared with the control groups. The CCI animals demonstrated a reduction of the nociceptive threshold and increased thermal hyperalgesia and cold allodynia compared to control groups. After pharmacological treatment with anti-NGF (CCI + anti-NGF), we observed a reduction of hyperalgesia and allodynia in these animals.Conclusions: Our results suggest that NGF is an important factor in the induction and maintenance of neuropathic pain, since increased NGF levels were observed in the DRG after injury. We also demonstrated the relevance of this mediator as a therapeutic target, since anti-NGF was able to reverse the often difficult to treat symptoms of this pathology. Studies for the characterization of anti-NGF as well as magnetic resonance imaging are underway to further elucidate these findings.

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Poster

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Trailblazer Award Department of Anesthesia at Boston Children's Hospital

NINDS K24 NS064050-08

Title: Functional alterations in resting state connectivity in rat pups following postnatal morphine exposure

Authors: D. BAJIC, M. M. CRAIG, D. BORSOOK, *L. BECERRA;
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Abstract: Our previous preliminary work described spontaneous, intrinsic brain activity in the absence of any stimulation as system-wide, resting state networks in the developing rat brain under light inhalational anesthesia. Whether patterns of resting state activity are affected by prolonged morphine administration in a developing rat model is unknown. Therefore, we used functional MRI and independent component analysis to map patterns of resting-state activity in 2-week old rat pups following either morphine or saline twice-daily injections for two weeks since postnatal day 1 (N=12/group). A total of 11 networks were identified, 6 of which displayed significant differences in connectivity between treatment groups. Morphine treated rats showed increased connectivity in the Default Mode Network, Basal Ganglia-Hypothalamic Network, Sensory (Exteroceptive) Network, Interoceptive Network, Auditory network, and Hippocampal Network. In contrast, morphine treated rats showed decreased connectivity in three of these networks: Basal Ganglia-Hypothalamic Network, Sensory (Exteroceptive) Network, and Auditory Network. Presented results suggest that resting-state networks driven by spontaneous BOLD signal fluctuations under light anesthesia are not only present in the developing rat brain at 2 weeks of age, but are also affected by prolonged morphine administration.

Disclosures: D. Bajic: None. **M.M. Craig:** None. **D. Borsook:** None. **L. Becerra:** None.

Poster

154. Pain Imaging and Perception

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Topic: D.08. Pain

Support: NIH K08 DA035972-01

Trailblazer Award Department of Anesthesia at Boston Children's Hospital

NINDS K24 NS064050-08

Title: Resting state networks in the infant rat brain under light isoflurane anesthesia

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Abstract: Resting state functional magnetic resonance imaging (fMRI) measures fluctuations in blood oxygenation level-dependent (BOLD) signal in the absence of stimuli and has become a powerful tool for mapping large scale brain networks in humans and animal models. Several fMRI studies have been conducted in anesthetized and awake adult rats, reporting an apparent similarity between the identified patterns of activity at the systems levels. The consistency of these resting-state networks, however, has not yet been evaluated and quantified in the developing rat brain. In this study, we used independent component analysis (ICA) to identify brain networks in lightly anesthetized 2-week old infant rats (N=11). An adult rat brain template from our lab (Becerra et al. Neuroimage 2011) was used for registration, comparison, and identification of infant rat group ICA results. The analysis identified 6 out of 7 template networks to be present in the infant rat. These include: Default mode network, Basal Ganglia-Hypothalamic, Basal Ganglia-Thalamic-Hippocampal, Autonomic, Sensory (Exteroceptive), as well as Interoceptive network. Some of these networks consisted of more than one component. Furthermore, infant rat networks appear underdeveloped, showing reduced connectivity with subcortical structures in comparison to the adult templates. ICA also revealed 5 additional components that did not correlate with our templates and include the following networks: Basal Ganglia, Hippocampal, Thalamic-Ventral Midbrain, Thalamic-Periqueductal Gray, as well as the Brainstem network. In summary, we demonstrate that (1) fMRI can be applied to study the functional connectivity of the infant rat brain at 2-weeks of age, and (2) that detected slow fluctuations in the BOLD signal correspond to functionally relevant resting-state networks.

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Poster

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Support: 103-CGN14

NSC 102-2311-B-002-034-MY3

NTU-ERP-104R892102

Title: FDG-PET imaging bone pain- and morphine analgesia- related brain changes in the mice

Authors: ***W.-H. CHU**[1], W.-Y. LIN[1,2], W.-Z. SUN[2], C.-T. YEN[1];
[1]Life Sci., Natl. Taiwan Univ., Taipei City, Taiwan; [2]Anesthesiol., Natl. Taiwan Univ. Hosp., Taipei City, Taiwan

Abstract: Metastatic cancer-induced bone pain (CIBP) is one of the most devastated symptoms throughout end-stage life. Bone metastasis is associated with unbearable pain, both in rest and during movement, which would lead to eventual disability and miserable quality of life. Morphine is the standard drug of choice in CIBP. However, the question whether morphine relieves CIBP through modulating neuropathic and nociceptive pain components is not well elucidated. In this study, we investigated morphine-induced effect on CIBP mice brain by 18F-fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PET/CT). We injected 4T1 mouse breast cancer cells into left femur bone marrow cavity of the BALB/c mice. Mice in control group were injected with phosphate buffered saline. Neuropathic pain behaviors, i.e. von Frey filaments and cold acetone, were measured on the day before surgery, Day 7, Day 10 and Day 14 after the surgery. Morphine doses (10, 15, 30 mg/kg, i.p.) were treated on Day 16 after the surgery. Behaviorally, 15 mg/kg morphine was sufficient to relieve mechanical and cold allodynia of the CIBP mice between 30 to 90 minutes post-treatment. In the PET imaging study, each mouse was scanned 3 times: before bone surgery, Day 14, and Day 16 after the surgery at 30 min after the 15 mg/kg morphine treatment. We found that glucose metabolic activity significantly increased in contralateral insula and bilateral primary somatosensory cortex (S1), primary motor cortex (M1), secondary motor cortex (M2), and hypothalamus; while decreased in anterior cingulate cortex (ACC), nucleus accumbens (NAc), striatum, ventral posterior nucleus (VP), periaqueductal gray (PAG) in CIBP, as compared to pre-surgery condition. In particular, morphine reversed the brain metabolic activity of bilateral M1, M2, ACC, PAG, contralateral insular cortex, S1, and VP; increased habenular nucleus (Hb) and interpeduncular nucleus (IPN) activity. Our data suggest that several brain regions are involved in the cancer-induced bone pain, and morphine analgesia may be produced by reversing most changes of the CIBP mice brain.

Disclosures: **W. Chu:** None. **W. Lin:** None. **W. Sun:** None. **C. Yen:** None.

Poster

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Topic: D.08. Pain

Support: Autism Speaks: Essick

Title: Aberrant processing of pain in autism spectrum disorder: FMRI evidence for immediate suppression of pain networks in ASD

Authors: *M. D. FAILLA[1], E. J. MOANA-FILHO[2], G. K. ESSICK[3], C. J. CASCIO[1]; [1]Psychiatry, Vanderbilt Univ., Nashville, TN; [2]Sch. of Dent., Univ. of Minnesota, Minneapolis, MN; [3]Sch. of Dent., Univ. of North Carolina, Chapel Hill, NC

Abstract: Individuals with autism spectrum disorders (ASD) exhibit aberrant behavioral responses to noxious stimulation compared to neurotypical controls, yet little is known regarding mechanisms of sensory processing in ASD. Thus, we investigated neural patterns of pain processing in ASD by applying a noxious heat stimulus (49°C) to the right calf during functional MRI. Subjects included 17 adults with ASD and 16 neurotypical controls. ADOS and ADI-R were administered to confirm ASD diagnosis and to estimate symptom severity. Sensory processing in daily life was assessed with the Sensory Profile Questionnaire. Blood oxygenation level-dependent (BOLD) signal in response to noxious heat was assessed with a whole brain and region of interest (ROI) approach. ROIs related to pain processing were selected based on previous literature: 5 mm spheres were created surrounding the peak voxel within the left primary somatosensory cortex (SI), left secondary somatosensory cortex (SII), and the left insular cortex in the control group. Mean activation was calculated for all voxels within the ROI. There were no differences in calf heat pain thresholds between the two groups ($p=0.551$). In whole brain analysis, individuals with ASD had hypoactivation in response to noxious heat, with controls exhibiting greater activation in cerebellum, thalamus, left putamen, left insula, and left premotor cortex compared to those with ASD. In ROI analysis, the ASD group had significantly less mean activation in the left SI ($p=0.047$), SII ($p=0.004$) and insula ($p=0.002$) ROIs. Additionally, BOLD response time series also differed between groups. The time course of BOLD responses in controls exhibited sustained activation during stimulus presentation, yet individuals with ASD had an early peak followed by an immediate undershoot. This pattern was consistent within left SI, left SII, and left insula ROIs. Left insula mean activation during noxious heat correlated with calf heat pain threshold ($r=0.39$, $p=0.04$) across groups. Mean activation in S1 correlated with restricted and repetitive behaviors (RRB) as measured on the ADOS ($r=-0.54$, $p=0.04$). A negative correlation between SI activation and RRB fits with

hypotheses that RRB may be a compensatory behavior for sensory hypo-responsiveness in ASD. Importantly, activation patterns of an immediate peak followed by possible active suppression in areas involved in pain processing may indicate suppression of pain networks immediately following onset of a painful stimulus in ASD. Future studies are needed to characterize pain processing in ASD, including neural networks for pain and general sensory salience, as they relate to behavioral symptoms.

Disclosures: M.D. Failla: None. **E.J. Moana-Filho:** None. **G.K. Essick:** None. **C.J. Cascio:** None.

Poster

154. Pain Imaging and Perception

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Topic: D.08. Pain

Support: Institut UPSA de la douleur

APICIL foundation

Title: Autonomic pain responses during an empathic and non-empathic context: a study of heart rate variability

Authors: C. FAUCHON[1], I. FAILLENOT[1], F. CHOUCHOU[1], C. BORG[2], A.-M. PERRIN[3], *R. PEYRON[4,1];
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Abstract: The autonomic nervous system (ANS) reacts to nociceptive stimulation and also to emotional events, which produce electroencephalographic (EEG) activation and concomitant changes of physiologic parameters under autonomic control such as RR intervals (RR). Empathy has been widely studied as a physiological phenomenon, and it's intuitively acceptable that other people can have a significant impact on one's pain. However, the mirror situation of how I receive empathic feedback from others and how I modulate the pain I am confronted to is still unexplored. Here we studied whether empathy or non-empathy of an observer can modulate subjective pain ratings, ANS responses to pain and whether one of the autonomic arms

(sympathetic/ parasympathetic) predominates. To this aim, we built experimental scenarios in which volunteers could hear comments from the observers on their ongoing pain made of hot stimulations delivered on the left hand via a heat thermode. Professional actors were recorded while playing roles of experimenters observing a subject. Some of them were empathic while others were non-empathic. Subjects received 90 stimulations at a constant temperature. They were asked to score their pain with a continuous online rating from 0 to 100 (VAS). We assessed ANS reactivity to nociceptive stimulation during the entire experiment through heart rate variability monitoring and correlated the results with the presence of empathic or non-empathic allegations. Autonomic parameters (LF, HF and LF/HF ratio) were obtained by spectral analysis. The experiment was conducted in 30 volunteers. We found that pain ratings was significantly attenuated (-12%) during empathic as compared to non-empathic conditions and this was also significant individually in 16/26 (61,5%) volunteers. Nociceptive-induced RR decrease was present whatever the conditions, mainly related to a sympathetic reactivity, which was significantly more important in non-empathic as compared to the empathic condition. In addition, RR was significantly decreased during non-empathic dialogues as compared to a baseline condition (=absence of pain and audio stimuli). To conclude, these results showed that for a constant painful stimulation, empathic and non-empathic contexts can influence significantly both pain ratings (VAS) and autonomic cardiac reactivity (RR and ratio LF/HF). Future investigations will help to clarify the neural nature of these pain modulations and their relationship with autonomic cardiac reactivity.

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Poster

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Topic: D.08. Pain

Support: University of Toronto Centre for the Study of Pain

Canadian Institutes of Health Research

Title: Intrinsic functional connectivity of the periaqueductal gray (PAG) is related to fibromyalgia clinical symptoms

Authors: *M.-A. COULOMBE[1], K. ST. LAWRENCE[2,3], D. E. MOULIN[3], P. MORLEY-FORSTER[3], M. SHOKOUHI[2,3], W. R. NIELSON[2,3], Y. BUREAU[2,3], K. D. DAVIS[1,4]; [1]Toronto Western Res. Inst., Toronto, ON, Canada; [2]Lawson Hlth. Res. Inst., London, ON, Canada; [3]Western Univ., London, ON, Canada; [4]Univ. of Toronto, Toronto, ON, Canada

Abstract: Introduction: Fibromyalgia syndrome (FMS) is characterized by chronic widespread pain, muscle tenderness, and distressful symptoms such as fatigue, sleep disturbances, anxiety and depression. Previous studies have found deficient endogenous pain modulation in FMS and suggested that this is linked to clinical symptoms. The periaqueductal gray (PAG) is a central node of the descending pain modulation pathway. In addition to its implication in pain and analgesia, this region is also involved in emotional and behavioral aversive responses (anxiety, fear, defensive reaction). In this study of FMS, we investigated 1) resting state functional connectivity (FC) of the PAG, 2) the relationship between PAG FC and FMS clinical symptoms, and 3) how FC of the default mode and salience networks (DMN, SN) relates to FMS clinical symptoms. Methods: Resting state 3T fMRI scans were acquired from 23 female FMS patients and 16 age- and sex- matched healthy controls (HC) after obtaining informed consent. FMS symptoms were assessed using the brief pain inventory (BPI), fibromyalgia impact questionnaire (FIQ), hospital anxiety and depression Scale (HADS), and pain catastrophizing scale (PCS). Seed-to-voxels analyses were performed using FMRIB's FEAT higher level cluster analysis ($z > 2.3$, $p > 0.05$). FC of PAG, DMN and SN were analysed in relation to clinical symptoms. Results: 1) Compared to healthy controls, the FMS group had greater PAG FC with the left lingual gyrus and left hippocampus, but decreased PAG FC with regions associated with motor and executive function as well as the SN and DMN (premotor and prefrontal cortex, temporoparietal junction, posterior cingulate cortex). 2) PAG FC in the FMS group with the PMC and dlPFC was negatively correlated with FIQ scores, and PAG FC with the PFC was correlated with the magnification subscale of the PCS. 3) Intra-network FC of the DMN and SN in the FMS group was similar with only subtle differences compared to healthy controls. 4) FMS subjects had disrupted FC between medial PFC and PMC. This decreased FC was negatively correlated with the global distress and anxiety components of the HADS. Conclusion: Patients with FMS exhibit significant disruptions in PAG FC, particularly with brain regions implicated in distress, threat-induced negative affect, self-awareness and saliency. These abnormalities were correlated with emotional and behavioral symptoms. This study thus implicates the PAG in FMS symptomatology.

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Poster

154. Pain Imaging and Perception

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Support: SFB936

Title: Influence of medication value on the nocebo effect and its mechanism assessed by cortico-spinal imaging

Authors: *A. TINNERMANN[1], S. GEUTER[2], C. SPRENGER[1], J. FINSTERBUSCH[1], C. BÜCHEL[1];
[1]Dept. of Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Inst. of Cognitive Sci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Background and aims The nocebo effect in pain research is characterized by an increase in pain perception after the administration of an inert treatment. In this study, we investigated the influence of a medication's price (cheap vs. expensive) on the magnitude of the nocebo response and the underlying modulation of the cortico-spinal pain network. Methods 52 subjects (25 females) underwent a heat pain stimulation protocol using a thermode on the left volar forearm while BOLD responses in the brain and spinal cord were recorded using functional MRI. The nocebo treatment was introduced as a medical cream that increases pain sensitivity as a negative side effect and was compared to a control cream. Subjects were randomly assigned to one of two groups, one receiving the cheap medical cream whereas the other group was tested with the expensive cream. One day before scanning and directly before scanning, subjects underwent a conditioning procedure which simulated the higher pain sensitivity (i.e. side effects of the cream) through a higher temperature. In the fMRI test phase, subjects received 36 identical heat stimuli (18 control/18 nocebo) with a duration of 20 seconds each and individual pain ratings were recorded after every trial. Additionally, physiological noise (pulse, respiration) was recorded during scanning. Results Pain ratings in the nocebo condition were significantly higher ($t = 3.11$, $p<0.01$) compared to the control condition. The interaction between the cheap and the expensive nocebo cream was significant ($t = -2.6$, $p<0.05$), indicating that the nocebo effect was increased in the expensive condition whereas in the cheap condition, the nocebo effect was mostly absent. Neural correlates of the nocebo effect were found in the left insula, the striatum and bilateral hippocampus. The comparison of the expensive and the cheap nocebo group revealed increased activations in the left insula, left anterior cingulate cortex and bilateral hippocampus. Conclusions This study shows that medication value modulates brain activity in the pain network. More specifically, an expensive medication can increase pain perception and lead to a greater nocebo effect in humans. These results indicate that an expensive medication elicits stronger side effects than a cheap medication.

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Poster

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NSF IGERT grant DGE-1069104

Title: Identifying chronic pain biomarkers using EEG informed fMRI analysis on sickle cell disease patients during wakeful rest

Authors: ***M. CASE**[1], C. H. ZHANG[1], Y. DATTA[1], S. C. NELSON[2], K. GUPTA[1], B. HE[1]; [1]Univ. of Minnesota, Minneapolis, MN; [2]Children's Hosp. and Clinics of Minnesota, Minneapolis, MN

Abstract: Sickle cell disease (SCD) is the most common inherited disorder characterized by sickling of red blood cells (RBCs) under low oxygen, leading to impaired oxygenation, organ damage and pain. As compared to other pain conditions, sickle pain is unique because it can start in infancy and continue to increase throughout life, and can be accompanied by frequent episodes of acute pain due to vasoocclusive crises caused by occlusion of venules by sickled RBCs. Pain treatment remains challenging because of the use of opioids as most common therapy. Patients often remain under-treated due to the fear of opioid addiction, partly due to the lack of an objective pain measurement system. Therefore, an unbiased imaging method to quantify pain is needed to help treat pain in SCD and other disorders. The goal of this study is to discover chronic pain biomarkers using non-invasive imaging methods. Eight SCD patients and five healthy controls were recruited and all subjects gave written informed consent. All experimental methods were approved by the IRB of the University of Minnesota. Functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) were simultaneously recorded while the subjects were in a wakeful resting state. EEG informed fMRI (EEG-fMRI) analysis using spontaneous power fluctuations and microstates was performed. A general linear model was used to calculate statistical activation maps from the EEG-fMRI analysis. Both power and microstate methods reflected that the healthy controls had default mode network (DMN) activity. DMN is active during the lack of a task and is one of the main resting state networks of

the brain. The alpha band was negatively correlated with DMN activity in healthy controls. SCD patients had reduced DMN activity compared to healthy controls; this was observed in both power and microstate results ($p<0.001$, uncorrected). The best activation of DMN negatively correlated with beta band for SCD patients. The results of this study suggest that DMN activity is a candidate for being a chronic pain biomarker and that EEG analysis can reflect DMN activity. An objective pain quantification method will be valuable for treating chronic pain objectively to maximize treatment outcomes and developing new analgesics. This work was supported in part by NIH grant U01-HL117664 and NSF IGERT grant DGE-1069104.

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Poster

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Support: Forest Laboratories (SAV-MD-09)

NIH Grant (K12-DE023574)

Title: Abnormalities in brainstem descending inhibitory structures could explain the deficient conditioned pain modulation commonly observed in fibromyalgia patients

Authors: *D. E. HARPER[1], E. ICHESCO[1], J. P. HAMPSON[1], D. J. CLAUW[1], T. SCHMIDT-WILCKE[2], R. E. HARRIS[1], S. E. HARTE[1];
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Abstract: Fibromyalgia (FM) is a chronic widespread pain disorder characterized by muscle tenderness, fatigue, poor sleep, and mood disturbance. Quantitative sensory testing has also identified deficient conditioned pain modulation (CPM) in this population. The present study used a combination of behavioral CPM testing with noxious pressure stimulation, voxel based morphometry (VBM), and resting state seed based functional connectivity (fcMRI) to identify structural and functional differences associated with CPM between FM patients (n=14) and age- and sex-matched healthy controls (HC, n=15). We performed a whole-brain VBM analysis using diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL), and resting

state connectivity analysis with the functional connectivity toolbox (v13; nitric.org/projects/conn), both in Statistical Parametric Mapping 8. Neuroimaging results were deemed significant at FWE p<.05 cluster-level corrected derived from a voxel-level threshold of p<.001. We observed significantly less gray matter volume in the periaqueductal gray (PAG) of FM patients [p<.001; small volume corrected] as compared to controls. This region was then used as a seed to determine whether the groups displayed different resting state fcMRI between this region and the rest of the brain. The degree of connectivity between the PAG seed and more caudal areas of the brainstem, including the locus coeruleus, was found to be significantly correlated with CPM magnitude in HCs [r=-.892, p=.007 FWE], with greater connectivity being related to more efficient CPM. In contrast, there was no significant relationship between PAG connectivity and CPM in FM patients, who as a group had significantly deficient CPM compared to controls [t(24)=-2.21,p=.037]. In summary, these results add to the growing literature on CPM deficiencies in FM, and they suggest that potential reasons for this abnormal endogenous pain modulation might be decreased gray matter volume in the antinociceptive PAG and altered connectivity of this structure to other descending pain modulatory regions.

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Poster

154. Pain Imaging and Perception

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NICT

Wellcome Trust

Title: Decoding pain from multimodal sensory stimuli in EEG

Authors: J. M. LANCASTER[1], H. MANO[2,1,3], D. CALLAN[2], *B. SEYMOUR[3,2,1];
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Abstract: Across neuroscience research, clinical diagnostics, and engineering applications in pain characterization and treatment, there is a desire for an objective measure of pain experience and detection when it occurs. This detector should be reliable across subjects in real-world settings using easily accessible, non-invasive data sources. We present a simple yet robust paradigm for decoding pain using neural and physiological data including electroencephalography (EEG), pulse, and skin conductance (GSR) measurements. The present study uses multivariate classification to distinguish painful events from non-painful multimodal sensory stimuli. In our experiment, pain was induced by cold stimulation which became noxious with prolonged exposure. Due to the long, ramp-and-hold nature of the stimulus, along with individual variability in sensitivity to pain, we suspected a lack of common time-locked events across participants. However, this format more closely resembles the experience of tonic pain conditions requiring intervention, which could be facilitated by the response of a decoding system. To classify the pain response and detect relevant data attributes, we employed a sparse logistic regression (SLR) machine learning protocol with automatic feature selection. EEG and physiological data is preprocessed and decomposed using independent component analysis (ICA). SLR is iterated using different high-performing feature vectors, including time series data in independent component space and power-frequency representations for each of the characteristic EEG activity bands. Classification performance exceeded 85% accuracy and selected between 5 and 15 features. These techniques allow us to address basic science questions by indicating which data characteristics most reliably identify pain events. Refining this approach will lead to the ability to objectively decode, detect, and quantify pain experience, as well as giving insight to important temporal, spectral, and spatial EEG events and physiological indicators of pain states. Success of a classifier protocol using these parameters should lead to the creation of a closed-loop system for decoding and intervention which can be applied in engineering and medical contexts.

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Poster

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Title: Fluctuations of pelvic pain intensity in men with urologic chronic pelvic pain syndrome (UCPPS) are modulated with successful treatment

Authors: *M. A. FARMER[1], D. DAVIS[2], A. J. SCHAEFFER[3], A. APKARIAN[4];
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Abstract: The pain experience of men with urologic chronic pelvic pain syndrome (UCPPS) remains poorly characterized. Whereas UCPPS severity has traditionally been assessed with a numeric rating scale, recent investigations have revealed that pelvic pain may manifest in one or more locations, each of which exhibit unique qualitative, spatial, and temporal qualities during basal conditions and periods of exacerbated pain (pain "flares"). As part of an ongoing double-blind randomized controlled trial to evaluate the impact of d-Cycloserine versus placebo on male pelvic pain, men were asked to rate their pelvic pain three times per day using a custom-developed smartphone application. The objectives of this report were to a) evaluate the spatial, qualitative, and time-varying properties of male pelvic pain prior to treatment; b) determine the impact of treatment response on pain properties; and c) investigate the interactions between pain intensity, urinary symptom severity, and mood over time. Men diagnosed with chronic prostatitis/CPPS and/or interstitial cystitis/bladder pain syndrome with moderate (>4/10) baseline pain intensity who met inclusion/exclusion criteria were consented and enrolled. Subject-wise daily pain intensity ratings (0-10 NRS) were extracted from the 3 week run-in period to characterize temporal variability and compared to rating vectors at 2, 3, and 4 months. Participants who exhibited a reduction > 20% from mean baseline intensity were classified as "treatment responders" and may include men taking d-Cycloserine or placebo (blind not broken during ongoing trial). Results indicate that men were > 90% compliant in providing three daily ratings; most men reported pain ≥ 2 locations, including testicles, perineum, urethra, bladder, prostate, anus, pelvic floor muscle, and nonspecific diffuse pelvic pain. At baseline, men bifurcated into two groups based on temporal properties of pain: a) a high pain variability group (with short periods of low or no pain) and b) a low pain variability group (stable mean pain intensity, pain always present). Following treatment these groups were conserved, yet treatment responders exhibited significant reduction in pain variability. Furthermore, pain reduction in treatment responders was evident across multiple sites of pain. These findings constitute the first longitudinal characterization of male pelvic pain intensity and establish that temporal variability

is a critical (and infrequently assessed) property of pelvic pain that is impacted by successful treatment of UCPPS. Future analyses will determine whether d-Cycloserine or placebo treatments differentially impact pain fluctuations.

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Poster

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Medical Research Council (MR/M006468/1)

Title: Non-invasive exploration of neurovascular coupling following acute noxious stimulation in the human term neonate

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Abstract: Aim: To explore the coupling between nociceptive electrophysiological and haemodynamic responses in the human infant brain. Background: Previous electroencephalography (EEG) studies [1] have demonstrated a specific pattern of cortical activity in term infants following noxious heel lance, and previous near-infrared spectroscopy (NIRS) studies [2] have shown a clear increase in total haemoglobin concentration following this stimulus. Here we have explored the relationship between these haemodynamic and electrophysiological responses using combined EEG and NIRS recordings in term neonates. Methods: Participants were 17 healthy term-born infants (8 females) studied at 0-13 days of age. Informed written parental consent was obtained prior to each study. The noxious stimulus was a clinically required routine heel lance. Brain activity was recorded with EEG electrodes placed according to the international 10:20 system and with a single NIRS emitter-detector pair centred over the primary somatosensory cortex (at C1 or C2, whichever was contralateral to the stimulation site). Ethical approval for this study was given by the UCLH ethics committee.

Results: It was possible to record artefact-free NIRS and EEG traces in most infants. Significant increases in oxyhaemoglobin concentration ([HbO2]) were observed in response to heel lance. EEG responses measured concurrently at the vertex (Cz) consisted of two waveforms, corresponding to the early sensory waveform and the later nociceptive specific waveform identified in previous work [1]. Of the 14 trials where both recordings were available, the EEG nociceptive specific waveform and NIRS HbO2 response were coupled in 9 cases (n=6, present in both; n=3 not present in both). In these trials there was a significant positive correlation between NIRS and EEG responses. By contrast, the EEG non-nociceptive specific waveform was coupled with the NIRS HbO2 response in only 5 of the 14 trials. Conclusion: Increases in oxyhaemoglobin concentration in the contralateral somatosensory cortex were more likely to be coupled with the nociceptive specific than with the non-nociceptive specific component of the electrophysiological response to tissue-breaking heel lances in term neonates. References: 1.Slater et al. (2010). Evoked potentials generated by noxious stimulation in the human infant brain. Eur J Pain, 14: 321-326. 2.Slater et al. (2006). Cortical pain responses in human infants. J Neurosci, 26: 3662-3666.

Disclosures: M. Verriotis: None. **L. Fabrizi:** None. **A. Lee:** None. **S. Ledwidge:** None. **J. Meek:** A. Employment/Salary (full or part-time):; University College London Hospital. **M. Fitzgerald:** None.

Poster

154. Pain Imaging and Perception

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 154.20/N38

Topic: D.08. Pain

Support: NCCAM AT007987-01A1

Title: Hippocampal volume is associated with placebo propensity in a blinded randomized clinical trial with chronic low back pain patients

Authors: *S. E. BERGER, E. VACHON-PRESSEAU, D. A. DAVIS, K. B. ZOSZAK, T. J. SCHNITZER, A. V. APKARIAN;
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Abstract: The placebo effect describes an improvement in symptoms caused by receiving an inert treatment disguised as an active treatment. It is a phenomenon rooted in underlying and identifiable neurobiology while simultaneously affected by psychological and social factors, and

it has been observed across a variety of systems, treatments, and conditions, including pain. The bulk of studies on placebo analgesia have been conducted in healthy, pain-free participants over a short period of time with acute stimuli. Thus, whether the placebo response differs in chronic pain populations and the extent to which is it repeatable and predictable remains unknown. The present research is part of a longitudinal trial investigating placebo propensity in individuals with chronic low back pain (CBP). The study lasted 8 weeks and had 6 visits, 4 with functional and structural magnetic resonance imaging. After a 2-week baseline monitoring period, participants were scanned and randomized into either a no-treatment (n=4) or treatment (placebo, n=20) group. There were two 14 day treatment sessions, each followed by a 1-week washout period to assess reliability and length of response. Behavioral measurements via questionnaires were collected at each visit, and pain and mood were monitored twice daily on a VAS scale using a smart phone app provided to participants for the study duration. Placebo response was defined as a 20% decrease in pain from baseline for at least 1 of the 2 treatment periods; using this criteria, n = 11 were classified as non-responders and n = 9 as responders. Responders experienced a decrease in reported pain ($p<0.001$) and a corresponding increase in reported mood ($p<0.05$) compared to non-responders, whose ratings were indistinguishable from those in no-treatment. Intriguingly, responders demonstrated a recall bias, remembering greater pain intensity than was reflected in their daily pain ratings ($p<0.05$), a finding not seen in non-responders. Subcortical structures were segmented for all participants at scan 1 (pre-treatment) and hippocampal volume was compared between groups; responders had a smaller left hippocampus than non-responders ($p =0.056$), with an AUC = 0.76. These initial results indicate that placebo response can be captured behaviorally in CBP and that hippocampal volume may be associated with propensity to respond to placebo in this patient population. This study is still active and expected to be completed by October 2015; thus by the conference, our sample size will greatly increase (no treatment, n = 20; treatment, n = 44) and additional analyses and results will be reported.

Disclosures: **S.E. Berger:** None. **E. Vachon-Presseau:** None. **D.A. Davis:** None. **K.B. Zoszak:** None. **T.J. Schnitzer:** None. **A.V. Apkarian:** None.

Poster

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Support: NIH Grant F32AT007800

Title: Functional magnetic resonance imaging of the spinal cord during thermal stimulation

Authors: *K. A. WEBER, Y. CHEN, X. WANG, T. KAHNT, T. B. PARRISH;
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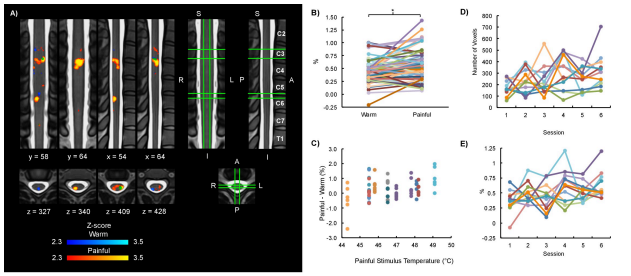
Abstract: Introduction Recent advancements have increased the potential use of fMRI to study neural processing in the spinal cord (SC). The purpose of this study was to expand on earlier work and demonstrate the feasibility of using fMRI to detect SC activity during thermal stimulation. Methods Twelve subjects were recruited. The cervical SC was imaged with a T2*-weighted sequence using ZOOMit selective field-of-view imaging on a 3T Siemens Prisma scanner (TR=2500 ms, TE=30 ms, resolution 1x1x3 mm^3) during thermal stimulation. For each session, 10 warm (43°C) and 10 painful (temperature producing 65/100 pain) 7.5 s thermal stimuli were applied to the right ventral forearm over 400 s, and each subject completed 6 sessions. The images were preprocessed, and statistical parametric maps were generated with the task vectors as explanatory variables and physiological noise vectors as covariates of no interest.[1,2] A fixed effects analysis was performed to generate average subject level activity maps. Spatial normalization was performed,[3] and average group activity maps were generated in a mixed-effects analysis. Results The extent of the activity exceeded that of a control analysis. Significant group activity was present for both the warm and painful stimuli (A). The signal change was greater for the painful stimulus than the warm stimulus and correlated to the painful stimulus temperature (B-C). The number of active voxels and signal change increased across the sessions for the painful stimulus, possibly due to sensitization (D-E). A trial-wise multi-voxel pattern analysis successfully classified the warm and painful stimuli based on activity patterns in the entire SC ($p<0.05$) and at the C3 level ($p<0.01$). Conclusions Both stimuli resulted in activity at the group and single-subject level. The activity was anatomically specific, and the signal change was proportional to the stimulus temperature. Moreover, SC fMRI may even have utility in studying phenomena such as sensitization. 1. Woolrich, MW. NeuroImage 14, 1370-1386, 2001 2. Brooks, JC. NeuroImage 39, 680-692, 2008 3. Cohen-Adad, J. OHBM 2014



A) Group average activity maps resulting from the warm (blue-light blue) and painful (red-yellow) thermal stimuli are shown. For the warm stimulus, the activity was more localized to the posterior hemicord, while the painful stimulus resulted in activity that extended across the anterior and posterior hemicords. The average activity maps were thresholded at a Z-score>2.3, which corresponds to a p<0.01 (uncorrected). The location of the sagittal, coronal, and axial slices on the standard spinal cord template with the corresponding vertebrae labeled is shown. B) The average signal change at the intersection of the subject level average activity maps for the warm and painful stimuli was calculated for each session. The percent signal change±1 standard deviation for the warm and painful stimuli was 0.44%±0.22% and 0.55%±0.29%, respectively. The painful stimulus resulted in a significantly greater signal change than the warm stimulus (paired two-tailed t-test, $t_5>0.001$). C) The signal change for the painful stimulus when baseline corrected for the warm stimulus signal change was significantly correlated to the painful stimulus temperature (Spearman's Rho=0.625, p<0.05). D-E) The total number of active voxels for each subject and the average signal change of the active voxels across the six sessions is shown for the painful stimulus. The number of active voxels and the average signal change significantly increased across the sessions for the painful stimulus, while no increase was present for the warm stimulus (repeated measures ANOVA, $p<0.01$ and $p<0.05$ for D and E, respectively). The increase in the number of active voxels and the average signal change may be due to peripheral or central sensitization from the repeated painful stimuli.

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Poster

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Topic: D.08. Pain

Title: Decreased functional brain connectivity during itch compared to pain

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Abstract: Histamine is well known as the prototypical itch mediator. If a smaller number of primary afferents fibers are activated by histamine stimuli the sensation is described as itch whereas activation of larger populations of afferent fibers activates the pain system. The aim of this study was to identify the cerebral networks with the help of functional imaging (fMRI). Since we expected a quite similar network during itch and pain stimuli the functional connectivity's within these networks were explored and compared. 18 healthy subjects were included in the study. They participated on two separated psychophysical pre-examination where pain and itch rating were recorded. Itch was applied by iontophoresis of histamine into the skin of the volar forearm. The recording started when the itch intensity passes 30% of the visual analogue scale (VAS, 0: no itch; 30: desire to scratch; 100: maximal conceivable itch). For pain the skin of the forearm was pretreated by topical application of capsaicin (0,05% for 30 minutes). Then two heat stimuli each 3 min with 5 minute break in between were applied to this site. This lead to a thermal hyperalgesia and heat pain could be induced by temperatures of less than 50 degree Celsius. The individual intensity was 1 degree above the pain threshold. Recording started when the pain passes 30% of the VAS. In two fMRI session itch and pain was assessed using a classical connectivity fMRI-design with EPI sequences on a 1.5 T Siemens Espree. The first run was without stimuli to detect the subject default mode network. During the second fMRI sequence the stimulus (itch or pain) was applied. The itching or painful sensation lasted during the whole fMRI. No earlier than 2 weeks the experiment was repeated with the other stimulus. Predefined anatomical regions (ROI) were used and the mean MRI time courses in these regions were extracted for each subject. These signals were z-transformed and Pearson's correlation coefficients (r) were calculated between the ROI for the periods baseline (B), itch (I) or pain (P) respectively. Nearly all connectivity values were higher during (P) than during (I). The highest r

values were found between S1 and S2 (BA 40) on both hemispheres (r>0.8, p0.6, p<0.00) between the areas insular cortex, anterior cingular cortex, amygdala for the painful stimuli. These findings support the “special contrast theory of itch” which assumes that itch could be induced by a focal but selective activation of a view sparsely distributed neurons innervating the skin. In contrast to that pain activates a larger population of skin afferents leading to a higher activity also in the CNS which facilitate synchronous activity between the affected brain regions.

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Poster

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Support: CIHR Grant MOP 10626

Title: Individual differences in temporal summation of pain reflect pro- and anti-nociceptive brain structure and function

Authors: *J. CHENG[1,2], N. ERPELDING[1], A. KUCYI[1,2], D. D. DESOUZA[1,2], K. D. DAVIS[1,3,2];
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Abstract: Introduction: Temporal summation of pain (TSP) is the perception of increasing pain evoked by repetitive noxious stimuli, and is highly variable between individuals. Individuals with facilitated pain processing and/or reduced pain-modulatory capabilities are characterized as pro-nociceptive, whereas individuals with reduced pain processing and/or facilitated pain-modulatory capacity are considered anti-nociceptive. Here, we tested the hypothesis that pro-nociceptive individuals have enhanced TSP compared to anti-nociceptive individuals, marked by facilitated ascending nociceptive processing and/or reduced capacity for descending pain modulation. **Methods**: 80 healthy subjects were tested with a TSP protocol as follows: 10 consecutive thermal stimuli were delivered to the left volar forearm at 0.5Hz, to a target temperature of 48°C at 10°C/s from a 32°C base. During each interstimulus interval (40°C) subjects rated pain from 0-100 (0 = no pain, 100 = worst pain imaginable). Subjects also underwent 3T imaging to collect a high resolution structural scan and a 5-min resting state fMRI

scan to measure functional connectivity (FC). Ascending nociceptive FC was determined between seeds in the right sensory thalamus and Brodmann Area 3a (thal-BA 3a), and correlated against TSP (significance: $P < 0.05$). In the descending pain-modulation system, a seed-to-voxel analysis (FSL's FEAT) with TSP as a regressor was performed between a seed in the RVM and a mask including the subgenual ACC (sgACC) (FWE-corrected $Z > 2.3$, cluster-based $P < 0.05$). **Results:** There were large inter-individual differences in TSP responses (% change from first to peak pain rating). Individual subject TSP was positively correlated with their thal-BA 3a FC, and with cortical thickness in the right insula and medial prefrontal cortex. In contrast, individual subject TSP was negatively correlated with RVM-sgACC FC. When subjects were grouped as pro- or anti-nociceptive based on whether they had greater thal-BA 3a or RVM-sgACC FC respectively, pro-nociceptive subjects showed greater TSP responses compared to anti-nociceptive individuals. Furthermore, TSP was positively correlated with the imbalance towards ascending nociceptive processing. **Conclusions:** We found that TSP in individual subjects reflects the balance of FC of their ascending nociceptive and descending pain-modulation pathways, with pro-nociceptive subjects demonstrating greater TSP than anti-nociceptive individuals. These findings provide insight into brain mechanisms that mediate central sensitization and a framework to examine chronic pain conditions.

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Poster

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DFG Grant PL 321/11-1

Title: Interactions between pain and motor processing in the human brain

Authors: M. POSTORINO, E. S. MAY, M. M. NICKEL, L. TIEMANN, *M. PLONER; TU Muenchen, Munich, Germany

Abstract: Pain is mostly conceptualized as a perceptual phenomenon. However, in order to be adaptive, pain not only includes a perceptual component but essentially depends on motor responses to avoid injury and promote recovery. The brain mechanisms of this motor component of pain are largely unknown yet. Here, we investigated how the preparation of an adaptive motor response functionally interacts with pain processing in the human brain. 20 healthy human subjects participated in an experiment, where thermal stimuli of increasing intensity were applied to the hand. Subjects were able to stop the painful stimulation by an adaptive motor response, i.e. by pressing a button. Button presses without pain, and pain without button presses served as control conditions. During the experiment, brain activity was recorded by using EEG. To test for the specificity of our results, a comparable control experiment with non-painful thermal stimuli was performed. The adaptive motor response was associated with reduced amplitude of the preparatory readiness potential as compared to a similar button press, which did not serve any protective function. Likewise, the adaptive motor response was associated with a weaker preparatory suppression of beta oscillations (14-30 Hz) over frontal premotor areas than the non-protective button press. Our results show that protective motor responses are associated with less preparatory brain activity than phenomenologically similar, but non-protective motor acts. This relative lack of voluntary motor preparation directly before an adaptive motor response indicates that motor preparation occurs involuntarily and continuously during pain. The results further suggest that motor preparation represents an inherent part of pain processing in the human brain.

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Poster

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Support: Office of Director, NIH. Grant number: 1DP2OD006469-01

Title: A Magnetoencephalography study of multi-modal processing of pain anticipation in primary sensory cortices

Authors: *R. GOPALAKRISHNAN[1], R. C. BURGESS[2], A. G. MACHADO[1];
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Abstract: Pain anticipation plays a critical role in pain chronification and results in disability due to pain avoidance. It is important to understand how different sensory modalities (auditory, visual or tactile) may influence pain anticipation, as different strategies could be applied to mitigate anticipatory phenomena and chronification. In this study, using a countdown paradigm, we evaluated with magnetoencephalography, the neural networks associated with pain anticipation elicited by different sensory modalities in normal volunteers. Testing was performed with 10 adult healthy controls seated upright in a 306 channel MEG array (Elekta AB). Conditioning stimulus were presented using visual, tactile and auditory modalities which uniquely signaled the imminent arrival of an unconditioned stimulus that was painful (PS), non-painful (NPS) or absence of stimulus (NOS). The paradigm consisted of 15 blocks of 63 pseudo-randomized trials consisting of conditions from all three modalities. Each trial had 1s of baseline, 2s of pre-stimulus anticipatory countdown and 5s post-stimulus period. The data were pre-processed to remove artifacts, trials parsed and band-pass filtered to 1-100 Hz. Trials were averaged to compute evoked activity. Min-norm source estimation was performed on a tessellated cortex extracted from MRI that was anatomically parcellated to ROIs. The average time series from ROIs was subjected to a wavelet analysis. Statistics was performed using cluster based permutation analysis to compare PS vs. NOS and PS vs. NPS within each modality. Time-frequency analysis was focused on beta (12 - 30 Hz) and gamma (30 - 90 Hz) bands because of their association with cognitive functions and related sensorimotor transformations during pain anticipation. Our results showed that the visual modality evoked significant gamma band oscillations during PS in V1 indicative of independent processing of contextual meaning of the cue after interacting with associative areas. Both tactile and auditory modalities engaged the associative areas during PS, however they did not present independent cue processing capabilities. Visual and tactile modalities evoked anticipation early in the countdown, whereas auditory cortex displayed delayed processing. Interestingly, cross-modal activation was also evident and strong when visual and tactile cues signaled upcoming pain. Dorsolateral prefrontal cortex and mid-cingulate cortex showed significant activity during PS regardless of modality. Our results show the highest degree of higher-order processing is modulated by context (pain) rather than content (modality) and rests within the associative limbic regions.

Disclosures: R. Gopalakrishnan: None. **R.C. Burgess:** None. **A.G. Machado:** 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.; Office of Director, NIH. Grant number: 1DP2OD006469-01. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); intellectual property and distribution rights in Enspire, ATI and Cardionomics. F. Consulting Fees (e.g., advisory boards); consultant to Spinal Modulation and Functional Neuromodulation. Other; Fellowship support from Medtronic.

Poster

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R21NS087472

Title: Detection of brain glial activation in chronic pain using [11C]PBR28 positron emission tomography imaging: occipital cortex as a pseudo-reference region

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Abstract: Using [[11]C]PBR28 PET, our group recently provided novel evidence that patients with chronic low back pain (cLBP) exhibit elevated brain levels of the translocator protein (TSPO) (Loggia et al., Brain 2015). As TSPO is upregulated in activated microglia and reactive astrocytes, this observation supports a role for activated glial cells in human pain conditions. For more widespread use of TSPO neuroimaging, it is preferable to work with simpler, and in some cases, more reliable SUVR analyses in lieu of traditional plasma kinetic analyses. In our previous analyses, we obtained standardized uptake value ratio (SUVR) values by normalizing SUV to whole brain. This method accounted for global signal differences across subjects, such as those introduced by the Ala147Thr *TSPO* polymorphism, which affects binding affinity. However, whole-brain normalization may reduce sensitivity to detect extended areas of signal increase throughout the brain. Thus, we evaluated pseudo-reference regions for SUVR. Nine cLBP patients and 9 age, sex, and *TSPO* genotype-matched controls were included in these analyses.

Integrated PET/MRI scanning began with IV injection of 11.1 ± 0.6 mCi [^{11}C]PBR28, and continued for 90 minutes. MPRAGE MRI scans were acquired for attenuation correction. 60-90 minute SUV images were reconstructed for each subject. SUV images were transformed to MNI space, spatially smoothed, and divided by average SUV from one of three pre-defined pseudo-reference regions: whole-brain, occipital lobe, and cerebellum, to create SUVR data. Region of interest (ROI) analyses and ROC curves characterized the ability of each pseudo-reference region to discriminate groups. In addition, voxelwise group analyses were performed to generate test statistic maps corresponding to these three regions. Occipital-normalized ROI SUVRs demonstrated the greatest ability to detect between-group differences, followed by whole-brain and cerebellum, respectively. The ROC curve was optimal for the occipital lobe normalization with AUROC=0.98, 0.89, and 0.77 for occipital lobe, whole brain, and cerebellum, respectively. In voxel-wise analyses, the use of occipital lobe as a pseudo-reference region confirmed the initial whole-brain results, and identified several additional regions of glial activation in cLBP patients, including bilateral posterior insulae, anterior cingulate cortex, and striatum. In our current sample, compared to whole-brain normalized SUVR analyses, the occipital lobe appears to be a viable pseudo-reference region for quantification of [^{11}C]PBR28 SUVR, and led to detection of glial activation in additional areas implicated in pain.

Disclosures: **D.S. Albrecht:** None. **S. Shcherbinin:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **A.J. Schwarz:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **D.B. Chonde:** None. **O. Akeju:** None. **C. Catana:** None. **R.R. Edwards:** None. **D. Izquierdo-Garcia:** None. **R. Ji:** None. **A.D. Wasan:** None. **N.R. Zurcher:** None. **B.R. Rosen:** None. **V. Napadow:** None. **V.N. Barth:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **J.M. Hooker:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **M.L. Loggia:** None.

Poster

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Title: Prestimulus EEG markers of subjective pain perception

Authors: ***P. TAESLER**, M. ROSE;
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Abstract: The perception of pain is strongly influenced by cognitive processes such as, for example, expectations toward the efficacy of pain medication . Such processes have been shown to significantly modulate the analgesic effects of opiates through a descending pain modulatory network. A possible correlate for this effect could be present in neural pre-stimulus activity. We utilized state of the art psychophysical methods to contrast pain perception with non-painful sensations at the threshold level without changes in stimulus intensity in an EEG paradigm. This way, the observed fluctuations in intensity ratings could directly be linked to oscillatory activity in the pre-stimulus time range. We observed strong differences at the contralateral Insula in theta-band (4-7Hz) co-varying with subjective pain perception, as well as more pronounced lateralization in fronto-lateral connectivity (theta band). A decrease in fronto-parietal connectivity for painful sensations was located in the low gamma band (39-42 Hz). These findings can help in developing an understanding of subjective pain sensitivity - a quantity closely related to effects such as placebo/nocebo and a potential modulator of drug efficacy.

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Poster

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DFG CEDER

Emerging Field Initiative FAU Erlangen-Nürnberg

Title: BOLD fMRI and it's predictive value for pain treatment success in inflammatory diseases

Authors: ***A. HESS**[1], J. RECH[2], R. ATREYA[3], A. DOERFLER[4], M. NEURATH[3], G. SCHETT[2];
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Abstract: Pain is the key symptom in patients with inflammatory diseases like rheumatoid arthritis (RA) or Morbus Crohn (MC). It is well known, that TNF-alpha is the primary molecule in these pathological processes. However the role of TNF-alpha for the pain perception remains unclear. Therefore, we hypothesized that the hypernociception due to chronic TNF

overexpression leads to an altered pain processing in the brain which should be true for different inflammatory diseases depending on TNF-alpha. Consequently we investigated patients suffering RA as well as from MC using standard BOLD fMRI and disease specific painful stimuli. For both diseases after treatment with an anti-TNF drug (Infliximab) a reduction of the hypernociception, i.e. reduced activated brain volume, in brain areas activated by the painful stimulation was found. For RA and MC this happened as fast as 24 h after the first drug application which is weeks before any established clinical score indicates an improvement. Most interestingly was the finding that by our fMRI approach we were able to retrospectively differentiate between therapy responders and non-responders, again for both diseases. In order to further validate, if this predictive value of fMRI for indicating treatment success was limited to the anti-TNF tested so far (Infliximab) we investigated the anti-TNF Certolizumab pegol in RA patients. Likewise for Certolizumab fMRI was able to demonstrate a fast but long lasting reduction of pain induced activity in the brain of the patients. Further graph theoretical connectivity analysis data showed rewiring within the pain matrix under chronic pain conditions i.e. tight clustering of brain activity in thalamus and periaqueductal grey. Neutralization TNF by antibodies rapidly reversed this hypernociception. This was reflected by an overall decrease of the functional activity in the brain pain matrix and by dissociation of the tight clustering. These dynamic changes in the brain happened long before anti-inflammatory effects were evident. Our results suggest profound functional changes of nociceptive brain activity in inflammatory diseases like RA or MC, which normalizes upon hTNF blockade very fast and strongly contributes to the well-being of the patients.

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Poster

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Topic: D.08. Pain

Support: Craig H. Neilsen Foundation

Title: Changes in pain processing in the spinal cord and brainstem after traumatic spinal cord injury characterized by means of functional MRI

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Abstract: Traumatic spinal cord injury (SCI) has a number of devastating consequences including high prevalence of chronic pain. The causes of pain vary depending on the injury, and are difficult to diagnose and treat. A better understanding of pain mechanisms after SCI is expected to lead to better diagnostic capabilities and improved treatments. The purpose of this study was to use functional magnetic resonance imaging (fMRI) to characterize responses in the brainstem and spinal cord to the same heat stimulus in a group of people who have previously had a traumatic spinal cord injury (SCI), in order to further our understanding of post-injury pain conditions. Functional MRI of the brainstem and spinal cord was carried out at 3 tesla and was used to determine the neuronal activity evoked by a series of repeated brief heat pulses at 49 °C applied to the right hand. A group of 16 participants was studied with a range of cervical and upper thoracic injuries, and participants provided ratings of the pain perceived from the thermal stimulation. Blood oxygenation-level dependent (BOLD) responses were detected with a General Linear Model (GLM) and effective connectivity was examined with Structural Equation Modeling (SEM). The pain perceived varied widely across participants from 0 to 52, out of 100, with a mean value of 25 ± 16, compared to a mean rating of 42 ± 18 in healthy participants. Functional MRI results were also observed to be highly variable in terms of location and magnitude of responses. However, the results were observed to vary in relation to the perceived pain and the level/severity of injuries, particularly in terms of hypothalamus connectivity with other regions, and descending modulation via the PAG-RVM-cord pathway. The results appear to provide sensitive indicators of each individual's pain response. We conclude that the results of this study demonstrate mechanisms of altered pain processing in the 16 participants that were studied. The ability to characterize changes in pain processing in individuals with SCI represents a significant technological advance.

Disclosures: P.W. Stroman: None. **H.S. Khan:** None. **D. Cadotte:** None. **R.L. Bosma:** None. **A. Cotoi:** None. **R. Leung:** None. **M.G. Fehlings:** None.

Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 155.01/N48

Topic: D.08. Pain

Support: Eli Lilly IIT F1J-US-X061

NCCAM RO1 AT007987-01A1

Title: Resting brain activity predicts placebo response in randomized clinical trials of knee osteoarthritis pain

Authors: *P. TÉTREAULT[1], A. MANSOUR[1], E. VACHON-PRESSEAU[1], T. SCHNITZER[2], A. APKARIAN[1], M. BALIKI[1];
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Abstract: Background and aims: Placebo response is identified to be mainly driven by central nervous system mechanisms, and it is thus reasonable to expect that specific brain states or traits can predispose individuals to respond to placebo treatment. Resting state functional magnetic resonance imaging (rs-fMRI) may provide such brain markers for placebo propensity. However, in a clinical context, no functional brain properties have yet been recognized that are able to identify and predict placebo responses. Methods: We studied placebo related brain functional connectivity (FC) in 2 separate clinical trials using rs-fMRI in knee osteoarthritis (OA) patients. Study 1 was used to identify brain circuits for placebo propensity, and study 2 to validate outcomes. Study 1 was a single blinded trial, where 17 OA patients underwent rs-fMRI before receiving placebo treatment for two weeks. Study 2 was a double-blinded trial, where 39 OA patients underwent rs-fMRI and then received either placebo or duloxetine for 3 months. Results: We prospectively defined an analgesic response as a 20% or greater decrease in pain. In both studies, approximately half of participants displayed placebo analgesia, exhibiting about 50% decrease in OA pain on average. The extent of right middle frontal gyrus (r-MFG) FC with the rest of the brain differentiated placebo responders and non-responders and predicted magnitude of placebo analgesia, and this area preferentially connected to anterior insula and periaqueductal gray. The density of FC of r-MFG discriminated placebo responders from non-responders in study 2 with an AUC of 0,92 (95% confidence interval, 0.78 to 1.1), but did not discriminate duloxetine responders from non-responders, implying that the latter response is not fully a placebo effect. Conclusion: This study suggests that propensity of placebo response to a treatment is embedded in functional properties of patients and represents a unique brain characteristic that may be a useful biomarker in targeted treatment approaches. Additionally, with functional brain properties, we were able to dissociate placebo response from a drug response of equal magnitude that was indistinguishable with behavioral outcome.

Disclosures: P. Tétreault: None. **A. Mansour:** None. **E. Vachon-Presseau:** None. **T. Schnitzer:** None. **A. Apkarian:** None. **M. Baliki:** None.

Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: NCCIH R01-AT004714

NCCIH R01-AT004714-02S1

NCCIH P01-AT002048

KIOM K15050

Title: Structural plasticity in primary somatosensory/motor cortex in carpal tunnel syndrome is improved following acupuncture

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Abstract: We have previously demonstrated maladaptive structural and functional neuroplasticity in primary sensorimotor (S1/M1) cortices for carpal tunnel syndrome (CTS). Previous clinical studies have suggested that acupuncture may improve both subjective and objective (nerve conduction latency) outcomes for CTS patients, the mechanisms are unknown. We investigated gray matter volume (GMV) changes following acupuncture in bihemispheric S1/M1 areas consistent with the cortical representations of median nerve innervated digits. White matter (WM) microstructural changes in the tracts between these regions were also investigated. We enrolled 60 CTS patients and 34 controls. CTS patients were randomized to a 2-month course of either local (n=21), distal (n=18), or sham (n=21) acupuncture. T1-weighted MPRAGE structural brain images were used to quantify GMV for S1/M1 ROIs defined by fMRI evaluation of brain response to vibrotactile stimulation for median nerve innervated digits from the more affected hand. ROIs were defined from contralesional S1 activation and ipsilesional S1/M1 deactivation clusters. DTI was performed to assess mean FA and MD in WM tracts obtained from probabilistic tractography. Clinical evaluation included nerve conduction studies and symptom/functional scales from the Boston CTS Questionnaire (BCTSQ). All acupuncture

interventions significantly improved symptom and function BCTSQ scales (P<0.05). As no differences were noted between local and distal acupuncture, these groups were combined into a single "verum" acupuncture group. Verum acupuncture improved nerve conduction latency more significantly than sham (P=0.04). At baseline, contralesional S1 GMV was reduced in CTS (P=0.01), consistent with our previous studies. GMV was also increased in ipsilesional M1 (P=0.03). Verum acupuncture reduced ipsilesional M1 volume more than sham (P=0.02). At baseline, mean diffusivity in WM tracts connecting contralesional S1 to ipsilesional M1 were reduced in CTS (P<0.05). Verum acupuncture increased mean FA in these tracts than sham acupuncture (P=0.03). Verum acupuncture improved both subjective (symptoms) and objective (peripheral nerve conduction) outcomes in CTS, which provided greater somatosensory afference compared to sham acupuncture, also improved structural brain plasticity in ipsilesional M1, and altered white matter microstructure in tracts connecting this region with contralesional S1. As GMV in the latter region was not altered immediately following acupuncture, future studies should explore longer-term changes and linkages with clinical outcomes.

Disclosures: H. Kim: None. **Y. Maeda:** None. **N. Kettner:** None. **J. Lee:** None. **J. Kim:** None. **S. Cina:** None. **C. Malatesta:** None. **J. Gerber:** None. **C. McManus:** None. **P. Mezzacappa:** None. **R. Ong-Sutherland:** None. **A. Libby:** None. **L.R. Morse:** None. **K. Park:** None. **J. Audette:** None. **V. Napadow:** None.

Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

Location: Hall A

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Program#/Poster#: 155.03/O2

Topic: D.08. Pain

Support: CIHR support for author RB

Pfizer Pain Research Grant

Title: Evidence of alterations to cerebral-midbrain-spinal mechanisms of pain control in Fibromyalgia

Authors: *R. BOSMA[1], E. AMELI MOJARAD[1], L. LEUNG[1], C. PUKALL[1], R. STAUD[2], P. STROMAN[1];
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Abstract: Fibromyalgia syndrome (FM) is a prevalent debilitating chronic pain condition, which afflicts primarily females. Although the etiology of this disease is not completely understood, FM pain is believed to be due to enhanced pain sensitivity maintained by central mechanisms. Central pain amplification can be evaluated by assessment of the temporal summation of second pain (TSSP). Here we use a TSSP paradigm and functional MRI (fMRI) to build upon previous brain fMRI studies and investigate the expected site of central sensitization in the spinal cord, and modulation from brainstem regions. Functional MRI of the brain, brainstem, and spinal cord, of pain-free female adults (NC) (N = 15) and FM patients (N = 14) was conducted while TSSP (0.33 Hz) and Control (0.17 Hz) heat pain paradigms were applied to the right hand. The stimulus intensity was adjusted to each participant's heat pain sensitivity. Data were analyzed by means of a General Linear Model and Structural Equation Modeling. As predicted, all participants demonstrated significant behavioral summation of pain in the TSSP condition. In FM patients compared to NC, fMRI responses to sensitivity calibrated pain stimuli identified similar brain activity; however, there were multiple areas in the brainstem (RVM and PAG) and spinal cord (DH) that showed greater activity. Altered effective connectivity differences between descending control regions was also detected in the FM group. Finally, increased after-sensations and enhanced dorsal horn activity was demonstrated upon cessation of the heat pain stimuli. In conclusion, our results suggest that the descending control mechanisms are altered in fibromyalgia and provide strong evidence for the involvement of central neural mechanisms in modulating fibromyalgia pain.

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Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

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Program#/Poster#: 155.04/O3

Topic: D.08. Pain

Support: NIMH Grant R21-MH103468

Title: Trigeminal sensory nucleus connectivity is modulated by respiratory-gated auricular vagus nerve stimulation in migraine patients

Authors: *R. G. GARCIA[1,2], R. LIN[3], J. LEE[3], H. JUNG[3], J. KIM[3], M. LOGGIA[3,4], A. D. WASAN[5], R. EDWARDS[4], N. HADJIKHANI[3], V. NAPADOW[3,4];

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Abstract: Objective Migraine is a primary brain dysfunction associated with increased sensitization of the trigeminal pain pathway. Respiratory-gated auricular vagal afferent nerve stimulation (RAVANS), an optimized version of transcutaneous vagus nerve stimulation, involves electrical stimulation at specific points of the respiratory cycle. While RAVANS has demonstrated improved outcomes in chronic pain patients, its mechanisms of action are unknown. Our aim was to use functional MRI (fMRI) to evaluate brain response to RAVANS and potential modulation of functional connectivity between trigeminal sensory nuclei (sp5) and the cortex in migraine patients. Methods Sixteen subjects with episodic migraine (interictal) and 16 age-matched healthy controls were randomized to receive RAVANS or sham stimulation during a 6 minute fMRI scan, with cross-over stimulation following 30 minutes. Electrodes were placed in vagal-innervated ear regions. RAVANS stimulation at 30Hz (0.5s duration) and gated to exhalation was delivered with current intensity set to achieve moderate (but not painful) sensation. FMRI was performed at 3T (TR/TE=2.5s/30ms, 43 slices, voxel size=2.6x2.6x2.6mm). Functional connectivity was computed using seed-based connectivity analysis for a nucleus tractus solitarius/sp5 ROI defined based on the peak activation voxel (-8,-38,-42 in MNI coordinates) from a permutation-based analysis contrasting RAVANS and sham stimulation. All group-level brain maps were cluster corrected for multiple comparisons ($Z>2.3$, cluster-size threshold=$p<0.05$). Repeated-measures ANOVA models were used for post-hoc ROIs, significant at $p<0.05$. Results During RAVANS, NTS/Sp5 connectivity was found to a network of brain regions, including anterior insula, putamen, midbrain, and thalamus. A significant INTERVENTION effect (RAVANS > sham) was found for NTS/Sp5 connectivity to left (peak voxel: -30,18,-6 in MNI coordinates; $F=27.51$, $p<0.001$) and right anterior insula (peak voxel: 44,12,-6 in MNI coordinates; $F=11.98$, $p<0.01$), and putamen (peak voxel: -30,0,2 in MNI coordinates; $F=9.32$, $p<0.01$). Significant INTERVENTION by GROUP interactions were found for left anterior insula ($F=4.52$, $p=0.04$) and putamen ($F=4.6$, $p=0.04$), with post-hoc analyses showing a significant increase in connectivity in migraine patients but not in controls. Conclusion RAVANS effectively activates NTS/Sp5 in the ipsilateral pontomedullary region of the brainstem. Regulation of NTS/Sp5 connectivity to known pain-modulatory brain regions may constitute an underlying mechanism supporting previously reported analgesic effects of RAVANS therapy.

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Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

Location: Hall A

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Program#/Poster#: 155.05/O4

Topic: D.08. Pain

Support: NIH 5R01DA035484

Title: Pain specific encoding of temporal dynamics of aversive stimuli in the ventral striatum

Authors: *S. GEUTER, E. A. REYNOLDS LOSIN, T. D. WAGER;
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Abstract: The nucleus accumbens and putamen have been shown to encode appetitive events and appetitive prediction errors (more reward than expected) in numerous studies. Recent studies in animals and humans suggest that the striatum may also respond to painful stimuli and aversive prediction errors (more aversive outcome than expected), though human findings have been inconsistent. Several functional magnetic resonance imaging (fMRI) studies in humans and animals have revealed phasic responses in the nucleus accumbens to pain onset and offset, but the sign of these responses (activation vs. deactivation) varies across studies. Activation to pain onset could be related to motivational properties of pain, and activation to offset could be related to the rewarding aspects of pain relief. Here, we investigated phasic responses to aversive stimuli (thermal pain and aversive sounds) in the basal ganglia and their functional significance in a large fMRI data set (N=60). Participants experienced painful heat and aversive sounds of varying durations and intensities. We observed phasic positive responses to pain onset in the putamen and caudate nucleus. Caudate nucleus also responded to cues that signaled upcoming painful stimulation. At stimulus offset, caudate, putamen and nucleus accumbens showed deactivation; we found no activity increases related to pain relief in the basal ganglia. These activity changes were strongly predictive of pain intensity ratings in a cross-validated multivariate regression analysis across individuals. Deactivation to pain offset in the basal ganglia explained 25% of the variance in pain ratings. To test for the functional significance of these bi-phasic responses, we regressed BOLD activity against the temporal derivative of ongoing pain. We found that caudate, putamen, and accumbens encoded the change in perceived pain and that these regions overlapped with the regions showing the bi-phasic responses. Importantly, accumbens and ventral caudate responses were specific for pain as the relationship was significantly stronger for changes in pain compared to aversive sounds. The positive responses to pain onset together with increased activity during periods of increasing pain and decreased activity with ongoing relief suggest that

the ventral striatum not only encodes the motivational significance of appetitive stimuli, but also the motivational demands imposed by pain and relief of pain along a single, bipolar dimension.

Disclosures: S. Geuter: None. **E.A. Reynolds Losin:** None. **T.D. Wager:** None.

Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 155.06/O5

Topic: D.08. Pain

Support: Canada Research Chair Program

Title: Cognitive behavioral therapy changes pain-evoked activity and intrinsic connectivity of the default mode network

Authors: A. KUCYI[1,2], T. V. SALOMONS[1,3], *K. D. DAVIS[1,4];
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Abstract: **Introduction:** We recently demonstrated that cognitive training modifies pain unpleasantness and secondary hyperalgesia (Salomons et al, 2014). A key question is how such training alters neural pain responses and network connectivity. We examined how cognitive behavioral therapy (CBT) impacts painful stimulus-evoked neural activity and changes intrinsic brain-network organization. **Methods:** Healthy subjects were assigned to a CBT (n=17) or inert treatment (Control) (n=13) group and underwent 8 experimental sessions of treatment and painful thermal stimuli. Before and after treatment, participants were scanned at 3T during painful stimulation (percept-matched) and at rest. Painful stimulus-evoked activity was analyzed both within- and between-groups. Data were preprocessed with FSL and Matlab as done previously (Kucyi et al, 2013). A region in right ventrolateral prefrontal cortex (R vlPFC) of the executive control network, that displayed group differences in pain responses was selected as a seed region for resting state functional connectivity analyses. Resting state and stimulus-evoked activity were examined using FSL, with significance set at family-wise error corrected (whole brain, $Z>2.3$, $p<0.05$). **Results:** In the Control group, repeated painful stimulation over time attenuated normal default mode network (DMN) deactivation during pain. This effect was

blocked by CBT, accompanied by a relative decrease in painful stimulation-evoked R vlPFC activation. Greater maintenance of DMN deactivation in the CBT group was associated with greater reduction in pain intensity and unpleasantness. Finally, the CBT group showed enhanced intrinsic functional connectivity between the executive control (based on a seed in the R vlPFC) and default mode medial prefrontal cortex) networks over time, compared to the Control group. **Conclusion:** This work highlights that the neural representation of pain is dynamic, in line with the concept of a "dynamic pain connectome" (Kucyi and Davis, 2015). Specifically, we show that cognitive training can impact behaviourally-relevant reorganization of neural responses to painful stimuli and intrinsic network communication. These findings point to the potential utility of cognitive training in pain therapy and also reveal a mechanism underlying its effect based on altering DMN activity and connectivity with the executive control network.

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Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 155.07/O6

Topic: D.08. Pain

Title: Neural and behavioral correlates to pain perception in nerve growth factor beta (NGFB) mutation carriers

Authors: *I. PERINI[1], M. TAVAKOLI[2], A. MARSHALL[3], J. MINDE[4], I. MORRISON[1]; [1]Dept. of Clin. and Exptl. Medicine, IKE, Linkoping, Sweden; [2]Ctr. for Endocrinol. & Diabetes, Inst. of Human Development, Univ. of Manchester;, MAnchester, United Kingdom; [3]Greater Manchester Neurosci. Centre, Salford Royal Hosp. NHS Trust, Stott Lane, Salford M6 8HD, Manchester, United Kingdom; [4]Dept. of Orthopaedics, Gällivare Hospital, Gällivare, Sweden, Gällivare, Sweden

Abstract: Implementing an adequate behavioral response to a noxious event is a crucial component of pain perception. Central mechanisms are important in forecasting whether a nociceptive input mediate a real threat for tissue damage. However, what happens when the nociceptive input is not efficient? We investigated a population of carriers of a rare mutation affecting the nerve growth factor beta (NGFB) gene, which causes a loss of unmyelinated C afferents. The carriers show different levels of impaired pain sensitivity although their thresholds

don’t differ from controls. To characterize the components of the carriers’ pain experience we designed a study that involved cerebral, psychophysical and peripheral measurements. At the brain level we investigated functional brain correlates to an action-based task that involved thermal stimulation as in Perini et al. 2013. This design allowed modeling of voluntary motor responses and painful stimulation separately. Using signal detection theory we calculated their sensitivity to the behavioral task (d'), that is their ability to distinguish painful from non-painful stimulations. At the behavioral level we looked at the carriers’ pain evaluation via the administration of the Situational Pain Questionnaire (SPQ) and their motivation to react to acute pain using a design in which they continuously rated their urgency to move away from a painful stimulation. To characterize the peripheral loss we carried out a quantification of the fiber density using corneal confocal microscopy (CCM), a non-invasive method that reliably detects and quantifies C-fibre loss in neuropathy. Carriers showed lower sensitivity to the task (d') and rated their urgency to move significantly lower than controls. Right AI and right inferior frontal gyrus were positively correlated to the carriers' sensitivity to the task (d') suggesting an involvement of those areas in discriminating painful versus non-painful stimulation. The mid cingulate cortex (MCC) was activated for both painful stimulation and motor response (button press) confirming previous finding on the action-related role of MCC during pain. In addition it was positively correlated to the urgency to react to painful stimulation during the response interval. Finally peripheral C-afferent loss correlated negatively with pain intensity evaluation, revealed by SPQ scores. In this study we show that NGFB mutation carriers have a lower ability to evaluate and react to pain that correlates to the extent of the loss of nociceptors. We show that the right AI has a role in the discrimination between different stimulation qualities, whereas the cingulate has a more action-related role during pain.

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Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 155.08/O7

Topic: D.08. Pain

Title: Intrinsic functional connectivity predicts task evoked responses and pain modulation in an experimental model of placebo analgesia

Authors: *J. A. HASHMI[1,2], R. YU[3], S. KHAN[3], R. L. GOLLUB[3], J. KONG[3];
[1]Harvard Med. Sch., Belmont, MA; [2]MGH, Boston, MA; [3]Harvard Med. School, MGH, Boston, MA

Abstract: Introduction: Placebo analgesia is a type of pain modulation mediated by the brain where pain intensity is reduced due to positive expectations engendered by the procedure of undergoing treatment. Several brain imaging studies have clearly shown task related activation during experimental pain modulation suggesting a role of the brain in placebo analgesia. We have shown that placebo analgesia can be predicted by mean local efficiency and clustering in resting state networks measured with functional MRI before treatment (Hashmi et al., 2013). Why local connectivity parameters predict placebo analgesia is not clear. Since greater network clustering represents higher number of local connections and more efficient information transfer within a network, we hypothesized that the regions that show a predictive relation between clustering and pain modulation are components of a local network that plays an important role in processing pain modulation. Methods: We investigated the relation between resting state network architecture and task related responses in an experimental placebo paradigm. Healthy subjects (n=43) were given two types of visual text cues that created expectancies for incoming heat pain stimuli. Cues that read either “high pain” or “low pain” preceded high or low intensity stimuli after which the subjects rated pain intensity. In some epochs, the engendered expectancies were tested by presenting high intensity stimuli after cues that induced expectations of low pain (placebo epochs) (Kong et al., 2013). We used Graph theory to identify connectivity patterns in resting state scans and BOLD signal dynamics in network nodes to observe activation patterns in task related scans. Networks were constructed at 5 different thresholds from a 131 ROI parcellation. Results: Network efficiency of regions in six medial prefrontal regions measured in resting state scans predicted the placebo analgesia induced during subsequently measured placebo task ($p<0.05$). The nodes that showed significant cue, pain, rating and placebo analgesia related activations (FDR corrected) showed 75% overlap with regions that were members of local networks of regions identified in the resting state scan. Conclusions: Here we show that greater clustering in resting state architecture predicts individual variability in placebo analgesia. The regions that most prominently show this affect are components of a local network that plays an important role in pain modulation. Thus, we confirm our previous findings and suggest a putative mechanism that explains the predictive relation between local functional connectivity and placebo analgesia.

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Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

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Program#/Poster#: 155.09/O8

Topic: D.08. Pain

Support: NIH NIDCR R01 DE022746

Title: The indirect pathway of nucleus accumbens shell amplifies tactile allodynia in rodent pain model

Authors: *W. REN, M. V. CENTENO, D. J. SURMEIER, M. MARTINA, A. V. APKARIAN; Dept. of Physiol., Northwestern Univ., Chicago, IL

Abstract: The forebrain limbic circuitry has long been hypothesized to play a critical role in the representation of pain, and the medial shell of the nucleus accumbens (msNAc) is a key node in this circuitry. msNAc has two parallel, opposing networks charged with affective evaluation of salient events. The direct pathway, which is linked to reward and positive affect, is anchored by spiny projection neurons (dSPNs) whose activity is enhanced by dopamine (DA) acting at D1 DA receptors. The indirect pathway, which is linked to aversion and negative affect, is anchored by spiny projection neurons (iSPNs) whose activity is suppressed by DA acting at D2 DA receptors. To investigate how these two networks respond to chronic pain, the electrophysiological adaptations in msNAc neurons were examined in a rodent peripheral nerve injury (spared nerve injury, SNI) model of chronic pain. Five days after the SNI (or sham) surgery, mice were tested for tactile allodynia and sacrificed to obtain msNAc brain slices for patch clamp recording. In iSPNs from SNI mice, the intrinsic excitability assessed by intrasomatic current injection was significantly increased both ipsilateral and contralateral to the nerve injury; moreover, rheobase current and first spike latency decreased, whereas input resistance increased. In contrast, early-SNI had no effect on neighboring dSPNs. To determine whether the cell type-specific adaptations in the nucleus accumbens have a causal correlation with neuropathic pain behavior, msNAc iSPNs were activated *in vivo* using a chemogenetic approach. Adora2-Cre mice had bilaterally stereotaxic msNAc injections of adenoassociated virus carrying Cre-dependent PSAML141F,Y115F-5HT3 HC/green fluorescent protein expression construct. In brain slices from infected mice, the PSAM ligand - PSEM89S - induced a reversible depolarization of iSPNs that led to increased spiking. 3 days post SNI surgery, treatment of PSAM expressing SNI mice with an intraperitoneal injection of PSEM89S (30 mg/kg) rapidly led to an exacerbation of tactile allodynia. The PSEM-induced decrease in tactile threshold was repeated and more significant at Day 5 post SNI surgery. However, PSEM89S had no effect on tactile threshold in sham animals. Our data suggested that SNI-induced adaptation of msNAc iSPNs drive descending segmental pathways that enhance the reactivity of segmental circuitry controlling withdrawal reflexes.

Disclosures: **W. Ren:** None. **M.V. Centeno:** None. **D.J. Surmeier:** None. **M. Martina:** None. **A.V. Apkarian:** None.

Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

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Program#/Poster#: 155.10/O9

Topic: D.08. Pain

Title: Generalized placebo improvement of both pain and pleasure evoked by specific suggestions of therapeutic benefit

Authors: *D.-M. ELLINGSEN[1,2], S. LEKNES[2], C. TRISCOLI[3], H. OLAUSSON[4], J. WESSBERG[3];
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Abstract: Although placebo effects are often assumed to arise from symptom-specific expectations of therapeutic benefit, the evidence for such explicit expectations as a defining factor is inconsistent. An emerging view, however, is that placebo effects (e.g. analgesia) builds on a more generic process of reward prediction, whereby the importance, and in turn the experience, of pain, is modified by the inferred predictive (positive or negative) value of available contextual cues. We recently showed that placebo improvement of pain (analgesia) and pleasure (hyperhedonia) employ a similar modulatory circuit. Here, we investigated whether modality-specific expectations, like “reduced pain” or “increased pleasure”, are necessary for placebo analgesia or hyperhedonia, respectively, or whether these effects can be induced by expecting “something good” more generally. In a crossover design, 47 healthy volunteers self-administered a saline nasal spray suggested to either (1) reduce pain (ANA group) or (2) enhance touch pleasantness (HYP group). To strengthen participants’ expectations of treatment benefit, they were, immediately before intranasal treatment, shown one out of two brief video documentaries that summarized scientific findings supporting either treatment-induced improvement of pain (ANA) or touch pleasantness (HYP). Next, they rated (un)pleasantness (Visual analogue scale -5 - 5) and sensory intensity of moderate heat pain and gentle stroking touch. After placebo treatment (PL), relative to a control condition without treatment (CO), both

groups reported reduced pain unpleasantness (ANA: PL: -1.56 ± 0.6 (mean ± SD); CO: -2.37 ± 0.9; HYP: PL: -1.5 ± 1.3; CO: -2.04 ± 1.2) and increased touch pleasantness (ANA: PL: 2.61 ± 1.3; CO: 2.21 ± 0.9; HYP: PL: 2.1 ± 1.4; CO: 1.79 ± 1.6). This was mirrored by placebo-induced improvement of sensory intensity, whereby pain intensity was reduced and touch intensity was increased, in both groups. Both trait optimism (LOT-R) and resting heart rate variability (HRV-HF), collected before the placebo manipulation, correlated positively with expectations of therapeutic benefit. However, neither placebo analgesia nor hyperhedonia were reliably associated with explicit expectations of improvement of pain or pleasure. The results indicate that modality-specific expectations are not necessary for beneficial placebo effects, but that these can be evoked by non-specific expectations of therapeutic improvement. This is consistent with a view of placebo responses as a generalized mechanism of reward prediction, by which a placebo-induced "affective state" affects both positive and negative hedonic feelings.

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Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 155.11/O10

Topic: D.08. Pain

Support: WSIB

Title: Changes in pain and cold sensitivity over time after peripheral nerve injury relate to pain catastrophizing and insula gray matter

Authors: *R. GOSWAMI[1], D. J. ANASTAKIS[2,3], J. KATZ[4,3,5], K. D. DAVIS[1,3];
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Abstract: Introduction: We do not know precisely why pain develops and becomes chronic after peripheral nerve injury (PNI), but it is likely due to a constellation of biological and psychological factors. We previously reported that a year after PNI, there are brain abnormalities linked to sensory deficits (Taylor et al., Brain 2009) and increased pain castastrophizing in patients who developed chronic pain (Davis et al., Neuroscientist 2011; Taylor et al., Pain 2010).

Here we conducted a longitudinal study to test the hypotheses that 1) high pain catastrophizing at the time of injury and repair is associated with pain and cold sensitivity during a 1 year recovery period, and 2) gray matter changes in the insula (a region implicated in pain and thermal sensation) reflect the course of injury and improvements over time. Methods: Ten patients with accidental or work-related transections of the median and/or ulnar nerve (2 F/ 8 M; 37 ± 15 yr), and primary surgical nerve repair within 3 days, and age/sex matched healthy controls provided informed consent. Testing including quantitative sensory testing, pain and personality questionnaires [MPQ, painDetect, Pain Catastrophizing Scale (PCS)]. and 3T MRI (Freesurfer cortical thickness analysis of the insula) at ~3 weeks after surgical nerve repair (Time 1) and again ~1 year later for 6 of the 10 patients (Time 2). Results: Overall, pain intensity and painDetect neuropathic pain scores were correlated with PCS, including the subscales of rumination, helplessness and magnification. Additionally, at time 1: 1) affected digits were insensate with an inability to detect thermal and pain stimuli, 2) pain present in the affected digits in all but 1 patient, and 3) cortical thinning of the right insula. The time 2 assessment showed: 1) partial recovery of sensorimotor function, 2) average pain in 5 of 6 patients followed from Time 1, 3) chronic pain was related to the Time 1 pain-PCS relationship, 4) cold sensitivity, 5) pain catastrophizing scores correlated with cold pain sensitivity, and 6) a reversal of the insula cortical thinning to control levels. Conclusion: This study provides evidence for the interplay between personality, sensory function and pain in patients following traumatic PNI and repair. The association between catastrophizing and pain suggests that a focus on affective or negative components of pain could render patients vulnerable to chronic pain. The common experience of cold sensitivity and the change in insula thickness may represent changes in the lamina 1-VMpo-insula thermosensory pathway implicated in cold and pain sensation, or the neural representation of sensorimotor awareness.

Disclosures: **R. Goswami:** None. **D.J. Anastakis:** None. **J. Katz:** None. **K.D. Davis:** None.

Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

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Program#/Poster#: 155.12/O11

Topic: D.08. Pain

Support: Harvard Catalyst Advanced Imaging Pilot Grant

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1UL1RR025758

Title: Glial activation in the brain and spinal cord of chronic pain patients

Authors: *M. L. LOGGIA[1], D. S. ALBRECHT[1], D. CHONDE[1], R. BORRA[1], O. AKEJU[2], N. KETTNER[3], C. CATANA[1], R. R. EDWARDS[4], D. IZQUIERDO-GARCIA[1], R.-R. JI[5], A. WASAN[6], N. ZÜRCHER[1], B. ROSEN[1], V. NAPADOW[1], Y. ZHANG[2], J. HOOKER[1]; [1]Radiology, Massachusetts Gen. Hosp. / Harvard Med. Sch., Charlestown, MA; [2]Anesthesia, Massachusetts Gen. Hosp. / Harvard Med. Sch., Boston, MA; [3]Logan Univ., Chesterfield, MO; [4]Anesthesiol., Brigham and Women's Hosp. / Harvard Med. Sch., Boston, MA; [5]Duke Univ., Durham, NC; [6]Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA

Abstract: While substantial evidence has established that microglia and astrocytes play a key role in the establishment and maintenance of persistent pain in animal models, the role of glial cells in human pain disorders remains unknown. Here we present the results of two studies providing evidence of glial activation in the brain and spinal cord of patients with chronic low back pain (cLBP). In the first study, 9 cLBP patients and 9 healthy controls received integrated brain PET/MR scans with [11C]PBR28, a recently-developed radioligand that binds to activated microglia and reactive astrocytes. The scanned participants were identified from a larger pool of subjects (n=44) so that each patient was matched to a healthy control for sex (5 M, 4 F in both groups), age (mean ± SD: cLBP = 48.8 ± 12.3, controls = 49.9 ± 12.7; p=0.37) and the Ala147Thr polymorphism in the TSPO gene (7 Ala/Ala, 2 Ala/Thr in both groups), which predicts binding affinity for [11C]PBR28 (Owen DR et al., J Cereb Blood Flow Metab 2012). [11C]PBR28 Standardized Uptake Values (SUVs) were computed from data collected 60-90 minutes post-injection and normalized to whole brain (SUVRs). Whole-brain nonparametric voxelwise analyses revealed that SUVRs were significantly higher in patients than controls in thalamus, the putative sensorimotor representations of the lumbar spine (in the central sulcus) and the leg (in the paracentral lobule). In a second, ongoing study, six chronic sciatica patients (2F; 48 ± 8 y.o.), and 4 healthy controls (3F; 40 ± 18 y.o.), were recruited for a spinal [11C]PBR28 scan. SUVs were computed from data collected 90-110 minutes post-injection. As all patients had L4/L5 and/or L5/S1 pathology, target ROIs were drawn on the lower lumbar/upper sacral spinal cord segments, which are situated at the T11-T12 vertebral levels. Results from preliminary (not genotype-corrected) nonparametric analyses on both SUVs and SUVs normalized by T12 vertebral signal (SUVR) suggest that patients with sciatica have higher spinal [11C]PBR28 signal compared to healthy control subjects (p=0.057 and 0.033, one-tailed, respectively). Together, these studies provide evidence for glial activation in the central nervous system of chronic pain patients. Given our emerging understanding of the importance of

activated glia in clinical pain management, the present findings offer far-reaching clinical implications that may serve to guide future studies on the pathophysiology and management of a variety of persistent pain conditions.

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Poster

155. Processing and Modulation of Pain: Neuroimaging, Psychophysiology, and Neurochemistry

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Topic: D.08. Pain

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NIH Grant P01AT006663

Title: Repeated verum but not placebo acupuncture normalizes connectivity in brain regions dysregulated in chronic pain

Authors: *N. EGOROVA, R. L. GOLLUB, J. KONG;
Massachusetts Gen. Hosp. / Harvard Med. Sch., Charlestown, MA

Abstract: Acupuncture, an ancient East Asian therapy, is aimed at rectifying the imbalance within the body caused by disease. Studies evaluating the efficacy of acupuncture with neuroimaging tend to concentrate on brain regions within the pain matrix, associated with acute pain. We, however, focused on the effect of repeated acupuncture treatment specifically on brain regions known to support functions dysregulated in chronic pain disorders. Transition to chronic pain is associated with increased attention to pain, emotional rumination, nociceptive memory

and avoidance learning, resulting in brain connectivity changes, specifically affecting the periaqueductal gray (PAG), medial frontal cortex (MFC) and bilateral hippocampus (Hpc). We investigated the relationship between chronic knee osteoarthritis symptoms measured with Knee injury and Osteoarthritis Outcome Score (KOOS) pain and sport subscales, and resting state connectivity between the PAG-MFC and PAG-Hpc in a group of 30 patients at baseline and after 6 sessions of either verum (n=20) or sham (n=10) acupuncture treatment. We found that the PAG-MFC and PAG-Hpc connectivity in patients with chronic pain correlates with baseline clinical severity scores - higher PAG-Hpc $r(30)=0.40$, $p=0.033$ (2-tailed) and lower PAG-MFC $r(30)=0.43$, $p=0.021$ (2-tailed) connectivity are associated with increased pain during regular and increased physical activity (pain and sport scores respectively). Repeated verum acupuncture produced a significant positive effect on sport (Mean=18.4, SE=5.7, $F(1,27)=4.252$, $p=0.049$) and pain (Mean=12.1, SE=3.0, $F(1,28)=5.596$, $p=0.025$) clinical outcomes compared to sham. Furthermore, we show that verum acupuncture-induced improvement in KOOS scores (compared to sham) is related to the modulation of PAG-MFC and PAG-Hpc connectivity. Specifically, following verum acupuncture PAG-Hpc connectivity is decreased ($p=0.002$), while baseline PAG-MFC connectivity is maintained. Following sham acupuncture PAG-MFC connectivity is decreased ($p=0.016$) with no changes to the PAG-Hpc connectivity. This study shows that repeated verum acupuncture might act by restoring the balance in the connectivity of the key pain brain regions, altering pain-related attention and memory.

Disclosures: **N. Egorova:** None. **R.L. Gollub:** None. **J. Kong:** None.

Poster

156. Somatosensory Neural Coding

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 156.01/O13

Topic: D.09. Tactile/Somatosensory Systems

Support: ARC Centre of Excellence for Integrative Brain Function

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Title: Contribution of layer 2/3 neurons to sensory adaptation in mouse somatosensory cortex

Authors: *M. ADIBI[1], E. KHERADPEZHOUH[1], L. R. FENLON[2], R. SUAREZ[2], L. J. RICHARDS[2,3], E. ARABZADEH[1];
[1]Eccles Inst. of Neurosci., Australian Natl. Univ., Acton, Australia; [2]Queensland Brain Institute, The Univ. of Queensland, Brisbane, Australia; [3]Sch. of Biomed. Sci., The Univ. of Queensland, Brisbane, Australia

Abstract: Exposure of cortical neurons to sustained sensory stimulation results in changes in their response function: a phenomenon known as sensory adaptation. Sensory adaptation in the primary somatosensory cortex is predominantly attributed to the depression of the thalamocortical synapses. However, the contribution of other intra-cortical circuits to adaptation is not well characterized. Here, we investigated the contribution of sustained activation of L2/3 neurons to adaptation in deeper cortical neurons located in deeper layers . We used the well-characterized mouse whisker-barrel system. To activate L2/3 neurons, channelrhodopsin-2 was expressed selectively in L2/3 by *in utero* electroporation at embryonic day 15.5. We juxtacellularly recorded single cell activity in the primary somatosensory cortex of urethane-anaesthetised mice (n=30) while applying mechanical stimuli of varying amplitudes (0-256 μm) to the vibrissae under two states: non-adapted and adapted. In the adapted state, 200ms optogenetic stimulation (454 nm light, 16 lm/W) of L2/3 preceded each vibrissal stimulation, while there was no optogenetic stimulation in the non-adapted state. In total, 240 neurons were recorded from granular and infra-granular layers. Histological reconstruction of labelled neurons (n=49) indicated that 38.8, 28.6, 26.5 and 4.1% of neurons were located in L4, 5a, 5b and 6, respectively. Optogenetic stimulation increased the average spiking rate of 56% of neurons, and decreased that of 32%. Overall, across all neurons, optogenetic stimulation elicited an average response rate of 6.33 spike/s, 2.06 times higher than the average spontaneous activity. This level of activation was 18% greater than the average neuronal response to the highest vibrissal stimulus (5.37 spike/s to 256 μm stimulus). We performed a linear regression analysis of the average neuronal response functions to vibrissal stimulation between adapted and non-adapted states. This revealed that sustained optogenetic stimulation of L2/3 neurons resulted in an attenuation of the response function by a factor of 0.92 with a 5.9% (0.18 spike/s) decrease in the average background activity. In contrast to a sustained vibrissal stimulation that produced prominent adaptation (rightward shift in neuronal response function), sustained optogenetic activation of L2/3 neurons caused minimal adaptation in downstream neurons.

Disclosures: M. Adibi: None. **E. Kheradpezhouh:** None. **L.R. Fenlon:** None. **R. Suarez:** None. **L.J. Richards:** None. **E. Arabzadeh:** None.

Poster

156. Somatosensory Neural Coding

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Topic: D.09. Tactile/Somatosensory Systems

Support: Bernstein center

BMBF, Förderkennzeichen 01GQ1001A

NeuroCure

Deutsche Forschungsgemeinschaft

Leibnitz prize

Title: Representation of egomotion in rat trident whisker cortex

Authors: *E. CHOREV, P. PRESTON FERRER, M. BRECHT;
BCCN, Berlin, Germany

Abstract: The submandibular trident whiskers, a set of three whiskers on the rat's chin, exhibit biomechanics that are consistent with speed sensing1. Moreover, these whiskers are in contact with the environment in particular contexts: exploring novel environments and while foraging for food in darkness, both instances which require probing ones location in the environment. These observations led to the hypothesis that the trident whiskers are used for monitoring egomotion. To test this hypothesis we identified the cortical area representing the medial trident whisker in the right hemisphere S1 area. This area differs in its anatomical connectivity pattern from facial whiskers, innervating both the spinal vestibular nucleus and the posterior parietal cortex, both areas involved in egomotion processing2, 3. Two thirds of units in the trident area showed speed tuning, a larger portion than the one fifth of speed tuned cells found in non-trident somatosensory areas. When electrically stimulating the trident area we were able to affect the locomotion speed of the animal. Stimulating non-trident areas was less efficient in driving such behavioral changes. These results show that the trident cortex activity represents aspects of egomotion and that this information is used to control locomotive behavior. 1.Thé, L., Wallace, M. L., Chen, C. H., Chorev, E. & Brecht, M. Structure, function, and cortical representation of the rat submandibular whisker trident. J. Neurosci. Off. J. Soc. Neurosci. 33, 4815-4824 (2013). 2.Nishiike, S., Guldin, W. O. & Bäurle, J. Corticofugal connections between the cerebral cortex and the vestibular nuclei in the rat. J. Comp. Neurol. 420, 363-372 (2000). 3.Reep, R. L., Chandler, H. C., King, V. & Corwin, J. V. Rat posterior parietal cortex: topography of corticocortical and thalamic connections. Exp. Brain Res. 100, 67-84 (1994).

Disclosures: **E. Chorev:** None. **P. Preston Ferrer:** None. **M. Brecht:** None.

Poster

156. Somatosensory Neural Coding

Location: Hall A

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Topic: D.09. Tactile/Somatosensory Systems

Support: BBSRC BB/L007282/1

MRC MR/L01064X7/1

Wellcome Trust (097820/Z/11/B)

Title: The somatosensory input to the brain during active sensation in awake behaving mice

Authors: *D. CAMPAGNER[1], M. H. EVANS[1], M. R. BALE[1,2], A. ERSKINE[1,3], R. S. PETERSEN[1];
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Abstract: Sensation is an active process where animals use motor control of their sense organs - eyes, fingers or whiskers - to inform their brains about the external world[1]. In any sensory system, it is fundamental to establish which sensory signals are transduced. Although it is well-understood how first order neurons respond to passive stimulation of sense organs under anaesthesia[2], it is not known whether these properties generalise to the awake, actively sensing state. We recorded the activity of single, well-isolated trigeminal primary afferents from awake, head-fixed mice (see the method described in [3]) as they explored a metal pole with their whiskers and, simultaneously, measured both kinematics (whisker position) and mechanics (forces on the whiskers) with high speed videography (1000 frames/s, total 1.5M frames). In each frame, we extracted key sensory parameters (whisker angle, whisker curvature, whisker-object contact) using a semi-automatic, custom tracking algorithm ('*WhiskerMan*')[,4,5,6]. We used Generalised Linear Models[2,7] to correlate kinematic/mechanic variables with neural activity. Consistent with theoretical predictions[5], we found that the best predictor of primary afferent activity during active sensation is the rotational force due to whisker bending ('bending moment')[6]. In contrast, the kinematic predictor (whisker position), known to predict activity well under passive conditions[2], performed poorly. We found that the relationship between kinematic and mechanical

variables is much richer in the awake, active sensing state compared to the passive state and that this difference can reconcile the apparent discrepancy in signalling between states. Our results identify a major somatosensory input to the brain in the awake, active sensing state and provide a framework for somatosensory encoding that unifies observations under active and passive conditions. 1. Gibson, J. J. (1962).Psychological. Rev., *69*, 477-491. 2. Bale, M. R. et al. (2013). J. Neurosci., *33*, 12003-12012. 3. O'Connor et al. (2010). The J. Neurosci., *30*, 1947-1967. 4. Bale, M. R. et al. (2015). J. Neurosci., *35* 5935-5940. 5. Birdwell, J. et al. (2007). J. Neurophysiol., *98,* 2439-2455. 6. Pammer, L. et al. (2013). J. Neurosci., *33*, 6726-6741. 7. Paninski, L. (2004). *15*, 243-262.

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Poster

156. Somatosensory Neural Coding

Location: Hall A

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Topic: D.09. Tactile/Somatosensory Systems

Support: TÜBİTAK 113S901

Title: Vibrotactile intensity coding by cortical neurons from the hindpaw representation in the rat SI cortex

Authors: *B. GUCLU;
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Abstract: Our previous work suggested that high-frequency vibrotactile intensity information is poorly encoded by individual neurons from the hindpaw representation in the rat SI cortex (Güçlü, 2013; *Soc. Neurosci. Abstr.* 39, program no: 72.16). To quantify this in detail, extracellular spike data from 24 neurons of 7 anesthetized Wistar albino rats were analyzed based on several measures: average firing rate, vector strength of the spike phases, mutual information, and information rate. After classification of single units, a stimulator probe (d = 1.9 mm) was placed on the center of each receptive field in the glabrous skin. Sinusoidal mechanical displacements (duration: 0.5 s, rise/fall times: 50 ms) were applied at 5, 40 and 250 Hz with various amplitudes (0.1 μm - 270 μm; 10 trials for each amplitude). The neurons mostly responded at the onset of the stimulus at 40 and 250 Hz. Average firing rate and vector strength were calculated for the spikes occurring within the entire duration (including rise/fall times) of

the stimulus (*rd*, *vd*) and the 0.1-s onset period (*ro*, *vo*). At 40 Hz, *rd* and *ro* were significantly correlated with stimulus amplitude only in 15 and 18 neurons respectively (avg. r = 0.76 and 0.79). Similar results were obtained at 250 Hz for *rd* and *ro* (14 and 10 neurons sig. correlated with avg. r = 0.77 and 0.81). Correlation was higher at 5 Hz (18 and 20 neurons sig. correlated with avg. r = 0.85 and 0.87). However, in all cases the change in the average firing rate was low as the stimulus amplitude increased. Nonlinear regression for power law did not necessarily yield better results. Vector strength (*vd*, *vo*) was only correlated with amplitude in a few neurons, but it was especially high in the onset period at 5 Hz. Mutual information was calculated by 3×3 and 4×3 stimulus-response matrices by using shuffle correction. Information rate was found as a function of time by neglecting associations between bins, and analyses were repeated at successive bin sizes (2.9, 5.8, 11.6, 29, 58, 116, 290, 580 ms). Due to the high variability, mutual information between stimulus and response was very low (< 1 bit) at all frequencies. Information rate peaked approximately 30-60 ms after the stimulus onset, which accounts for the latencies in the somatosensory pathway and the rise time of the stimulus. Therefore, the results show that single neurons in the hindpaw representation mostly act as onset detectors especially for high-frequency vibrotactile stimuli. Intensity information is probably represented in a population of activated neurons.

Disclosures: **B. Guclu:** None.

Poster

156. Somatosensory Neural Coding

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Topic: D.09. Tactile/Somatosensory Systems

Support: NIH Grant 2R56NS045130-10A1

Brain Research Foundation

Title: Subthreshold optogenetic modulation changes the representation of sensory stimuli by layer 6 corticothalamic neurons and disrupts neocortical processing of sensory change

Authors: *J. VOIGTS[1], C. A. DEISTER[2], C. I. MOORE[2];
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Abstract: Neocortex learns predictive models of sensory input and detects and represents novel stimuli differently from expected or repeated stimuli. How this detection of stimulus deviations

is implemented in the layered neocortical circuitry however, and how the novelty of a stimulus can be represented independently of the stimulus content is currently not understood. Here, using single-neuron recordings across neocortical layers and calcium imaging in awake mice, we find that layer 2/3 neurons encode heterogeneous, history dependent change signals, in contrast to layer 4 and 6 neurons that represent stimuli faithfully. Using calcium imaging in layer 6, we find that instead of selectively reacting to stimulus changes, layer 6 cells represent stimuli faithfully, similar to neurons in the main cortical input layer 4. This finding can explain why layer 2/3 neurons, which are driven by layer 4 and suppressed by layer 6 can represent stimulus changes without being constrained to represent the content of the most recent stimulus. Weak optogenetic stimulation of layer 6 disrupts the heterogeneous change encoding in layer 2/3 neurons, causing them to linearly represent current stimuli, without changing overall firing rates. Using simultaneous bidirectional optogenetic manipulation and 2-photon imaging in L6 corticothalamic neurons, we find that weak de- or hyperpolarization of these neurons does not change their overall baseline or vibrissa-stimulus evoked firing rates, but heterogeneously made neurons stimulus driven that were not without stimulation, or suppressed stimulus responses in otherwise driven neurons. The receptive fields of individual neurons were also changed, with optogenetic depolarization generally removing the neural representation of small changes in stimulus amplitude, while hyperpolarization preferentially made the L6 neuron firing rates reflect stimulus amplitude changes more faithfully. These findings suggest a central role in feature-independent change detection by normalization of neural responses to previous or expected stimuli through layer 6 mediated inhibition.

Disclosures: J. Voigts: None. **C.A. Deister:** None. **C.I. Moore:** None.

Poster

156. Somatosensory Neural Coding

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.09. Tactile/Somatosensory Systems

Support: Einstein Stiftung Berlin

Title: Air-Track: a novel physical and virtual environment for sensorimotor behavior

Authors: *M. N. ABDELHAMID[1,2], H. ORABY[2], R. SACHDEV[2], Y. WINTER[2], M. LARKUM[2];
[1]Berlin Sch. of Mind and Brain, [2]Neurocure Cluster of Excellence, Dept. of Biol., Humboldt Univ. of Berlin, Berlin, Germany

Abstract: Natural behaviour occurs in multiple sensory and motor modalities. These multimodal behavioural interactions are embodied in the various iterations of the head-fixed rodent stepping on a treadmill or walking on an air ball while navigating a virtual reality, or discriminating between somatosensory stimuli. A goal of these new developments is to elicit natural behaviour by mimicking a quasinatural environment and to study the effect of locomotion on cortical responses at the cellular level. Here we have developed an "Air-Track" where head-fixed mice navigate a physical environment - an environment rich in visual, auditory and somatosensory cues. The environment has walls in the shape of a small plus maze, and surfaces for the animal to discriminate with its forepaws or whiskers or both. The Air-Track system combines a virtual reality - the animal is moving an Air-Track, but is not physically moving itself - and a physical reality, where movement by the mouse positions physical discriminanda that mouse uses in making decisions. In a two alternative forced choice task, the animal moves the Air-Track back and forth and rotates the maze from lane to lane, performing contextual behaviour. We are developing methods for tracking feet and whiskers, while monitoring the neocortical physiologic response in S1 and M1 forepaw and vibrissal cortex. This compact, light weight, low cost, and open source method for studying complex decision making is amenable to both head-fixed, and freely moving mice, and can be used with more complicated radial mazes, Y-mazes or with gap crossing paradigms using different modalities. With Air-Track, we come closest to providing a physical environment for eliciting the natural behaviour of mice in closed spaces.

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Poster

156. Somatosensory Neural Coding

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Topic: D.09. Tactile/Somatosensory Systems

Support: DARPA

Title: Area S1 and S2 mapping of vibrotactile stimulation using microECoG electrodes

Authors: *J. C. TANNER[1], T. HEARN[2], S. HELMS TILLERY[3];
[1]SBHSE, Arizona State Univ., Tempe, AZ; [2]SBHSE, ASU, Tempe, AZ; [3]ASU, SBHSE, AZ

Abstract: A dire prosthetic need remains in providing natural and discernible tactile feedback. Mapping the differences in cortical response to mechanical stimuli versus electrical nerve stimuli

is the first step in rectifying the differences. Our goal is to observe both responses and obtain a methodology for stimulating the peripheral nerve in a manner that elicits neural responses mimicking physical stimuli. The secondary goal is to observe any potential adaptation or reorganization of the peripheral stimulation response towards the natural response. A 64 channel 8x8 microECoG array was surgically placed over S1 and S2 on the surface of the right anterior parietal cortex in an anesthetized Rhesus macaque, NHP-M. A pneumatic mechanical stimulator was designed and built in order to provide specific frequencies of punctate stimulation that does not cause electrical noise interference with neurophysiological recording equipment. Using vibrotactile stimulation, specific locations on the NHP's dorsal and palmar left hand were stimulated. All channels were recorded using Tucker Davis Technology equipment and software at a sampling rate or 4000 Hz, with a bandpass filter of 2-1000 Hz and a notch filter at 60Hz. Preliminary principal component analysis was performed on two sessions' data to determine if individual digital stimulation could be decoded and there is substantial success. Experiments are undergoing and we plan on having a much more detailed response map from multiple NHP subjects.

Disclosures: **J.C. Tanner:** None. **T. Hearn:** None. **S. Helms Tillery:** None.

Poster

156. Somatosensory Neural Coding

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 156.08/O20

Topic: D.09. Tactile/Somatosensory Systems

Support: NIH Grant R01NS072416

NSF GRFP DGE1106400 to B.R.I.

Title: Whisker-based encoding of grating stimuli in head-fixed mice

Authors: ***B. R. ISETT**[1], D. E. FELDMAN[2];
[1]Helen Wills Neurosci. Inst., [2]Mol. & Cell Biol., Univ. of California Berkeley, Berkeley, CA

Abstract: Rodents use facial vibrissae to gather tactile information about their environment, including location, texture, and shape of nearby objects. While whisker sensory coding of location and texture are well studied, how the whisker system encodes shape is poorly understood. We studied how whiskers encode the spatial frequency of tactile gratings in head-fixed mice performing grating-smooth discrimination during a virtual foraging task. Mice ran on

a rotary treadmill while a smooth wall moved past, carrying either grating stimuli (raised ridges of 2, 4, 8 or 10 mm spatial period) or smooth stimuli (the curved wall alone), randomly interleaved. Stimulus movement was yoked to the mouse's locomotion, thus allowing for naturalistic stimulus presentation and self-initiated trials. Animals learned to lick for a water reward when a grating was present, and to suppress licking for smooth stimuli in a Go/No-Go design. 50% of mice learned to discriminate grating from smooth with 80-90% accuracy after ~3 weeks of training (d' = 1.5-1.7, n= 3 mice). All gratings were equally discriminated from smooth. To ask whether grating features are sensed similarly to texture, we examined high-acceleration whisker micromotion events (slip-sticks), which occur as a whisker skitters across a surface, and whose frequency and magnitude have been proposed to encode texture. We tracked 2 whiskers with high-speed imaging and found that average slip-stick rate and amplitude were higher on grating stimuli than smooth surfaces. Meanwhile, mean whisker velocity lowered, which is likely due to a reduction in whisking amplitude during grating contact. Despite these findings, linear classification of stimulus identity using slip-stick frequency, amplitude, or mean whisker speed substantially underperformed the observed mouse detection rates. This indicates that these features alone are not sufficient to explain behavioral performance. We next asked whether slip-sticks could encode grating period. Calculating whisker position along the stimulus surface for each slip-stick, we found that many slip-sticks were spatially aligned with ridge edges, and therefore the mean spatial interval between slip-sticks matched the spatial frequency of the underlying stimulus. We are currently recording spikes in primary somatosensory cortex (S1) to learn how grating period is represented in S1 spike patterns. We hypothesize that grating representations are transformed across cortical layers in S1, and that different neurons may exhibit different spatiotemporal receptive field properties analogous to those observed in primate area 3b when embossed letters and dots are scrolled over the fingertips.

Disclosures: **B.R. Isett:** None. **D.E. Feldman:** None.

Poster

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Topic: D.09. Tactile/Somatosensory Systems

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Bernstein Center for Computational Neuroscience

Bundesministerium für Bildung und Forschung (Förderkennzeichen 01GQ1001A)

Neurocure

Gottfried Wilhelm Leibniz Prize

Title: Affective social touch: Electrophysiological correlates of “ticklishness” in juvenile rats

Authors: *S. ISHIYAMA, M. BRECHT;
Bernstein Ctr. For Computat. Neurosci. Berlin, Humbold-Universität zu Berlin, Berlin, Germany

Abstract: The phenemenon of “ticklishness” has fascinated humans for more than two millennia. In rats much like in humans, heterospecific play and “tickling” body contacts induce vocalizations. Theses 50 kHz ultrasonic vocalizations (USVs) indicate positive emotions or “laughter” in these animals (Panksepp & Burgdorf, 1999). However, the neurophysiological correlates of ticklishness are poorly understood. We aimed to study ticklish “laughter” as well as its somatosensory neural correlates in rats. Male juvenile rats were implanted with an eight-tetrode microdrive in the trunk region of the primary somatosensory cortex. Rats received tickling and gentle touch stimulation by experimenter’s hand on the dorsal/ventral trunk and the tail, or experimenter’s hand was placed in the test box so that the rats chased the hand to initiate play. Bouts of 50 kHz USVs were emitted especially during tickling on the ventral trunk and chasing hand. Electrophysiological recordings revealed various neural response patterns to ‘tickling’ and gentle touch. Some cortical units increased the firing rate during ‘tickling’, while others were suppressed. Most interestingly, some tickling-activated cells also responded strongly when the rat chased the experimenter’s hand, even though this experimental condition did not involve physical interaction or direct tactile stimulation. Our data point to an involvement of somatosensory cortical responses in ‘tickling’ and play related behaviors.

Disclosures: S. Ishiyama: None. **M. Brecht:** None.

Poster

156. Somatosensory Neural Coding

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Title: Two-photon calcium imaging depicts population encoding of vibrotactile information within excitatory and inhibitory networks of the limb associated mouse somatosensory cortex

Authors: *M. V. BANDET[1,2], I. R. WINSHIP[1,2,3];
[1]Neurosci. and Mental Hlth. Inst., [2]Neurochemical Res. Unit, [3]Psychiatry, Univ. of Alberta, Edmonton, AB, Canada

Abstract: To distinguish between varying somatosensory stimuli, the somatosensory cortex should process dissimilar stimuli with different patterns of neuronal activation. Intrinsic optical signal (IOS) imaging reveals activation in the mouse somatosensory cortex in response to limb stimulation and allows functional segregation of cortical regions activated by the contralateral hindlimb (cHL) and contralateral forelimb (cFL). IOS imaging and electrophysiological recordings of small numbers of single cells have been used in the past to demonstrate "tuning" of cortical regions and of individual cells within the somatosensory cortex of monkeys to different modalities of cutaneous stimulation. However, a large scale population based examination of somatosensory tuning to complex somatosensory stimuli has never been reported within the limb associated somatosensory cortex of rodents. Further, the differential role of excitatory and inhibitory neuronal responses to the processing of somatosensory information has never been elucidated. Here we used *in vivo* two-photon Ca2+ imaging after IOS to measure HL and FL somatosensory neuron responsiveness and response dynamics of populations as large as 250 neurons per optical section. We show that individual neurons within the somatosensory cortex can be precisely tuned to particular frequencies of vibrotactile limb fluctuation or broadly tuned to multiple frequencies of fluctuation, thereby forming a population code for sensory processing. We further demonstrate that higher frequency vibrotactile fluctuation of the limbs results in a larger percentage of neuron populations responding and with greater neuronal response strength. These population codes may result from preferential activation of different subsets of cutaneous and musculoskeletal receptors that respond to particular stimuli features and encode a sensory percept of somatosensory information needed for fine adjustment of motor control during limb movement. Here we also expressed an AAV for GcAMP6S under a neuron specific promoter and an AAV for tdTomato selectively in parvalbumin positive (PV+) inhibitory interneurons under CRE dependent control in PV-CRE mice. We demonstrate that long duration high frequency limb fluctuation results in increased PV+ cell activity within the somatosensory cortex as a potential means to actively inhibit prolonged responses of non-PV+ cortical neurons. As PV+ cell dysfunction is thought to be a contributor to sensory abnormalities in disorders such as schizophrenia and stroke, these results will be highly useful as a comparison for cell population specific changes in activity in these disorders.

Disclosures: M.V. Bandet: None. **I.R. Winship:** None.

Poster

156. Somatosensory Neural Coding

Location: Hall A

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Program#/Poster#: 156.11/O23

Topic: D.09. Tactile/Somatosensory Systems

Title: Stimulus feature coding by trigeminal ganglion neurons during natural whisking against an object

Authors: *K. S. SEVERSON, D. XU, D. H. O'CONNOR;
Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: The rodent whisker system is a common model for sensorimotor processing, yet the stimulus features encoded by primary afferent neurons during active sensing remain poorly understood. To fill this gap, we aimed to explain variance in spiking of mouse trigeminal ganglion (Tg) neurons in terms of the forces and moments exerted during natural whisking against an object. We recorded 29 Tg single units, 15 rapidly adapting (RA) and 14 slowly adapting (SA), while simultaneously capturing high-speed video of whiskers (>1500 minutes at 500 frames/s) as head-fixed mice actively whisked in air and against a thin pole. Single unit spike rates ranged from 0.6 spikes/s to 47 spikes/s over the entire recording session, with instantaneous rates often surpassing 1000 spikes/s. Using measurements of whisker curvature, we calculated axial force, lateral force, and moment exerted on the follicle at each time point. Analysis to relate these quasistatic force/moment components to contact-evoked spiking is currently ongoing.

Disclosures: **K.S. Severson:** None. **D. Xu:** None. **D.H. O'Connor:** None.

Poster

156. Somatosensory Neural Coding

Location: Hall A

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Program#/Poster#: 156.12/O24

Topic: D.09. Tactile/Somatosensory Systems

Title: Decoding acupuncture point specificity using multi-voxel pattern analysis

Authors: *W. JUNG, Y. CHAE;
AMSRC / Kyunghee Univ., Seoul, Korea, Republic of

Abstract: Objectives: Despite a number of previous fMRI studies, strong and consistent evidence supporting the neural representations of acupoint specificity have not been introduced. The present study applied multi-voxel pattern analysis (MVPA) and investigated the predictive capacity of fMRI data for decoding the neural representations of two different acupoints. **Methods:** Fourteen participants received acupuncture needles at both PC6 (median nerve) and HT7 (ulnar nerve) acupoints on their left hand. Twenty manual stimulations were randomly given at one of two acupoints. Using Freesurfer Desikan-Killiany Atlas, we defined region of interest (ROI) masks in individual brain. After constraining trial-wise data in anatomical ROIs, the prepared images were preprocessed by standardization of the parameter estimates within each voxel and by Euclidean normalization of image within each trial. We trained and tested a Gaussian Naive Bayes classifier on trial-wise fMRI data for discriminating accuracy in each ROI. **Results:** Our analysis showed a rank order of brain regions: activity in the primary somatosensory cortex and primary motor cortex yielded the most accurate trial-by-trial discrimination between acupuncture stimulations at PC6 and HT7. **Conclusions:** The present study demonstrated that distributed spatial patterns of brain activations encode two different acupoints. Our findings suggest that characteristics of acupoint specificity could be revealed with the application of MVPA approach.

Disclosures: W. Jung: None. **Y. Chae:** None.

Poster

156. Somatosensory Neural Coding

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Support: DFG Grants to HJL

BaCoFun Research Unit

Title: High stimulus-related information in inhibitory fast-spiking interneurons of the adult rat barrel cortex *in vivo*

Authors: *V. REYES-PUERTA, S. KIM, J.-J. SUN, B. IMBROSCI, W. KILB, H. J. LUHMANN;
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Abstract: A major goal in current neuroscience is to understand how sensory information is represented and processed in the central nervous system. In this respect, the manner in which populations of inhibitory (INH) and excitatory (EXC) neocortical neurons collectively encode stimulus-related information is a fundamental, yet still unresolved question. In the present study we address this question by simultaneously recording with 128-channel multi-electrode arrays the activity of cell ensembles (of up to 74 neurons) upon sensory stimulation in the anesthetized adult rat barrel cortex *in vivo*. The recorded cells were distributed along all cortical layers of 3-4 neighboring columns, and further classified as fast-spiking (presumed INH) and putative pyramidal EXC neurons according to their distinct extracellular spike waveforms. Using two different whisker stimulus modalities (location and frequency) we estimated the stimulus encoding performance of identified neurons by means of mutual information and linear classifiers. These analyses revealed that in granular and infra-granular layers individual INH neurons discriminate better between restricted sets of stimuli (containing ≤6 stimulus classes) than EXC neurons. In addition, assemblies containing all INH cell provided as much information about such stimuli as comparable large ensembles including the ~20% most informative EXC neurons, however, presenting a lower amount of information redundancy. Moreover, the information provided by the remaining ~80% least informative EXC neurons was majorly redundant to that conveyed by the most informative ones, advocating for a sparse stimulus encoding scheme employed by EXC cortical neurons. Our results were consistent when applying both linear discriminant analysis classifiers and theoretical information measurements, thus indicating that a rather similar amount of information might be represented by the total population of EXC neurons as compared to that represented by INH neurons, however with a higher degree of redundancy. Taken together, these data suggest that a consortium of INH neurons dominates the information content about sensory stimuli within the neocortical network, thereby efficiently shaping the processing of incoming sensory information. This conclusion extends our view on the role of the INH system to orchestrate cortical activity.

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Poster

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C.I.M. NIH Grant# NS045130

Title: Functional dynamics of heterogenous interneuron and pyramidal cell subnetworks over multiple time scales

Authors: *C. A. DEISTER, K. SALEHI, A. TRIEDMAN, C. I. MOORE;
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Abstract: Dynamic sensory processing in neocortex relies on interactions between populations of pyramidal cells and interneurons. The general activity of pyramidal cells and key interneuron subtypes, such as parvalbumin-positive (PV+) and somatostatin-containing (SOM) neurons, has been studied across cortical sensory areas and is reasonably understood. However, there is remarkable heterogeneity (both functional and genetic) within each of these well-described cell classes whose behavioral relevance is largely unknown. We studied the activity of PV+, SOM and pyramidal cells in layers 2/3 and 5a within the vibrissal region of mouse primary somatosensory cortex (barrel cortex) over multiple days using two-photon calcium imaging, before and after animals learned to report the detection of weak tactile stimuli. Using the genetically encoded calcium indicators GCaMP6f and 6s along with an array of cre-driver and bac-transgenic lines, we imaged the activity of each interneuron population alone or in combination with pyramidal cells. For each cell class there are distinct alterations in the trial-to-trial reliability of sensory-evoked responses for 'threshold-level' stimuli, with the overall trend being a stable increase. Of note, many SOM neurons showed very large increases in reliability, with most appearing unresponsive before training and ultimately many showing even higher reliability than PV+ and pyramidal cells. We also examined the degree of trial-to-trial correlation between the evoked-responses of cells within each class, and across classes, during detection. For PV+ and pyramidal cells these correlations were relatively weak in trained and untrained animals, but small subgroups of cells showed strong correlations that were highly indicative of successful perception. In contrast, we found negligible correlations among SOM cells in untrained animals, but once animals were trained, SOM cells showed high correlations with each other. Further, highly informative pyramidal neurons showed large, and significant, positive and negative correlations with SOM neurons. Overall, our data support the notion that interneurons form distinct and functionally heterogeneous subnetworks with pyramidal cells to help represent weak or ambiguous sensory information, and potentially use that representation to better inform action. Ongoing studies are aimed at characterizing these dynamics with competing representations by using an active tactile discrimination task.

Disclosures: **C.A. Deister:** None. **K. Salehi:** None. **A. Triedman:** None. **C.I. Moore:** None.

Poster

156. Somatosensory Neural Coding

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Topic: D.09. Tactile/Somatosensory Systems

Support: NIH Grant 1R01NS086859-01 (MG)

Training grant 2T32MH067564 (DDF)

Title: Temperature representation in the *Drosophila* brain

Authors: *M. GALLIO[1], D. D. FRANK[1], G. C. JOUANDET[1], P. J. KEARNEY[1], L. J. MACPHERSON[2];
[1]Neurobio., Northwestern Univ., Evanston, IL; [2]Departments of Biochem. and Mol. Biophysics, Columbia Univ., New York, NY

Abstract: In *Drosophila*, rapid temperature changes are detected at the periphery by dedicated receptors forming a simple sensory map for hot and cold in the brain. However, flies show a host of complex innate and learned responses to temperature, indicating that they are able to extract a range of information from this simple input. Our work aims at defining the anatomical and physiological repertoire for temperature representation in the *Drosophila* brain. Here, we use a photolabeling strategy to trace the connections that relay peripheral thermosensory information to higher brain centers, and show that they largely converge onto three target regions: the Mushroom Body, Lateral Horn (well-known centers for sensory processing) and the Posterior Lateral Protocerebrum, a region we now define as a major site of thermosensory representation. Then, using *in vivo* calcium imaging, we describe the thermosensory projection neurons selectively activated by hot or cold stimuli. Fast-adapting neurons display transient “ON” and “OFF” responses and track rapid temperature shifts remarkably well, while slow-adapting cell responses better reflect the magnitude of simple thermal changes. Unexpectedly, we also find a population of 'broadly-tuned' cells that respond to both heating and cooling, and show that they are required for normal behavioral avoidance of both hot and cold in a simple 2-choice temperature preference assay. Taken together, our results uncover a coordinated ensemble of neural responses to temperature in the fly brain, demonstrate that a broadly tuned thermal-line contributes to rapid avoidance behavior, and illustrate how stimulus quality, temporal structure, and intensity can be extracted from a simple glomerular map at a single synaptic station.

Disclosures: **M. Gallio:** None. **D.D. Frank:** None. **G.C. Jouandet:** None. **P.J. Kearney:** None. **L.J. Macpherson:** None.

Poster

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Support: NIH R01NS089652

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Title: Choice- and stimulus-related activity mapped across layer 2/3 of mouse s1 and s2

Authors: *S. E. KWON, H. YANG, G. MINAMISAWA, D. H. O'CONNOR;
Neurosci., Brain Sci. Inst. / Johns Hopkins Univ., Baltimore, MD

Abstract: During perceptual decisions, the activity of single sensory cortex neurons and behavioral choices often co-vary, even when the stimulus is identical. “Choice probability” (CP) is the probability with which an ideal observer can predict the choice of the animal based on a single-trial neuronal response. CP is frequently studied because it links neuronal activity and perception. CP is often higher for neurons with greater stimulus sensitivity. Intuitively, this is consistent with more informative neurons contributing more to perceptual decisions. However, theoretical studies suggest a key role for inter-neuronal “noise” correlation (Rsc) in determining CP. The exact relationship between CP, neuronal sensitivity, and Rsc is unclear. Also unknown are how CP is mapped at cellular resolution across multiple sensory cortex areas, and to what degree choice is predicted by population activity patterns unobservable when recording from one or a few neurons at a time. We trained mice to detect weak deflections of a single whisker. Using cellular resolution 2-photon calcium imaging during the task, we measured activity across hundreds of L2/3 neurons in whisker areas of primary (S1) or secondary (S2) somatosensory cortex (S1: 4 mice; S2: 4 mice). Ideal observer analysis quantified how well each neuron discriminated stimulus presence/absence (“Stimulus probability”, SP) and the choice of the mouse. SP is a measure of neuronal sensitivity to our stimulus. We also quantified Rsc among neuron pairs. The fraction of neurons with significantly above-chance SP was greater in S1 than S2 (S1: 47%, S2: 30% of responsive neurons). S1 neurons also had higher SP (S1 median: 0.57; S2: 0.53, $p<0.001$, K-S test). Thus, stimulus representation was somewhat stronger in S1. In contrast, S2 and S1 neurons predicted choice similarly (S1 median CP: 0.56, S2: 0.55, $p=0.065$, K-S test). Mean Rsc with other responsive neurons was higher for neurons in S2 vs S1 (0.17 vs 0.09; $p<0.001$, Wilcoxon rank sum test). Partial correlations revealed that CP depended

separately on a neuron's stimulus sensitivity and its mean Rsc. We used a machine learning algorithm to decode the stimulus and choice from populations of hundreds of S1 or S2 neurons. S1 populations yielded better decoding of the stimulus (S1: 93 ± 3% correct; S2: 75 ± 4%; mean ± SEM across mice). Mouse performance (78 ± 1%) was similar to that of the S2 decoder. Decoding of choice was similar in the two areas (S1: 75 ± 4%; S2: 77± 2%). Could widespread choice- and stimulus-related activity reflect direct S1<->S2 communication? We imaged S1->S2 axons (2 mice), and S2->S1 axons (4 mice), and found stimulus- and choice-related activity propagating bidirectionally.

Disclosures: **S.E. Kwon:** None. **H. Yang:** None. **G. Minamisawa:** None. **D.H. O'Connor:** None.

Poster

156. Somatosensory Neural Coding

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Topic: D.09. Tactile/Somatosensory Systems

Support: EU Project VERE WP1

Title: TMS over V5/hMT+ disrupts tactile direction discrimination

Authors: ***T. AMEMIYA**[1,2], B. BECK[2], H. GOMI[1], P. HAGGARD[2];
[1]NTT Communication Sci. Labs., Kanagawa, Japan; [2]Univ. Col. London, London, United Kingdom

Abstract: Several human imaging studies have found that visual motion area V5/human medial temporal complex (hMT+) responds to tactile motion. A multivariate pattern analysis found specific patterns of activity in V5/hMT+ corresponding to leftward and rightward tactile directions. Some studies have also reported activations in the primary somatosensory cortex (SI) and posterior parietal cortex (PPC) during tactile motion, but they have not established a causal involvement of these areas in tactile direction processing. Here, we created an ecological tactile motion stimulus by varying the direction of a single object moving across the fingertip. We disrupted activity in SI, PPC (Brodmann's areas 7/40) and V5/hMT+ using online double-pulse transcranial magnetic stimulation (TMS) while participants judged tactile motion direction. TMS over both SI and V5/hMT+, but not over PPC, reduced tactile direction discrimination. Our results demonstrate, for the first time, that V5/hMT+ plays a causal role in tactile direction processing, extending previous studies that found directionally sensitive patterns of activity in

V5/hMT+. Further, our findings are consistent with a serial model of cortical tactile processing, in which processing by higher-order perceptual areas depends upon the quality of input received from SI. By contrast, our results do not provide clear evidence that the PPC is causally involved in discriminating tactile direction. This suggests that the pathway for tactile motion processing is not routed through inferior regions of the PPC.

Disclosures: **T. Amemiya:** A. Employment/Salary (full or part-time):; NTT. **B. Beck:** None. **H. Gomi:** A. Employment/Salary (full or part-time):; NTT. **P. Haggard:** None.

Poster

156. Somatosensory Neural Coding

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Program#/Poster#: 156.18/O30

Topic: D.09. Tactile/Somatosensory Systems

Title: Locus coeruleus activity patterns modulate coding properties of the ventral posteromedial nucleus in the thalamus of the rat vibrissae pathway

Authors: *C. RODENKIRCH, Y. LIU, Q. WANG;
Columbia Univ. Dept. of Biomed. Engin, New York, NY

Abstract: The locus coeruleus (LC) is the sole source of norepinephrine (NE) to the forebrain. The varying firing patterns of the LC have a large effect on behavioral state, with certain patterns shown to be associated with optimal performance in sensory-initiated behavioral tasks. However, how LC activity modulates neural coding in the early stage of the sensory pathways remains poorly understood. We recorded single-unit activity in the ventral posteromedial (VPM) nucleus of the thalamus, an early stage of the rat's vibrissae pathway, in pentobarbital-anesthetized rats. During each recording, varying microstimulation patterns (Tonic 1, 2, and 5 Hz as well as Phasic) were delivered to the LC. For these varied patterns, the feature selectivity of each recorded neuron was then estimated using spike-trigged reverse correlation analysis. Our preliminary data showed that stimulation of the LC resulted in a moderately decreased firing rate for neurons in the VPM. More interestingly, we found that LC stimulation caused a dramatic reduction in the occurrence of bursting activity within the VPM while concurrently causing an increase in the temporal precision of events in the peristimulus time histogram. Feature selectivity of the VPM neurons was not significantly altered by LC stimulation. However, on average, spikes were found to encode significantly more information about the feature during LC stimulation. Taken together, these results suggest that LC activity modulates the coding properties of neurons in the early stage of sensory pathways.

Disclosures: **C. Rodenkirch:** None. **Y. Liu:** None. **Q. Wang:** None.

Poster

156. Somatosensory Neural Coding

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Intramural program of the NIH/NINDS

Title: Context-dependent information decoding of sensory-evoked responses in the rat vibrissa pathway

Authors: *H. J. ZHENG[1], C. WAIBLINGER[1], B. J. HE[2], G. STANLEY[1];
[1]Coulter Dept. of Biomed. Engin., Georgia Inst. of Technol. & Emory Univ., Atlanta, GA; [2]Natl. Inst. of Neurolog. Disorders and Stroke, Natl. Inst. of Hlth., Bethesda, MD

Abstract: A fundamental pursuit of neuroscience is to understand how external stimuli are encoded in the brain and how the information about the stimuli is extracted to form sensory perception. A confounding problem in sensory coding is that the representation of the same stimulus in the primary sensory cortex is highly variable. This variability arises at least partially from the internal state of the brain, which is spontaneously dynamic, and can also be influenced by bottom-up mechanisms such as sensory adaptation and top-down mechanisms related to level of arousal, attention, etc. We hypothesize that the internal state of the cortex can be at least partially extracted from the spontaneous activity and provide the context under which the stimulus is encoded. Therefore, given access to the spontaneous activity, an ideal observer can reduce the uncertainty about the stimulus despite the variable cortical response. In order to uncover the interaction between spontaneous and sensory-evoked activity, we recorded the cortical activity simultaneously with a genetically-encoded voltage indicator (ArcLight) and local field potential (LFP) in the same cortical column of rat barrel cortex in response to a single, punctate whisker deflection. We find that the frequency content in the pre-stimulus spontaneous activity predicts the magnitude and the variability of the stimulus-evoked response. In trials where the pre-stimulus spontaneous activity exhibits less low frequency fluctuation (quantified with a frequency ratio, which is the ratio of total power in 0-5 Hz band to the total power in 0-50

Hz), a whisker stimulus evokes a smaller but less variable thus more easily discriminable response. We find that sensory adaptation, where repetitive whisker stimulation (10 Hz deflection) attenuates the cortical response over hundreds of milliseconds, changes the frequency content in the spontaneous activity in a dynamic manner. Adaptation shifts low frequency trials into a higher-frequency regime and vice versa, suggesting that sensory adaptation may serve to stabilize the frequency content in the cortex and may have dynamic effects on the detection and discrimination of the stimulus depending on the initial state of the cortex.

Disclosures: **H.J. Zheng:** None. **C. Waiblinger:** None. **B.J. He:** None. **G. Stanley:** None.

Poster

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NSF Graduate Research Fellowship Program

NIH T-32

Title: Altered sensory processing following cortical spreading depression in awake mice

Authors: ***P. D. PARKER**[1,2], J. J. THERIOT[1], J. M. MENDEZ[1], K. BRENNAN[1];
[1]Dept. of Neurol., [2]Interdepartmental Program in Neurosci., Univ. of Utah, Salt Lake City, UT

Abstract: Migraine is a pervasive neurological disorder affecting 12% of the population. For many individuals, the disorder exceeds the hallmark headache and includes maladaptive distortions of sensory perception and motor function. Cortical spreading depression (CSD), the physiological correlate of many of these distortions, has the capability of inducing plasticity in neural networks that might underlie the sensory features of individual migraine attacks, and of chronic migraine. To better understand the effects of CSD on sensory processing, we used 2-photon microscopy to characterize neural response patterns in awake mice to whisker stimulations prior to and after CSD. This includes both wild type mice and knock-in mice carrying the genetic mutation for familial hemiplegic migraine, type 2 (FHM2). CSD entails a major shift in the ionic environment in the extracellular space, including dramatic increases in the concentrations of extracellular potassium and neurotransmitters, such as glutamate. Intracellularly, cytosolic Ca^{2+} levels remain elevated in neurons for several minutes and neurons

remain depolarized for 3-5 minutes after the passing of the wave. A subset of neurons within the barrel cortex showed a potentiated response to trains of whisker stimulations after CSD. More interestingly, additional subsets of neurons showed a depressed response following CSD, as well as alterations in the timing of responses to whisker stimulation. This provides evidence that CSD induces complex changes in neural network processing and provides potential network level mechanisms that underlie the changes in sensory perception seen in migraine.

Disclosures: P.D. Parker: None. **J.J. Theriot:** None. **J.M. Mendez:** None. **K. Brennan:** None.

Poster

156. Somatosensory Neural Coding

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 156.21/O33

Topic: D.09. Tactile/Somatosensory Systems

Support: NSERC Discovery Grant

Title: Establishing the link between single afferent firing and perception across the foot sole

Authors: *N. STRZALKOWSKI, R. MILDREN, L. BENT;
Univ. of Guelph, Guelph, ON, Canada

Abstract: Introduction: Four classes of cutaneous afferents (FAI, FAII, SAI, SAII) mediate tactile sensibility across the soles of the feet. Each class is sensitive to unique features of tactile stimuli, and may provide different functional cues to aid in the control of posture and gait. Monofilament (light touch) testing is a common clinical and research tool used to determine perceptual and afferent firing thresholds. Previous work in the hand found light touch perceptual thresholds to most closely couple with the firing threshold of FAI afferents. FAI afferent thresholds were consistent across the hand and similar to perceptual threshold in the fingers, however perceptual thresholds were found to be elevated in the palm [1]. The purpose of the present study was to establish the link between afferent firing and perceptual thresholds across the foot sole, and to investigate the influence of skin hardness on these measures. Methods: Microneurography was used to identify and record from 102 foot sole cutaneous afferents in 21 subjects (12 male, 20-27 yrs). Once identified, afferent monofilament firing thresholds (minimum force to evoke an action potential) were measured. Monofilament perceptual thresholds (minimal force to evoke a percept) were also recorded and compared to afferent firing thresholds. Skin hardness was measured at each receptive field using a hand held durometer

(arbitrary units; au). Results: FAI and FAII afferents had significantly lower firing thresholds than SAI and SAII afferents. Perceptual thresholds were not found to be different from FAI or FAII afferent firing threshold but were significantly lower than the firing thresholds of SAI and SAII afferents. Both perceptual threshold and FAI afferent threshold were significantly lower in the arch compared to the heel, forefoot and toe regions. Positive correlations were found between both FAI ($r=0.357$, $p<0.018$) and FAII ($r=0.758$, $p=0.007$) firing threshold and receptive field hardness. Perceptual threshold was also found to correlate with receptive field hardness ($r=0.433$, $p<0.0001$). Conclusions: Across the foot sole, the firing of FA afferents appears to mediate monofilament perceptual threshold, while SAI and SAII afferents do not contribute. Receptive field hardness was shown to influence FAI and FAII firing thresholds, and is thought to help explain observed afferent firing and perceptual threshold differences across the foot sole. Establishing the link between foot sole afferent firing and perceptual threshold is necessary to fully understand the contributions of cutaneous afferent classes to perception and motor control. References: [1] Johansson and Vallbo (1979) J. Physiol 286:283-300

Disclosures: **N. Strzalkowski:** None. **R. Mildren:** None. **L. Bent:** None.

Poster

156. Somatosensory Neural Coding

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 156.22/O34

Topic: D.09. Tactile/Somatosensory Systems

Title: Mechanisms of illusory continuous force sensation induced by asymmetric vibration - A computational approach to sensory processing -

Authors: ***H. GOMI**, T. AMEMIYA, S. TAKAMUKU, S. ITO;
NTT Communication Sci. Labs, Kanagawa, Japan

Abstract: Force sensation is one of the important information in various interactions with environments. Several recent attempts have succeeded to elicit illusory perception of tangential continuous force only by applying asymmetric vibration without any application of continuous force (e.g, Amemiya et al. 2004; Tappeiner et al. 2009; Amemiya & Gomi 2014), but its perception mechanism is not yet well understood. The present study, therefore, focuses on the following questions. How do we perceive an illusory force while temporal mean force is physically zero? Which mechanoreceptor contributes in yielding this illusory sensation? To examine these questions, we have developed a sensory processing model which can fit the illusory force perception performance obtained from a psychophysical experiment. In the

experiment, subjects (n = 10) pinched a small hexahedron object (19 g) in which a linear actuator was embedded, so as to apply the vibration in tangential direction at the skin contact. We used 50 patterns of vibration stimuli (6 sec with sign reversal twice) generated by bipolar rectangular driving patterns. The subjects answered the perceived force direction (2-alternative) of the last phase of each stimulus (100 trials in random order). The selection probability of perceived force direction for each vibration stimulus, which corresponds to the force direction clarity, was greatly varied by the stimulus patterns. To find dominant factors in characterizing the force-direction clarity, we introduced a model which calculates temporal average of detected sensory signal over a threshold. Then, we tested position, velocity, acceleration, and interaction force patterns during the application of vibration stimuli as candidates of the dominant sensory signal for the direction perception. As a result, selection probability function of the force directions for all the stimulus patterns was successfully modeled (correlation coefficient: 0.83) by the interaction force signal. The force threshold obtained by the fitting optimization was 10 mN, which is comparable to the threshold of the slowly adapting type II (SA II) mechanoreceptor measured previously (Johansson et al. 1980). These results suggest that SAII signal strongly contributes to induce the illusory continuous-force perception caused by asymmetric vibration. This implication is consistent to the functional role of the SA II expected in the previous studies, and therefore the model would bridge the gap between the tactile sensory processing and force perception.

Disclosures: **H. Gomi:** None. **T. Amemiya:** None. **S. Takamuku:** None. **S. Ito:** None.

Poster

156. Somatosensory Neural Coding

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Topic: D.09. Tactile/Somatosensory Systems

Support: Career Award at the Scientific Interface from the Burroughs Wellcome Fund (JTR)

Quantitative Biology and Physiology Training Program (JBS)

Title: Closed-loop optogenetic stimulation reveals primary somatosensory cortex participation in whisk timing

Authors: ***J. B. SCHROEDER**, V. J. MARIANO, G. I. TELIAN, J. T. RITT;
Boston Univ., Boston, MA

Abstract: Active sensing incorporates closed-loop behavioral selection of information during sensory acquisition. The rodent whisker tactile system provides an ideal platform for studying these processes. We examined coordinated head and whisker motions of unconstrained mice performing tactile search for a randomly located reward, and found that mice select from a diverse range of available active sensing strategies, based at least in part on the behavioral context of whisker contacts. In particular, mice selectively employed a strategy we term contact maintenance, where whisking is modulated to counteract head motion and sustain repeated contacts, but only when doing so is likely to be useful for obtaining reward. The context dependent selection of sensing strategies, along with the observation of whisker repositioning prior to head motion, suggests the possibility of higher level control, beyond simple reflexive mechanisms. In order to further investigate a possible role for primary somatosensory cortex (SI) in whisk-by-whisk motion, we delivered optogenetic feedback to SI, time locked to whisking estimated through facial electromyography. We found that stimulation regularized whisking (increasing overall periodicity), and shifted whisking frequency, changes that emulate behaviors of rodents actively contacting objects. We found different effects for stimulation on protractions versus retractions, possibly encoding that contact is more likely during forward motion of the whiskers, and downstream areas may show greater sensitivity to SI activity during protractions. Additionally, stimulation induced small, short latency motions similar to those in a previous study (Matyas, et al., Science, 2010) in head fixed animals, except that we induced protractions rather than retractions. These findings support a role for sensory cortex in driving whisk-by-whisk motor outputs, but suggest a coupling that depends on behavioral context. Elucidating any role for sensory cortex in motor outputs is important to understanding active sensing, and may provide novel insights to guide the design of sensory neuroprostheses that exploit active sensing context.

Disclosures: **J.B. Schroeder:** None. **V.J. Mariano:** None. **G.I. Telian:** None. **J.T. Ritt:** None.

Poster

156. Somatosensory Neural Coding

Location: Hall A

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Program#/Poster#: 156.24/O36

Topic: D.09. Tactile/Somatosensory Systems

Support: EU Grant CP-FP-INFSO 224012 TIME project

EU GRANT FP7-HEALTH 602547 EPIONE project

Title: Sensory substitution in amputees: intraneural versus transcutaneous nerve stimulation for the bidirectional control of prostheses

Authors: *F. M. PETRINI[1], E. D'ANNA[1], S. RASPOPOVIC[1,2], S. MICERA[1,2];
[1]EPFL, LAUSANNE, Switzerland; [2]The BioRobotics Inst., Scuola Superiore Sant'Anna, Pisa, Italy

Abstract: Sensory feedback is essential for sophisticated and effortless hand control. Appropriate sensations from the periphery and their interaction with the external world are essential for correct agency and ownership of the hand. Sensory feedback in amputees can be conveyed both by direct neural stimulation (DNS) via implantable devices or by means of superficial transcutaneous electrical nerve stimulation (TENS). In this work, we systematically compared DNS and TENS, both in open (only electrical stimulation) and in closed-loop (i.e., stimulation integrated in a bidirectional prosthesis). The same transradial amputee was stimulated with TIME electrodes implanted in the median and ulnar nerves (DNS) and with TENS during two different sessions of experiments providing monopolar and multi-polar electrical stimulation. We showed that intraneural single channel stimulation elicits close-to-natural touch sensations over discrete areas of the phantom hand, while TENS provides a fuzzy paresthesia. For the first time, we studied the effects of multipolar stimulation, which evoked, in some cases, linear combinations and, in others, non-linear summation of the sensations resulting from mono-polar stimulation. Unexpectedly, in the latter condition, the resulting perceived area was completely different from those related to the mono-polar sensations. Finally, the performance of the control of the force exerted by the prosthesis, during bidirectional prosthesis control was evaluated when TENS feedback was used, and compared to the results reported with intraneural stimulation. We showed that similar performance can be achieved with the two approaches. Overall, these results establish clear guidelines regarding the use of non-invasive and invasive solutions for sensory feedback restitution in the next generation of prosthetic devices.

Disclosures: **F.M. Petrini:** None. **E. D'Anna:** None. **S. Raspopovic:** None. **S. Micera:** None.

Poster

156. Somatosensory Neural Coding

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Topic: D.09. Tactile/Somatosensory Systems

Support: NIH grant R01ES03299

Michigan State University College of Veterinary Medicine

Title: The role of extracellular calcium concentration ($[Ca^{2+}]_e$) on methylmercury (MeHg)-induced toxicity in mouse dorsal root ganglia (DRG) primary cultures

Authors: *E. FORMILLER[1], H. HANNON[2], W. D. ATCHISON[3];
[1]Neurosci. Progam, [2]Comparative Med. & Integrative Biol., [3]Pharmacol. & Toxicology, Michigan State Univ., East Lansing, MI

Abstract: MeHg is a neurotoxicant that alters normal nervous system function in distinct neuronal populations. One of the first symptoms of MeHg poisoning is sensory neuropathy, corresponding to dysfunction and degeneration of DRG. Previous studies have shown that MeHg poisoning causes a biphasic increase in $[Ca^{2+}]_i$, with phase 1 attributed to the release of Ca^{2+} from intracellular stores and phase 2 due, in part, to the influx of Ca^{2+}_e; these perturbations in $[Ca^{2+}]_i$ contribute to MeHg-induced cytotoxicity. To examine the extent to which Ca^{2+}_e contributes to decreased viability in MeHg toxicity of DRG, we exposed primary mouse DRG to MeHg in the presence and absence of Ca^{2+}_e. DRG (T11-L6) were isolated from 4-6 week-old C57BL/6J mice, enzymatically and mechanically dissociated, and plated as heterogeneous cultures for experiments; experiments were carried out within 24 hours of isolation to prevent dedifferentiation of neuronal subtypes. Cells were exposed to 200 nM, 500 nM, 1μM, or 2 μM MeHg in Hepes Buffered Saline (HBS) for 30 minutes, and viability was assessed at either 1 hr or 4 hrs following MeHg exposure using a commercial cell viability assay. For experiments carried out in the absence of Ca^{2+}_e, Ca^{2+} was excluded from the HBS and EGTA was added to chelate trace amounts of Ca^{2+}. Viability of DRG was reduced 1 hr following exposure only at high [MeHg], as compared to controls. In contrast, all [MeHg] reduced DRG viability at 4 hrs post-exposure. Loss of DRG viability was both [MeHg]- and time-dependent in standard HBS. With the removal of Ca^{2+}_e, [MeHg]-dependence remained at only high [MeHg] and time-dependence was lost; viability remained significantly reduced at high [MeHg] 1 hr post-exposure. However, viability at 4 hrs was reduced only at 2 μM MeHg. Removal of Ca^{2+}_e increased DRG viability at intermediate [MeHg] 4 hrs post-exposure. In summary, reduction of DRG viability occured at high [MeHg] at 1 hr post-exposure in a Ca^{2+}_e-independent manner. In contrast, the loss of DRG viability at 4 hrs post-exposure is measureable at all [MeHg] and is Ca^{2+}_e-dependent. These results show that decreased DRG viability due to Ca^{2+}_e only occurs at later time points, and there may be another mechanism that is contributing to reduced DRG viability at earlier time points. Supported by NIH grant R01ES03299 and Michigan State University College of Veterinary Medicine.

Disclosures: E. Formiller: None. **H. Hannon:** None. **W.D. Atchison:** None.

Poster

157. Spinal Cord Injury: Motoneuron Excitability

Location: Hall A

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Topic: D.10. Spinal Cord Injury and Plasticity

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NIH COBRE Grant 1P20GM103642-01A1

Title: The modulatory effects of caffeine on the intrinsic properties of spinal lateral motoneurons

Authors: *M. S. RIVERA OLIVER[1], Y. ALVAREZ-BAGNAROL[2], M. DIAZ-RIOS[3]; [1]Univ. of Puerto Rico-Institute of Neurobio., San Juan, PR; [2]Anat. and neurobiology, Inst. of Neurobio., San Juan, Puerto Rico; [3]Anat. and Neurobio., Inst. of neurobiology, San juan, Puerto Rico

Abstract: Caffeine is the most consumed psychoactive drug worldwide. It produces similar behavioral effects as other classical psychostimulants, such as cocaine and amphetamines, mainly increasing motor activation, arousal, and reinforcing effects related to neural reward systems. Caffeine is known to be a non-selective adenosine receptor antagonist (A1/A2a mainly). Most of the studies assessing the effects of caffeine and/or adenosine receptor agonists and antagonists on locomotor behavior have been performed on freely behaving rodents using systemic administration of these drugs that can activate multiple neural pathways making extremely difficult to study specific mechanisms of action. We propose to elucidate the cellular mechanisms by which caffeine modulates the intrinsic membrane properties of spinal lateral motoneurons, which are an essential component of the lumbar neural network producing hindlimb locomotion in mammals, through bath perfusion onto spinal cord slices, which have significant components of the spinal network controlling locomotion. Recent data from our laboratory from extracellular recordings on spinal lumbar nerves in the presence of serotonin (5-HT), NMDA and dopamine (DA), which are known to elicit a fictive locomotor pattern, shows that caffeine modulates motor activity by enhancing the bursting properties of motoneurons (Acevedo et al. 2015). Thus, we studied the neuromodulatory effects of caffeine and other adenosine receptor antagonists on the intrinsic properties of spinal lateral motoneurons using pharmacological blockade and perforated patch clamp recordings. The application of 5-HT and NMDA, in the presence of synaptic blockers of inhibitory neurotransmission depolarized the membrane potential of most motoneurons reversibly. The addition of caffeine (50μM), in the

presence of 5-HT and NMDA, significantly depolarized the membrane potential (~10-15mV), hyperpolarized the action potential (AP) threshold and decreased the AP after-hyperpolarization (AHP) in 70% of the recorded lateral motoneurons. In 17% of the recorded lateral motoneurons caffeine showed an inhibitory effect by increasing the AHP and no effect in the AP threshold. Finally, in 13% of the recorded lateral motoneurons tha application of caffeine did not produce any significant effects on any of the parameters measured. The excitatory effects produced by caffeine on most of the lateral motoneurons suggest that they could be a primary target for the neuromodulatory effects of caffeine in the lumbar region of the spinal cord.

Disclosures: M.S. Rivera Oliver: None. **Y. Alvarez-Bagnarol:** None. **M. Diaz-Rios:** None.

Poster

157. Spinal Cord Injury: Motoneuron Excitability

Location: Hall A

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NINDS R01 NS079751

Foundation for Physical Therapy - PODS II Scholarship

Title: Supraspinal changes following human spinal cord injury contribute to altered neural activation strategies during dynamic contractions

Authors: *H. E. KIM[1], L. M. ROGERS[2], D. M. CORCOS[3], T. G. HORNBY[4];
[1]Grad. Program in Neurosci., Univ. of Illinois At Chicago, Chicago, IL; [2]Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [3]Physical Therapy and Human Movement Sci., Northwestern Univ., CHICAGO, IL; [4]Physical Therapy, Univ. of Illinois, CHICAGO, IL

Abstract: *Introduction:* Recent data suggest subjects with incomplete spinal cord injury (SCI) generate greater central drive of both the knee extensors and plantarflexors during lengthening maximal voluntary contractions (MVCs) than during isometric or shortening MVCs. Several lines of evidence link these unexpected gains in central drive during lengthening contractions to spinal mechanisms, including increased Ia excitation of agonists. The degree to which supraspinal changes following SCI may contribute to unique activation strategies during dynamic contractions is still unknown. The current study's aims were to investigate cortical and corticospinal contributions to specific control of dynamic contractions following SCI. *Methods:* Single-pulse transcranial magnetic stimulation (TMS) was used to probe modulation of peak-to-

peak motor evoked potential (MEP) amplitudes and cortical silent period (CSP) durations during lengthening and shortening contractions of the tibialis anterior in 8 SCI and 8 healthy control subjects. *Results:* Active motor thresholds during low-level contractions (10-30% MVC) were higher in SCI than controls (46.5 ± 10.5% vs 31.4 ± 14.6% of maximum stimulator output; $p < 0.05$). SCI subjects demonstrated smaller MEPs (normalized to maximal M-waves) than controls during submaximal (75% MVC) lengthening (0.41 ± 0.24 vs 0.66 ± .21 for SCI and controls, respectively; $p < 0.05$) and shortening contractions (0.36 ± .20 vs 0.64 ± 0.16; $p < 0.01$). Contraction type-dependent differences in modulation of MEPs were not detected within either group. However, there was a trend for larger lengthening than shortening MEPs in the SCI group ($p = 0.07$). SCI subjects demonstrated similar CSP durations during lengthening and shortening submaximal contractions (140 ± 29 and 152 ± 34 ms for lengthening and shortening, respectively; $p > 0.05$). Consistent with previous literature, however, controls demonstrated shorter CSPs during lengthening than shortening contractions (187 ± 79 vs 210 ± 85 ms; $p < 0.05$), indicating decreased intracortical inhibition during lengthening. Similar comparisons of CSPs were observed during MVCs. *Conclusion:* Overall, corticospinal excitability was decreased in SCI subjects compared to controls, most likely due to impaired transmission between pyramidal and spinal neurons. Differences in modulation of CSPs across contraction types between SCI and controls also suggest specific cortical changes post-chronic SCI. In the context of recent findings, the current study suggests changes at spinal and supraspinal levels following SCI alter the neural activation strategies used to control dynamic contractions.

Disclosures: **H.E. Kim:** None. **L.M. Rogers:** None. **D.M. Corcos:** None. **T.G. Hornby:** None.

Poster

157. Spinal Cord Injury: Motoneuron Excitability

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Topic: D.10. Spinal Cord Injury and Plasticity

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MEXT/JSPS KAKENHI 23500617

MEXT/JSPS KAKENHI 26120002

Title: Galvanic vestibular stimulation is available to induce long-term potentiation of indirect cortico-motoneuronal excitation in a relaxed arm muscle in humans

Authors: *S. SUZUKI[1], T. NAKAJIMA[1], S. IRIE[1], Y. MASUGI[2], T. KOMIYAMA[3], Y. OHKI[1];
[1]Kyorin Univ. Sch. of Med., Tokyo, Japan; [2]Univ. of Tokyo, Tokyo, Japan; [3]Chiba Univ., Chiba, Japan

Abstract: We previously reported that repetitive combined stimulation (RCS) of pyramidal tracts and peripheral nerve can induce long-term potentiation (LTP) of indirect cortico-motoneuronal (C-M) excitation, which could be mediated by cervical propriospinal neurons (PNs) in humans. However, this procedure has a limitation for clinical application, since the LTP could be induced only when the target muscle was voluntarily contracted. We hypothesized that vestibular stimulation, which is known to activate cervical interneurons in animals, can be substituted for voluntary contraction to induce LTP in the indirect C-M excitation. Healthy volunteers, who all gave written informed consent, were seated with recording surface electromyogram of the right biceps brachii (BB). For RCS, transcranial magnetic stimulation (TMS) to the contralateral motor cortex (arm area) was delivered, in conjunction with electrical stimulation of the right ulnar nerve, at 0.2 Hz for 10 min (120 pairs). Interstimulus interval was set at 10 ms (TMS behind), which gave converging inputs in upper cervical segments. During RCS, BB was usually kept relaxed, and bilateral bipolar galvanic vestibular stimulation (GVS) was sometimes delivered. Four types of the interventions were conducted in separate sessions; (1) RCS alone, (2) RCS with cathodal GVS (right electrode cathode), (3) RCS with anodal GVS, and (4) RCS with weak voluntary contraction of BB. To evaluate effects of the interventions, motor-evoked potentials (MEPs) induced by TMS were obtained from BB. After the intervention with RCS alone, MEPs in BB were significantly suppressed, and this suppression lasted for ~60 min. In contrast, RCS with cathodal or anodal GVS induced LTP in MEP, which was very similar to that after RCS with BB contraction. The potentiation was not observed in the initial part of MEP. The spatial facilitation effect induced by the combined stimulation was significantly enhanced after RCS with GVS intervention. Our observations suggest that RCS with GVS could efficiently induce LTP in the indirect C-M excitation, which may be mediated by cervical PNs, even without voluntary contraction.

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Poster

157. Spinal Cord Injury: Motoneuron Excitability

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Title: Effects of sensorimotor rhythm modulation on the flexor carpi radialis H-reflex

Authors: *A. K. THOMPSON[1], H. CARRUTH[2], R. HAYWOOD[2], N. J. HILL[3], W. A. SARNACKI[4], D. MCFARLAND[4];
[1]Dept. of Hlth. Sci. and Research, Col. of Hlth. Professions, Med. Univ. of South Carolina, Charleston, SC; [2]Univ. of Glasgow, Glasgow, United Kingdom; [3]Burke Med. Res. Inst., White Plains, NY; [4]Ctr. for Adaptive Neurotechnologies, Wadsworth Ctr., Albany, NY

Abstract: Over the past several decades of non-invasive brain-computer interface (BCI) research, it has become apparent that people can learn to control sensorimotor rhythms (SMR) of the electroencephalogram through a series of training sessions; people can voluntarily modulate SMR amplitudes in the mu (8-12 Hz) and/or beta (18-26 Hz) frequency bands over the sensorimotor cortex (Electroencephalogr Clin Neurophysiol 1991:78: 252-259; 1994:90: 444-449). Such BCI-based training of SMR might help to improve motor function recovery in people with CNS disorders by guiding activity-dependent brain plasticity (Lancet Neurol 2008;7:1032-1043). Since activity-dependent brain plasticity is thought to guide spinal cord plasticity in motor skill learning (Neuroscientist 2010;16:532-549), it is possible that BCI-based SMR training could affect spinal reflex excitability. If so, it could provide a potential therapy for people with CNS disorders affecting motor control. To test this hypothesis, we are currently investigating the effects of SMR (mu-rhythm) modulation on the H-reflex of the flexor carpi radialis (FCR) muscle. To date, 4 adult subjects with no known neurological conditions and 2 subjects with chronic incomplete spinal cord injury (SCI) have been studied. Subjects are trained using a BCI-based cursor control task to increase (SMR-up) and decrease (SMR-down) the mu-rhythm amplitude over the right hand/arm area (i.e., at/around the C3 or CP3 electrode locations over the left hemisphere). Once fully trained to control the SMR amplitude over >10 training sessions, subjects then perform the same SMR cursor task while the H-reflex is elicited in the right FCR muscle using electrical median nerve stimulation. H-reflex trials occur during SMR-up trials, SMR-down, or in-between SMR trials at random intervals. So far, in all four healthy subjects, the H-reflex was significantly larger during SMR-up trials, and smaller during SMR-down trials, as compared with the in-between trials. In one of the two subjects with SCI, the results were similar to those in healthy subjects, whereas in the other subject with SCI there was no clear

difference in the H-reflex between SMR-up and -down trials. Interestingly, in both subjects with SCI, spastic EMG activity in the FCR (i.e., clonus and spontaneous low-frequency firing) progressively decreased during the cursor task. The results to date suggest that SMR control training can influence spinal reflex excitability. They also support the possibility that SMR training may be developed as a therapeutic technique to aid motor control recovery in people with spinal cord injury or other central nervous system disorders.

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Poster

157. Spinal Cord Injury: Motoneuron Excitability

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University of Oklahoma Undergraduate Research Opportunity Program awards

M. Blanche Adams and M. Frances Adams Scholarship

Title: Innocuous flexion reflex shows windup, mediated partly by L-type calcium channels

Authors: K. P. JOHNSON, S. M. TRAN, E. A. SIEGRIST, M. S. ELSON, *A. BERKOWITZ; Dept. of Biol., Univ. Oklahoma, Norman, OK

Abstract: Windup is a form of multisecond temporal summation in which identical stimuli, delivered seconds apart, trigger increasingly strong neuronal responses. Windup is correlated with activation of a class of sensory neurons (C fibers) that have unmyelinated, slow-conducting axons. Most, but not all, such sensory neurons are nociceptive. Windup of spinal cord dorsal horn neurons and of leg withdrawal reflex (flexion reflex) has generally been linked to nociceptive processing. In these neurons, L-type calcium channels have been suggested to play an important role in the production of windup. We attempted to evoke windup using innocuous stimuli and examined the role of L-type calcium channels in producing windup *in vivo*, using the

L-type Ca2+ channel antagonist, nifedipine. We evoked flexion reflex *in vivo* in spinal cord-transected and immobilized red-eared turtles (Trachemys scripta elegans), using taps to the dorsal hind leg foot with a von Frey filament or electrical stimulation of the foot skin. The hip flexor nerve was recorded during flexion reflex and the flexion reflex response integrated amplitude was used for analysis. We found that we could consistently evoke windup using innocuous (4-g) mechanical or weak electrical stimuli. Application of 100 μM nifedipine to the spinal cord significantly reduced the amplitude of a single flexion reflex (evoked with a 10-g von Frey filament or stronger electrical pulses) and also significantly reduced windup of innocuous flexion reflex evoked by weak mechanical and electrical stimuli. We have shown that innocuous flexion reflex exhibits windup, using both mechanical and electrical stimulation of the foot skin, and that L-type calcium channels contribute to this windup, as they have previously been shown to do for noxious stimulation. Our findings suggest that multisecond temporal summation mechanisms may contribute to normal sensory processing of both noxious and innocuous stimuli. This work was supported by Oklahoma Center for the Advancement of Science and Technology and National Science Foundation awards to A.B., University of Oklahoma Undergraduate Research Opportunity Program awards to E.A.S., K.P.J., and S.M.T., and an M. Blanche Adams and M. Frances Adams Scholarship to M.E.

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Poster

157. Spinal Cord Injury: Motoneuron Excitability

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Support: NIH 1P41EB018783

NS069551

Title: Characteristics of the soleus h-reflex in middle-age people

Authors: *L. MCCANE[1], A. K. THOMPSON[2], Y. MAKIHARA[3], W. B. HAUG[1], J. R. WOLPAW[1,4];
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Abstract: Many studies have examined changes in the soleus H-reflex with age. Most have focused on H-reflex differences between young and old subject groups; characterization of H-reflexes in middle-aged (i.e., ~40-60 year-old) people is generally lacking. CNS damage commonly occurs in this age group; the current average age at which spinal cord injury occurs is 42 (www.mscisc.uab.edu), and up to 10% of strokes may occur in individuals under age 45. Thus, knowledge of H-reflexes in this group may contribute to development and/or evaluation of therapeutic methodologies. Thus, we are studying the soleus H-reflex in healthy, neurologically normal middle-age adults. To date, we have studied 14 middle-aged healthy adults (7 women and 7 men, mean age 45.9±7.5 SD, range 35-57). The soleus H-reflex/M-wave recruitment curve is obtained while the subject stands and maintains a defined level of EMG activity (typically natural standing level) (Thompson, et al., J Neurosci 2009:29:5784-5792). H-reflex and M-wave are measured at ~10 different intensities (range 0.75-7mA) from below H-reflex threshold to just above maximum M-wave (Mmax). Four trials are averaged at each intensity to determine the recruitment curve, the maximum H-reflex (Hmax), and the maximum M-wave (Mmax) (measured peak-to-peak). The results in the 14 subjects to date are: mean(±SD) Mmax 6.31(±2.5) mV; and mean(±SD) Hmax 3.20(±1.84) mV (similar to those during standing for a middle-age group in another recent study (mean age 56.4 years; mean Mmax 7.79 mV; mean Hmax 3.75mV) (Raffalt, et al., Muscle&Nerve 2015:51:419-425)). While the mean values are similar to those in previous aging studies (e.g., Kido et al., Can J Physiol Pharmacol 2004:82:238-248), Mmax and Hmax vary widely (i.e., Mmax range: 1.60-10.1 mV; Hmax range: 0.44-6.5 mV), resulting in a wide range of Hmax/Mmax ratios (0.12-0.87; mean±SD:0.50±0.20) with no significant dependence on age (Pearson's r = -0.02). These results indicate high variability in spinal reflex excitability in normal middle-aged subjects; this may result from differences in physical activity, life-style, and aging. They further suggest that studies using the H-reflex to investigate spinal plasticity in the normal and the damaged CNS should take such variability into consideration in protocol development and in data evaluation. Indeed, differences in baseline reflex values may correlate with differences in responses to specific therapeutic interventions, and thus may help to guide the design of individual treatment protocols.

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Poster

157. Spinal Cord Injury: Motoneuron Excitability

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 157.07/O44

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH grant K01 HD079584

Title: Effect of split-belt walking on cortical and spinal excitability

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Abstract: Split-belt walking is a well-studied locomotor adaptation paradigm that has been tested in children, young and elderly adults, as well as patients with stroke and Parkinson's disease. Split-belt walking involves treadmill walking with one belt going twice as fast as the other. Asymmetric stepping practice during split-belt walking may challenge the neural circuitry beyond existing gait interventions through error-based motor adaptation. In a recent study, a single session of split-belt walking was shown to enhance cortical excitability of lower extremity muscles, suggesting that split-belt walking may have potential as a rehabilitation paradigm to up-regulate the corticomotor pathways. However, whether split-belt walking modulates spinal excitability is unknown. The aim of this study was to evaluate changes in cortical excitability (measured using transcranial magnetic stimulation (TMS)) and spinal excitability (measured using H-reflexes) of the soleus muscle in response to a single session of split-belt gait training compared to a control walking task (regular or tied-belt treadmill walking). Thirteen able-bodied individuals (3 males and 10 females, ages 24-31) participated in 2 test sessions. During each session, measurements of cortical and spinal excitability were obtained before and after 20 minutes of split-belt treadmill walking or regular walking (tied-belts). EMG data were recorded from the Soleus muscle of the right leg (fast leg during split-belt walking). The primary measure of cortical excitability was the peak to peak amplitude of motor evoked potential in response to TMS delivered to the soleus hotspot at 125% active motor threshold. The primary measure of spinal excitability was the ratio of the maximum H-reflex and peak M-wave (H-max/M-max ratio) obtained via peripheral electrical stimulation of the tibial nerve. Our preliminary results to date show a trend toward decreased cortical excitability ($p=.07$) and significant reduction in H-max/M-max ratio ($p=.04$) of the soleus muscle following a single-session of split-belt walking. To our knowledge, our study is the first demonstration of changes in spinal excitability after a single-session of split-belt walking. The depression in spinal excitability observed after a single-session of split-belt walking may be reflective of the recalibration of neural pathways controlling locomotion and increase in segmental (local) and descending (supraspinal) inhibitory influences on the spinal segmental reflex circuitry. Ongoing studies are investigating the mechanisms and clinical implications of the modulation of corticospinal circuitry induced by split-belt walking.

Disclosures: T.M. Kesar: None. **M.R. Borich:** None. **M. Sabatier:** None.

Poster

157. Spinal Cord Injury: Motoneuron Excitability

Location: Hall A

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Program#/Poster#: 157.08/O45

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH R21 NS087320

The Grainger Foundation

Title: *In vivo* intraspinal neurochemical monitoring for characterization of underlying mechanisms of stimulation-evoked motor responses following spinal cord injury

Authors: *A. A. MENDEZ[1], E. NICOLAI[2], P. J. GRAHN[3], J. K. TREVATHAN[3], K. E. BENNET[2,4], S.-Y. CHANG[2], J. L. LUJAN[2,5], K. H. LEE[2,5];
[2]Dept. of Neurologic Surgery, [3]Mayo Grad. Sch., [4]Div. of Engin., [5]Dept. of Physiol. and Biomed. Engin., [1]Mayo Clin., Rochester, MN

Abstract: Introduction: Intraspinal microstimulation (ISMS) has been used to evoke motor responses following spinal cord injury. However, functional recovery remains far from optimal. In order to optimize stimulation-evoked limb movement, it is paramount to understand the neurochemical interactions associated with stimulation-evoked motor responses. To date, neurochemical monitoring within the spinal cord has focused on analysis of neurotransmitter presence using *in vitro* or off-line *in vivo* techniques (e.g., microdialysis). In this study, we demonstrate the feasibility of fast scan cyclic voltammetry (FSCV) to measure real-time stimulation-evoked and spontaneous (stimulation-OFF) neurotransmitter dynamics in the spinal cord of anesthetized, spinalized rodents. Methods: Rodents underwent a bilateral laminectomy to expose the lumbar enlargement (L1-L5) of the spinal cord. A stimulating electrode was implanted into the ventral horn at the L2 vertebral level to evoke hind limb extension. Additionally, a carbon fiber microelectrode was implanted within the ipsilateral ventral horn and approximately 5mm caudal to the stimulating electrode. ISMS required to evoke hind limb extension was applied for 2s, followed by 30s of rest, for up to 15 minutes. This ISMS ON phase was followed by a stimulation OFF phase for 10 minutes. After completing the described stimulation sequence, the carbon fiber microelectrode was repositioned within the ventral horn to detect additional neurochemical responses. Neurochemical monitoring was performed using FSCV for the duration of the experiment. Results: Neurochemical responses detected during both the ON and OFF-stimulation phases demonstrated seemingly random spontaneous release of electroactive analytes with characteristics similar to adenosine, as indicated by two current oxidation peaks at 1.4 and 1 V. In addition, neurochemical activity during the ON-stimulation phase demonstrated electrically evoked chemical changes that have yet to be identified.

Conclusions: These preliminary results, although inconclusive for specific analytes involved in ISMS evoked movement, demonstrate the feasibility of detecting real time *in vivo* spinal neurochemical events during stimulation-evoked restoration of movement. Thus, further electrochemical and pharmacological work is required to characterize neurochemical dynamics underlying stimulation-evoked restoration of movement.

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Poster

157. Spinal Cord Injury: Motoneuron Excitability

Location: Hall A

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant HD32571

Georgia Institute of Technology

NWO-ALW grant 864-10-011

Title: Preservation of reflex pathways following muscle adaptations accompanying tendon transfer

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Abstract: Tendon transfer surgery has been used to improve motor function in patients with weak or paralyzed muscles. In some cases function does not adequately recover. We asked whether this failure is due to increased myofascial force transmission from the transferred muscle or due to failure of neural circuits to adapt to the altered function of the transferred muscle. We transferred distal tendons of the plantaris muscle (PLANT) using the Pulvertaft Method to the severed distal tendons of tibialis anterior muscle (TA) in eight cats. In half of the animals TA was partly (approx. 50%) resected and in the other half TA and extensor digitorum longus (EDL) were denervated during the transfer surgery. Following a four-month survival period, we investigated the strength and distribution of inhibitory force feedback among muscles of the hindlimb as well as the anatomy of PLANT and TA. The organization of force dependent

feedback was not distinguishable from the distribution observed in the contralateral limb and in control animals. Preservation of normal feedback organization was observed despite the substantial atrophy of PLANT in the four cases where the transferred tendon had ruptured. Substantial alterations were observed, however, in the connective tissue elements associated with the donor and recipient muscles. In cases with partial resection of TA, the muscle regenerated, including a new tendon that joined the transferred tendon near its original insertion. The regenerated TA also provided strong reciprocal inhibition to the gastrocnemius muscle as observed in control animals. In addition, we found strong connective tissue linkages between PLANT and the calcaneous. We concluded that the response to the agonist-to-antagonist tendon transfer was to restore previous mechanical connections of the muscles. Despite a variety of tendon and muscle adaptations, no evidence of alterations in neural circuits was observed.

Disclosures: **H. Maas:** None. **M.A. Lyle:** None. **E. Kajtaz:** None. **T.R. Nichols:** None.

Poster

157. Spinal Cord Injury: Motoneuron Excitability

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH grant NS082463

Barrow Neurological Foundation

Title: Changes in Ia EPSPs and effective synaptic currents in hindlimb motoneurons following incomplete spinal cord injury

Authors: V. V. TURKIN, D. O'NEILL, *T. M. HAMM;
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Abstract: What adaptations occur in spinal cord motor networks following incomplete spinal cord injury (SCI)? Do synaptic projections from muscle proprioceptors to motoneurons increase in strength following injury, compensating for loss of descending drive? To answer this question, we have compared Ia postsynaptic potentials and effective synaptic currents (I_N; Heckman and Binder, 1988) in gastrocnemius-soleus (GS) motoneurons of rats with chronic SCI and sham-operated controls. Female Long-Evans rats received a mild-moderate (170 kdyne) impact injury at thoracic vertebra 9 or a sham surgery. Terminal experiments were performed 8-10 weeks following injury/surgery after recovery of locomotor performance had reached a plateau. Tonic

Ia EPSPs were produced by vibration of the GS muscle at 200 Hz. In sham-operated controls, Ia EPSP amplitude increased with input resistance ($r = 0.74$, $p = 0.005$); I_N was not correlated with input resistance in the small sample used in this analysis ($N = 12$). The amplitudes of tonic Ia EPSPs and I_Ns recorded in low-input resistance motoneurons of SCI animals were more variable than those recorded in sham animals ($F = 6.27$, $p = 0.03$; $F = 5.65$, $p = 0.04$, respectively) and included larger values of Ia EPSPs and I_Ns than observed in the sham-operated animals. Mean Ia EPSPs and I_Ns tended to be larger at comparable values of R_N in SCI than in sham-operated animals, although these trends did not reach statistical significance ($p = 0.07$, $p = 0.11$, respectively). Too few cells were recorded in large R_N-cells of SCI animals to assess the size-related distribution of Ia synaptic inputs. These preliminary results suggest that Ia postsynaptic currents and their associated postsynaptic potentials increase in large, low-input resistance motoneurons following moderate spinal cord injuries associated with substantial recovery of locomotor function.

Disclosures: **V.V. Turkin:** None. **D. O'Neill:** None. **T.M. Hamm:** None.

Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH/NINDS grant R21NS084528

NIH/NINDS grant P01NS084974

Mayo Clinic Foundation

Mayo Graduate School

ALS Association

Target ALS

Title: Expression of C9ORF72 repeat expansions in mice causes behavioral impairments mimicking c9FTD/ALS clinical features

Authors: ***J. CHEW**[1,2], K. JANSEN-WEST[2], T. GENDRON[2], M. PRUDENCIO[2], C. W. LEE[2], Y.-J. ZHANG[2], M. CASTANEDES-CASEY[2], A. KURTI[2], M. YUE[2], L. ROUSSEAU[2], M. M.

MURRAY[2], J. TONG[2], L. DAUGHRITY[2], E. PERKERSON[2], D. W. DICKSON[1,2], J. D. FRYER[1,2], L. PETRUCELLI[1,2];
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Abstract: The C9ORF72 hexanucleotide repeat expansion (GGGGCC) is the major genetic cause underlying both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Extensive efforts to study the pathogenic mechanisms associated with "c9FTD/ALS" have been hindered by a lack of animal models recapitulating key disease features. Here, we report the development and characterization of a novel model in which mice express an expanded repeat expansion ((G4C2)66) or non-expanded repeat ((G4C2)2) as control via somatic brain transgenesis mediated by adeno-associated virus (AAV). After 2 and 6 months of age following intracerebroventricular (ICV) injection on post-natal day 0, mice were subjected to a battery of behavioral and motor performance tests. Two months old (G4C2)66-mice did not exhibit any significant behavioral abnormalities compared to control (G4C2)2-mice, however, behavioral and motor deficits were detected in the 6 month cohort expressing expanded repeats. In addition, 6 months old (G4C2)66-mice exhibited decreased brain weight compare to controls suggestive of brain atrophy while this feature was not observed between mice of younger cohorts. Consequently, brains and spinal cord of 2 months and 6 months old mice will be evaluated for key c9FTD/ALS pathological features including the accumulation of RNA foci, repeat-associated non-ATG (RAN) translated polypeptides, phosphoTDP-43 inclusions and neuronal loss to further examine whether the neuropathological burden will correlate with behavioral deficits. In summary, these data suggest that the expression of expanded repeats in the murine CNS induces progressive behavioral abnormalities mimicking c9FTD/ALS clinical phenotypes and that further characterization of this model will be a useful tool in elucidating the pathogenic role of expanded repeat expression in c9ALS/FTD.

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Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 158.02/P1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NS077863

NIH NS063535

Craig H. Neilsen Postdoctoral Fellowship

NIH AR053608

Title: Chronic EMG activity reveals early changes in muscle activation in treadmill running SOD1 mice

Authors: *K. A. QUINLAN[1], E. KAJTAZ[1], J. D. CIOLINO[4], R. D. MANUEL[1], M. C. TRESCH[1,5,2], C. J. HECKMAN[1,3,2], V. M. TYSSELING[3,1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease. A common model of ALS, the SOD1 mouse, presents with prominent hindlimb deficits beginning at postnatal day (P)90. To improve early diagnosis and understanding of disease progression, this study measured activity in hindlimb muscles during treadmill running over 8 weeks leading up to overt symptom onset, postnatal days (P) 55-100. Chronic electromyogram (EMG) electrodes were implanted into vastus lateralis (VL), biceps femoris posterior (BFP), lateral gastrocnemius (LG), and tibialis anterior (TA). Results were assessed using linear mixed models to account for numerous variables and repeated measures. Effects of the SOD1 mutation were mainly observed in three parameters: burst peak amplitude, mean intermuscular phase, and burst shape (skew). Peak amplitude is larger in BFP in mutant mice, while burst peaks in TA and LG increased in combination with other factors (mutation interacts with both treadmill incline (TA and LG), and age (LG)). Phase and skew are related parameters that indicate changes in the timing of muscle activation during locomotor behavior. In SOD1 mice, BFP and LG advanced in phase and skew shifted toward the early portion of the burst, while both parameters shifted in SOD1 VL in combination with treadmill incline. TA was relatively unaffected, suggesting extensors are better suited for detecting early disease-related changes. Future investigations should pursue exploiting these parameters in humans for early diagnosis of ALS, as well as revealing the underlying mechanistic changes in the neural control of muscle activation during disease progression.

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Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 158.03/P2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Les Turner ALS Foundation

Title: Connecting genes to pathways and networks in an effort to reveal the basis of selective motor neuron vulnerability

Authors: *P. KURU, P. H. OZDINLER;
Neurol., Northwestern Univ. Sch. of Med., Chicago, IL

Abstract: Even though motor neurons undergo progressive degeneration in motor neuron diseases, they differ by the motor neuron pools that display primary vulnerability. For example in ALS, different from hereditary spastic paraplegia (HSP) or primary lateral sclerosis (PLS) and spinal muscular atrophy (SMA), both the cortical and the spinal motor neurons are affected. To date numerous genetic mutations are identified as “causative” for ALS, SMA and HSP/PLS. We hypothesize that if a mutated gene causes a disease, then its protein product must be extremely important for the function and health of the motor neurons that show primary vulnerability. This may explain why some mutations lead to upper and some lead to lower motor neuron death. The goal of this study is to investigate the link between genes, proteins, and networks in an effort to understand the cellular and molecular basis of selective vulnerability in motor neuron diseases. In order to identify the networks and cellular pathways that are affected we focus on the binding partners of the proteins that are encoded by the mutated genes. We first generated an extensive data set for binding partners of proteins, whose mutated versions were reported to “cause” ALS, HSP, PLS and SMA. We performed systems analysis to reveal how these proteins interact,, which canonical pathways they are mainly involved in, and which cellular networks are critically important. We then identified common and unique pathways and networks in motor neuron diseases. Our preliminary studies reveal that underlying cellular events for upper and lower motor neuron vulnerability are distinct, and there are novel interaction domains for upper and lower motor neurons. However, common events, such as glucocorticoid receptor signaling occurs in all motor neuron diseases, which suggests the involvement of immune reaction, especially towards end-stage. This systems level analysis, using mutations that cause diseases in which upper and lower motor neurons are primarily affected will shed light onto common and unique mechanisms that are responsible for their degeneration. Our findings have the potential to reveal key signaling pathways responsible for selective neuronal vulnerability, and it will also define new targets for drug discovery efforts.

Disclosures: **P. Kuru:** None. **P.H. Ozdinler:** None.

Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 158.04/P3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Les Turner ALS Foundation

Les Turner ALS Foundation/Herbert C. Wenske Foundation Professor

Vena E. Schaff ALS Research Fund

Ride for Life

S.W. Ranson Fund in ALS Research

Foglia Family Foundation

NS050641Genetics of ALS

Title: CHCHD10 gene mutation causes ALS with complex mechanisms

Authors: *J. YAN[1], K. B. AHMETI[2], N. A. SIDDIQUE[2], E. B. RYAN[2], T. J. LUKAS[2], L. M. KINSLEY[2], Y. SHI[2], Y. YANG[2], N. MILLER[2], N. J. CORBETT[3], D. NICHOLSON[3], Y. MA[2], H.-X. DENG[2], T. SIDDIQUE[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal large motor neuron degenerative disorder. Using whole exome sequencing and linkage analysis, we found R15L mutation of CHCHD10 gene in a large FALS pedigree, then 4 more FALS out of 308 FALS families by Sanger sequencing, but not in 84 SALS with disease duration equal to or more than 5 years. R15L was not present in 1480 controls we sequenced. The five CHCHD10 R15L families had a total of 55 ALS patients, 16 obligate carriers. Onset of site were available from 22 ALS patients, 4 had lower extremities onset, 1 had trunk onset, and 17 had upper extremities onset, none had bulbar onset. Age of onset were recorded in 23 ALS patients, the average was at 55 years. 34.8% of the 23 Affected developed ALS in their 50's, 21.7% in 60's, 13% in 40's, 17.4% in 30's.The average of disease duration of 14 deceased ALS was 81.2 months .9 affected are still living. Muscle biopsy of a male R15L ALS patient had two typical ragged red fibers and two myofibers with rimmed vacuoles which were stained for NADH-TR. Confocal microscopy showed both wild type and R15L CHCHD10 mutant were expressed diffusely in the cytoplasm as well as the extended neuronal processes and mostly colocalized with mitochondria. Immunohistochemistry

showed remarkable, tangle like CHCHD10 immunoreactive protein aggregates in the cytoplasm and neuritis of spinal cord motor neurons. EM of R15L skin fibroblast showed swollen mitochondria body, broken cristae, homogeneous matrix and multivesicular bodies and vacuoles . R15L fibroblast genomic DNA showed multiple additional bands in Southern Blotting comparing to controls. R15L fibroblast mitochondria DNA copy number by real-time PCR was significantly less than those of controls. The mitochondria movement was analyzed in 137 mitochondria of 9 motor neurons expressing wild type CHCHD10 and 180 mitochondria of 12 motor neurons expressing R15L CHCHD10. The backward moving frequency of R15L mitochondria was significant less. R15L fibroblast Mitochondria oxygen consumption rate (OCR) by Seahorse XFe Analyzer was 296.3 ± 24 compared to 534.0 ± 21.90 in controls. Complex IV and V activity were significantly decreased in R51L fibroblast. It is suggested that CHCHD10 mutation causes ALS with very complex mechanism involving mitochondria. (Yan and Ahmeti made equal contribution to the work)

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Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

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Program#/Poster#: 158.05/P4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: UAMS startup funds

UAMS College of Medicine Research Council Pilot Grant

NIGMS IDeA award P30 GM110702

Title: Mutant profilin1 toxicity in the transgenic mouse model for ALS

Authors: *M. KIAEI[1], A. BASNAKIAN[2], M. COZART[2];
[1]Neurobiology/Dev Sci., [2]Pharmacology/toxicology, Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: The mechanism of neuronal degeneration and muscle atrophy in amyotrophic lateral sclerosis (ALS) is poorly understood. Current animal models have been helpful in defining disease development and identifying pathways and molecules potentially involved. This suggests

more models are needed to increase our understanding of molecular mechanisms responsible for motor neuron death. Recent discoveries of new genes linked to ALS will likely lead to new disease models and therapeutic targets. There are currently seven mutations in the profilin1 (PFN1) gene linked to fALS1-3. This argues that mutation in PFN1 causes ALS in humans. However, the mechanism of mutant profilin-1 toxicity and ALS pathogenesis remains unknown. To explore this relationship we have developed mouse models overexpressing mutant and wild-type (WT) human profilin1 (hPFN1) driven by mouse prion promoter. We chose the glycine to valine mutation at residue 118 (G118V) for our mutant hPFN1 mouse. These hPFN1G118V mice faithfully recapitulate ALS phenotype. We have now generated mice overexpressing WT human profilin1 (hPFN1WT). We found proteinopathy and multiple other abnormalities in our hPFN1G118V model while hPFN1WT mice are as viable and healthy as nontransgenic littermates. Here we report that lumbar spinal cords of symptomatic hPFN1G118V mice demonstrate a reduced F/G-actin ratio relative to WT mice, suggesting mutant profilin-1 may dysregulate actin polymerization. DNase I, which is another G-actin-binding protein and the most active apoptotic endonuclease, translocated to the nucleus in the hPFN1G118V mice. This DNase I redistribution in spinal cords correlated with the increase of nuclear DNA fragmentation measured using TUNEL assay indicating elevated cell death. We show mutant profilin-1 exists in the insoluble fractions of total spinal cord homogenates of PFN1G118V mice and most of proteins in the insoluble fraction are ubiquitinated. This confirms *in vitro* data published by Landers and colleagues and further suggests that aggregation of mutant profilin1 may contribute to neurotoxicity in ALS. In conclusion, we have developed two novel mouse models to investigate the role of mutant profilin-1 in ALS. In characterizing these models we have found multiple ALS-like pathologies in the hPFN1G118V mice while hPFN1WT mice show no pathology as expected. Supported by UAMS startup funds, UAMS College of Medicine Research Council, and NIGMS IDeA award P30 GM110702. References: 1Wu, C. H. et al.. Nature 488, 499-503 (2012). 2Ingre, C. et al. Neurobiology of aging 34, 1708 e1701-1706 (2013). 3Smith, B. N. et al. Neurobiology of Aging (2015) 36(3). 1602e17-27.

Disclosures: **M. Kiaei:** None. **A. Basnakian:** None. **M. Cozart:** None.

Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

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Program#/Poster#: 158.06/P5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: R01 NS088645

MDA294842

Title: New evidence for the role of dna repair defects in motor neuron diseases

Authors: *M. L. HEGDE;
Houston Methodist, Houston, TX

Abstract: Accumulation of genome damage including oxidized bases, single- and double-strand breaks, in affected brain cells has been linked to Motor neuron diseases including ALS whose underlying cause(s) are not completely understood. The transactive response DNA-binding protein-43 **(TDP-43),** is an hnRNP family protein whose intracellular aggregation has been etiologically implicated in Amyotrophic Lateral Sclerosis (ALS), a motor neuron degenerative disease affecting two per 100,000 people worldwide, and about 40% of other neurodegenerative diseases including Alzheimer's (AD) and Parkinson's disease (PD). Primarily involved in RNA processing, TDP-43 and related protein FUS also bind to DNA, but its DNA binding functions have not been investigated. While hereditary mutations in TDP-43/FUS have been linked to ALS, the molecular mechanism(s) of its pathology contributing to neuronal death are still unclear. *The unique feature of TDP-43 pathogenesis in ALS is its nuclear clearance and simultaneous cytoplasmic aggregation in affected motor/cortical neurons.* Furthermore, significant accumulation of genomic damage is observed in TDP-43-linked diseases and previous studies identified a key DNA repair protein 'Ku' in TDP-43 immunocomplex from human cells, raising the possibility of TDP-43's involvement in DNA damage repair, which led us to investigate this. We demonstrate that (1) TDP-43 stably interacts with DSBR proteins in neuroblastoma SH-SY5Y cells, which was enhanced after treatment with DSB-inducing radiation/bleomycin. (2) TDP-43 is recruited to the DSB sites neuronal cells, and (3) TDP-43's overall as well as nucleus-specific depletion markedly increased accumulation of DSBs in SH-SY5Y as well as motor neurons differentiated from hNSCs cells and sensitized the cells to radiation. (4) TDP-43 controls the recruitment of DSB repair protein 53BP1 at DSB sites and thus regulates non-homologous end joining pathway of DSB repair in neurons. These results are consistent with the dramatic accumulation of unrepaired DSBs in postmortem brains of ALS-affected human patients and a distinct nuclear clearance of TDP-43 in these affected neurons. Our more recent studies show that FUS, unlike TDP-43 is required for single strand break repair in neurons via activation Ligase III protein. In summary these results reveal that TDP-43 and FUS are critical components of genome damage response in the neurons whose loss of function(s) causing DNA repair deficiency is a key etiological factor in ALS and possibly other neurodegenerative diseases. (Supported by NIH/NINDS and Muscular Dystrophy Association).

Disclosures: M.L. Hegde: None.

Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 158.07/P6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Target ALS

Taube and Koret Foundation

Title: Identification of therapeutic targets for cytoskeletal defects in ALS

Authors: *A. JAVAHERIAN[1], P. GOYAL[1], M. ANDO[1,2], E. DANIELSON[2], C. FALLINI[2], J. LANDERS[2], S. FINKBEINER[1];
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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. Cytoskeletal regulation represents a good candidate for therapeutic intervention, as it plays an essential role in a variety of cellular processes including axonal transport and neuronal plasticity.

Disclosures: **A. Javaherian:** None. **P. Goyal:** None. **M. Ando:** None. **E. Danielson:** None. **C. Fallini:** None. **J. Landers:** None. **S. Finkbeiner:** None.

Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 158.08/P7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Target ALS

The Robert Packard Center for ALS Research

Amyotrophic Lateral Sclerosis Association

JHU Neuropathology Pelda fund

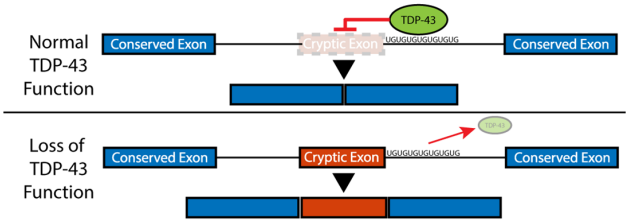
NIH Grant P50AG05146

Samuel I. Newhouse Foundation

Title: Tdp-43 suppression of non-conserved cryptic exons is compromised in amyotrophic lateral sclerosis and frontotemporal dementia

Authors: *J. LING, O. PLETNIKOVA, J. TRONCOSO, P. WONG;
Johns Hopkins Univ., Baltimore, MD

Abstract: Cytoplasmic aggregation of TDP-43, accompanied by its nuclear clearance, is a key common pathological hallmark of amyotrophic lateral sclerosis and frontotemporal dementia (ALS-FTD). However, a limited understanding of this RNA-binding protein (RBP) impedes the clarification of pathogenic mechanisms underlying TDP-43 proteinopathy. In contrast to RBPs that regulate splicing of conserved exons, we report here that TDP-43 suppresses the splicing of non-conserved cryptic exons. When TDP-43 is depleted from the cell, these cryptic exons are spliced into mRNAs, often disrupting their translation and promoting nonsense-mediated decay. Moreover, enforced repression of cryptic exons prevents cell death in TDP-43 knockout cells. Importantly, our work demonstrates that suppression of cryptic exons is impaired in brains of ALS-FTD, suggesting that this splicing defect underlies TDP-43 proteinopathy. TDP-43 may be the first member of a class of RBPs that serve to maintain the integrity of introns. Thus, our discovery not only has mechanistic and therapeutic implications for ALS-FTD and other human diseases that share TDP-43 pathology, but also reveals more fundamental principles regarding RNA splicing and the evolution of exon-intron structure.



Disclosures: **J. Ling:** None. **O. Pletnikova:** None. **J. Troncoso:** None. **P. Wong:** None.

Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 158.09/P8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: VA Career Development Award (CDA2) I01BX007080

Title: Identification of CDC7 mediated pathways regulating TDP-43 phosphorylation in simple models of ALS and FTLD-TDP

Authors: ***N. LIACHKO**[1,2], J. KUSHLEIKA[1], T. BIRD[1,2], B. KRAEMER[1,2];
[1]GRECC, VA Puget Sound Hlth. Care Syst., Seattle, WA; [2]Univ. of Washington, Seattle, WA

Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) are severe progressive neurodegenerative diseases characterized by lesions containing aggregated, hyperphosphorylated TDP-43. To study the cellular, molecular, and genetic underpinnings of TDP-43 mediated neurotoxicity in tractable model systems, we have developed *C. elegans* and mammalian cell culture models of TDP-43 proteinopathy. Expression of familial ALS-mutant TDP-43 in all *C. elegans* neurons causes severe motor dysfunction, and recapitulates some characteristic features of ALS and FTLD-TDP including decreased lifespan, neuronal degeneration, hyperphosphorylation and ubiquitination of TDP-43, and accumulation of detergent insoluble aggregates. We have shown that in *C. elegans*, phosphorylation of TDP-43 at serine residues 409/410 (S409/410) drives mutant TDP-43 toxicity. Using these models, we found the kinase CDC7 directly phosphorylates TDP-43 at S409/410. To identify genes upstream of CDC7 controlling TDP-43 phosphorylation, we have assembled and screened an RNA interference (RNAi) library targeting known CDC7 interacting, regulating and pathways genes. This library includes genes involved in cell cycle regulation, DNA damage response and repair, and chromatin maintenance. 85 candidate genes were

individually tested for modification of TDP-43 dependent behavioural phenotypes, and for changes in the phosphorylation status of TDP-43 by immunoblot. From this primary screen we have confirmed 4 genes that suppress TDP-43 phenotypes. Additional work on these genes will provide mechanistic insight into the environmental and cellular triggers of TDP-43 phosphorylation, and provide potential novel avenues for therapeutic interventions into TDP-43 proteinopathies such as ALS and FTLD-TDP.

Disclosures: **N. Liachko:** None. **J. Kushleika:** None. **T. Bird:** None. **B. Kraemer:** None.

Poster

158. Amyotrophic Lateral Sclerosis: Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 158.10/P9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Indian council of medical research, Govt. of India

Department of Bitechnology, Govt. of India

Title: Chitotriosidase, a putative biomarker augments neuroinflammation in sporadic amyotrophic lateral sclerosis

Authors: ***T. R. RAJU**[1], A. M. VARGHESE[2], M. GHOSH[2], P. S. MISHRA[2], K. VIJAYALAKSHMI[2], P. A. ALLADI[2], T. N. SATHYAPRABHA[2], A. NALINI[3];
[2]Neurophysiol., [3]Neurol., [1]Natl. Inst. Mentl Hlth. Neurosci, Bangalore, India

Abstract: Profuse increase (~20 fold; n=70) in the levels of Chitotriosidase (CHIT-1) in the cerebrospinal fluid (CSF) of patients with sporadic Amyotrophic Lateral Sclerosis (SALS; ALS-CSF) marks it a biomarker status. Additionally, CHIT-1 was found to be biologically active in view of the up-regulated enzyme activity (~16 fold; n=73). Expression of CHIT-1 specifically by microglia suggests a definitive role for the enzyme in the disease pathogenesis. The current study is aimed at investigating the effect of the recombinant CHIT-1 protein on spinal motor neurons and glial cells. Intrathecal administration of CHIT-1 (50, 100 and 200pg) to rat neonates resulted in microgliosis and astrogliosis in both white and grey matter of the spinal cord similar to what was seen following ALS-CSF administration. Qualitative observations revealed presence of several amoeboid microglial cells around the central canal. Several GFAP immunopositive astroglia bearing long processes were also seen around the central canal following CHIT-1 administration. CHIT-1 also induced loss of Choline Acetyl Transferase (ChAT) positive

neurons, similar to ALS-CSF at a dose of 500pg. Further, exposure of astroglial as well as the microglial cultures to ALS-CSF for 48hours resulted in a significant up-regulation of pro-inflammatory markers like IL-6, TNF-α, PGE-2 and COX-2, while the trophic factors namely, VEGF and GDNF were down regulated. These findings suggest that CHIT-1 in ALS-CSF induces microglial and astroglial activation accompanied by enhanced expression of inflammatory markers, resulting in neuroinflammation, which accentuates degeneration of motor neurons. In view of an initial surge of CHIT-1 activity which gradually decreases with progression of disease and its multifold increase observed in the definite ALS cases, it could be a potential target for therapeutic intervention at the early stages of the disease.

Disclosures: **T.R. Raju:** None. **A.M. Varghese:** None. **M. Ghosh:** None. **P.S. Mishra:** None. **K. Vijayalakshmi:** None. **P.A. Alladi:** None. **T.N. Sathyaprabha:** None. **A. Nalini:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.01/P10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NHMRC

Title: Untangling the dendritic network degeneration of upper and lower motor neurons in amyotrophic lateral sclerosis

Authors: ***M. J. FOGARTY**[1], E. W. H. MU[1], N. A. LAVIDIS[1], P. G. NOAKES[1,2], M. C. BELLINGHAM[1];
[1]Sch. of Biomed. Sci., Univ. of Queensland, Brisbane, Australia; [2]Queensland Brain Inst., Brisbane, Australia

Abstract: The progressive death of both upper and lower motor neuron (MN) populations in the brain and spinal cord is pathognomic for amyotrophic lateral sclerosis (ALS). Although evidence from animal and clinical studies suggest that elevation of excitatory synaptic inputs onto upper and lower MNs occurs prior to neuron loss, the timing and structural correlates of this degenerative pathology remains unclear. We examined pre-symptomatic (postnatal [P] day 25-35) , disease onset (P60-70) and mid-disease (P100-120) heterozygote hSOD1G93A(SOD1) and wild type (WT) control mice using Golgi-Cox impregnation to visualise the dendritic arbors of upper or lower MNs (10 animals per age/genotype). Golgi-impregnated neuronal arbors were

traced in Neurolucida using a 63x oil (1.4NA) objective on a Zeiss Axioplan 2 microscope with an automated stage. All analyses were two-way ANOVAs with Bonferroni post-tests and $p<0.05$ determined significant results. Spine densities were calculated as spines per 100 μm. We report significant dendritic degeneration and spine loss of layer V pyramidal neurons in the motor cortex from SOD1 mice at pre-symptomatic ages (31% reduction in dendritic length and a 51% decrease in spine density) and lumbar MNs by mid-disease (71% decrease in dendritic length, and 29% decrease in spine density). By contrast, increases in neuronal arborisation and increased spine density of lumbar MNs occurred at pre-symptomatic ages in SOD1 mice (dendritic arbors increased by 26% and spine density increased by 21%). Our results suggest that prior to degeneration, the lower MN network is perturbed structurally, as illustrated by excessive arborisation and increased spine density. Concomitant with excessive lower MN arborisation, motor cortex layer V pyramidal neurons exhibited a regressive morphologic phenotype. By mid-disease, lower lumbar MNs also developed similar regressive degenerative characteristics. Thus, our findings correlate with recent clinical studies, where functional and network disturbances were seen in the cortex before overt lower motor symptoms. Our results pose two intriguing quandaries in the interpretation of our lower MN data. Firstly, are pre-symptomatic changes in lower MNs an adaptive or pathogenic response? Secondly, as the timing of regressive dendritic degeneration differs between upper and lower MNs, is the mechanism driving these changes similar or disparate in these neurons?

Disclosures: **M.J. Fogarty:** None. **E.W.H. Mu:** None. **N.A. Lavidis:** None. **P.G. Noakes:** None. **M.C. Bellingham:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.02/P11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Hydrogen sulphide: a double edged glial factor endogenously overproduced in Amyotrophic Lateral Sclerosis that enhances motor neuron death

Authors: *P. LONGONE[1], A. SPALLONI[1], V. GRECO[1], E. GUATTEO[1], A. DAVOLI[2], G. RICCIARDO RIZZO[2], A. URBANI[2,1], N. B. MERCURI[2,1];
[1]Fondazione Santa Lucia, Rome, Italy; [2]Univ. of Tor Vergata, Rome, Italy

Abstract: Hydrogen sulphide (H2S) is now considered, with nitric oxide and carbon monoxide, the third gaseous neurotransmitter. H2S can be generated both through enzymatic and non-

enzymatic ways. Enzymatically H2S is produced via the action of three enzymes cystathionine-beta synthase (CBS), cystathionine-gamma lyase (CSE) and 3-mercaptopyruvate sulphur transferase (3-MST). In the brain the main enzyme responsible for the production of H2S is CBS. H2S is a double-edged sword. Although the majority of the studies, looking at its role in brain function, have described neuromodulatory and neuroprotectant actions, when it reaches levels in the high microM to milliM concentrations, it turns to a poisonous agent. In a study performed in Amyotrophic Lateral Sclerosis (ALS) sporadic patients and in a familial ALS (fALS) mouse model, the classical SOD1G93A mouse, we have found toxic liquoral levels of H2S in the patients, higher H2S content in the brain tissues of the fALS mouse, and in the media from SOD1G93A primary spinal cord cultures (Davoli et al., AON 2015). In the same work we have described an increased toxicity of H2S toward motor neurons (compared to GABAergic neurons) and an increased Ca2+ concentration in spinal cord neurons following H2S treatment. We have also determined that glial cells release H2S. Here we describe its effects on the motor neurons demeanor at low (neuroprotective) and high (neurotoxic) concentrations. Moreover, we propose H2S as a modulator of the glial resting state and one of the possible mediators of the non-cell autonomous degeneration described in ALS.

Disclosures: **P. Longone:** None. **A. Spalloni:** None. **V. Greco:** None. **E. Guatteo:** None. **A. Davoli:** None. **G. Ricciardo Rizzo:** None. **A. Urbani:** None. **N.B. Mercuri:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.03/P12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NHMRC Australia Project Grants

MND Research Institute of Australia

Bethlehem Griffiths Research Foundation

Title: Dysfunction of Rab1-dependent ER-golgi transport is a common pathogenic mechanism in ALS

Authors: ***J. ATKIN**[1,2], M. HALLORAN[1], V. SUNDARAMOORTHY[1], J. SULTANA[1], K. SOUTHAM[3], A. KING[3], K. SOO[2,3];

[1]Biomed. Sci., Macquarie Univ., Sydney, Australia; [2]La Trobe Inst. for Mol. Sci., Melbourne, Australia; [3]Univ. of Tasmania, Hobart, Australia

Abstract: Several diverse proteins are linked genetically or pathologically to neurodegeneration in amyotrophic lateral sclerosis (ALS) including SOD1, TDP-43 and FUS, but currently, no common pathogenic mechanism has been described. Using a variety of cellular and biochemical techniques, we demonstrate that ALS-associated mutant forms of each protein inhibit protein transport between the endoplasmic reticulum (ER) and Golgi apparatus in neuronal cells. ER-Golgi transport was also inhibited in embryonic cortical and motor neurons obtained from a widely used animal disease model (SOD1G93A mice), validating this mechanism as an early event in motor neurons of mice. Each protein inhibited transport by distinct mechanisms, but each of these processes was dependent on Rab1. Mutant TDP-43 and mutant FUS both inhibited the incorporation of secretory protein cargo into COPII vesicles as they bud from the ER. We detected TDP-43 on the cytoplasmic face of the ER membrane whereas FUS was present within the ER. In contrast, mutant SOD1 destabilised microtubules and was not present within the ER. Furthermore, over-expression of Rab1 restored ER-Golgi transport and rescued inclusion formation, ER stress and apoptosis triggered by ALS-associated mutant SOD1. Rab1 also formed inclusions in motor neurons of spinal cords from sporadic ALS patients. Hence, these results describe a central and novel pathogenic mechanism shared by mutant TDP-43, mutant FUS, and mutant SOD1. These data thus implicate inhibition of Rab1-mediated ER-Golgi transport as a possible novel therapeutic target in ALS.

Disclosures: J. Atkin: None. **M. Halloran:** None. **V. Sundaramoorthy:** None. **J. Sultana:** None. **K. Southam:** None. **A. King:** None. **K. Soo:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.04/P13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: JSPS KAKENHI Grant Number 23111006

MEXT KAKENHI Grant Number 25293020

Title: Roles of osteopontin and matrix metalloproteinase-9 in the subtype-selective motor neuron vulnerability in ALS

Authors: Y. MORISAKI[1], A. TSUBOTA[1], M. WATANABE[1], Y. MORIWAKI[1], K. YAMANAKA[2], *H. MISAWA[1];
[1]Keio Univ. Fac Pharm, Tokyo, Japan; [2]Neurosci Pathol, Res. Inst. Env Med. Nagoya Univ., Nagoya, Japan

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive death of α-motor neurons (MNs) and consequent skeletal muscle denervation. α-MNs are classified into three subtypes: FF (fast-twitch-fatigable), FR (fast-twitch fatigue-resistant), and S (slow-twitch fatigue-resistant) MNs. In ALS, it is generally accepted that FF MNs are more vulnerable than FR and S MNs; the reason for this selective vulnerability remains enigmatic. In order to elucidate the mechanism of the subtype-specific neurodegeneration, we focused on two proteins: osteopontin (OPN) and matrix metalloproteinase-9 (MMP-9). OPN was originally discovered as a bone matrix protein, but later recognized as a multifunctional protein involved in tissue inflammation and remodeling. We have previously reported that OPN is selectively expressed in α-MNs. Kaplan et al. (Neuron, 81, 333-348, 2014) recently reported that MMP-9 is a marker for FF MNs and selectively conditions them to be vulnerable to mutant SOD1-induced toxicity. Other studies showed that OPN regulates the transcription and activation of MMP-9. In the spinal cord of wild type (WT) mice, we found that OPN and MMP-9 were expressed in different MNs; OPN was selectively expressed in FR and S MNs. In the spinal cord of ALS model mice (SOD1G93A-Tg), we observed that OPN-positive granules were accumulated in the extracellular matrix as the disease progressed. Around the period of disease onset, we detected OPN-MMP-9 double positive MNs, which were barely observed in WT mice. In the double positive MNs, increased ER stress and αvβ3 integrin (an OPN receptor) expression were observed. Furthermore, the analysis of neuromuscular junction revealed that double positive MNs are ‘remodeled’ MNs as a consequence of functional compensation after denervation of FF MNs. The OPN-induced expression of MMP-9 through αvβ3 integrin was also observed in mouse neuroblastoma cells stably expressing choline acetyltransferase cDNA (Neuro-2a-ChAT cells). From these results, we hypothesize as follows: MMP-9 positive MNs (FF MNs) first undergo denervation from their targeting muscle fibers and then OPN positive MNs (FR or S MNs) compensate for the denervated muscle endplate by sprouting and innervation. Such remodeled (FR or S) MNs obtain FF-like characteristics by expressing MMP-9, and gain selective vulnerability to the mutant SOD1-linked cellular stresses through OPN-αvβ3 integrin axis by an autocrine or paracrine manner. Collectively the present results suggest that OPN and MMP-9 are involved in the mechanism of the subtype-selective neurodegeneration in the mouse model of ALS.

Disclosures: Y. Morisaki: None. **A. Tsubota:** None. **M. Watanabe:** None. **Y. Moriwaki:** None. **K. Yamanaka:** None. **H. Misawa:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.05/P14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: C9ORF72 knockout mice show amyotrophic lateral sclerosis-like motor abnormalities

Authors: *B. IKIZ, S. D. CROLL, V. LAI, N. STAHL, A. J. MURPHY, L. E. MACDONALD, M. L. LACROIX-FRALISH;
Neurosci., Regeneron Pharmaceuticals, Inc, Tarrytown, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is the most frequent adult-onset paralytic disorder, characterized by the loss of upper and/or lower motor neurons. GGGGCC hexanucleotide repeat expansions in the non-coding region of C9ORF72 are the most common mutations found in familial, as well as sporadic, ALS cases. However, the mechanism through which these mutations cause the disease, whether through a loss- or gain-of-function of toxicity, is not known. To test whether the genetic silencing of C9ORF72 would result in any ALS-like motor behavioral abnormalities, we have generated C9orf72 knockout mice by replacing the entire coding sequence of the mouse homolog (3110043O21Rik) with a LacZ reporter. We studied the motor behaviors of these mice using rotorod, open field and CatWalk assays up to 60 weeks of age. We also examined the mice for neurological deficits throughout this period. We observed that only 40% of C9orf72 knockout mice survived past 60 weeks of age and that they stopped gaining body weight beginning at 36 weeks of age. While rotorod tests did not show any significant changes due to C9orf72 deletion, these mice started showing significant hind limb paresis, motor impairment, decreased mobility and gait abnormalities at around 40 weeks of age. We have found that the genetic silencing of C9orf72 results in multiple motor and neurological abnormalities in the mouse similar to those found in human motor neuron diseases. These findings suggest that loss-of-function mutations in C9ORF72 warrant further examination as they may play a role in the development and progression of motor neuron diseases in patients.

Disclosures: **B. Ikiz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **S.D. Croll:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **V. Lai:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **N. Stahl:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **A.J. Murphy:** E.

Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **L.E. Macdonald:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **M.L. Lacroix-fralish:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.06/P15

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: AKC Canine Health Foundation Clinician-Scientist Fellowship

University of Missouri Phi Zeta Grant

Title: Lumbar spinal cord neuroprotective microglia and fractalkine are increased with disease progression in canine degenerative myelopathy, a model for amyotrophic lateral sclerosis

Authors: *C. SIBIGTROTH, M. JONES, V. GARCIA, E. VILLALÓN, J. COATES, M. GARCIA;
Univ. of Missouri, Columbia, MO

Abstract: Canine degenerative myelopathy (DM) is an adult-onset neurodegenerative disorder. Mutations in the superoxide dismutase 1 gene (*SOD1*) are causal for disease, similar to some forms of amyotrophic lateral sclerosis (ALS). SOD1-mutant microglia have a central role in ALS progression. Their behavior is dynamic and is characterized by neuroprotective (M2) or neurotoxic (M1) phenotypes. The mechanism(s) underlying microglial phenotype determination within ALS is not known. Fractalkine, a neuronally produced chemokine, has been shown to suppress *SOD1* mutant microglial-mediated neurotoxicity. Thus, it is a possible contributor to microglial phenotype determination. Progressive microglial accumulation has been documented in DM-affected dogs. My goal was to define the phenotype of microglia within close proximity to lumbar ventral horn motor neurons in DM-affected dogs and to correlate this with fractalkine expression. Preliminary data indicate a progressive increase in total number of M2 microglia closely associated with lumbar motor neurons in DM-affected dogs compared to age-matched controls without an increase in the number of M1 microglia. Additionally, lumbar spinal cord fractalkine levels increase with disease progression, correlating with increased M2 microglia.

Taken together, these data suggest that M2, but not M1, microglia are progressively recruited to motor neurons during disease progression.

Disclosures: **C. Sibigtroth:** None. **M. Jones:** None. **V. Garcia:** None. **E. Villalón:** None. **J. Coates:** None. **M. Garcia:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.07/P16

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Academy of Finland

Jane and Aatos Erkko Foundation

Title: Role of CDNF in SOD1-G93A mouse model of amyotrophic lateral sclerosis

Authors: ***F. DE LORENZO**[1], M. H. VOUTILAINEN[1], E. MONTONEN[1], A. SAUKKONEN[1], M. AIRAVAARA[1], R. K. TUOMINEN[2], D. LINDHOLM[3], M. SAARMA[1]; [1]Inst. of Biotech., [2]Div. of Pharmacol. and Pharmacotherapy, [3]Inst. of Biomedicine, Univ. of Helsinki, Helsinki, Finland

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive degeneration of motor neurons (MN) in the ventral horn of the spinal cord, brainstem and motor cortex, which leads eventually to paralysis and muscular atrophy in the affected individuals. Patients usually die within 3-5 years of symptom onset and death is caused, in most cases, by the paralysis of respiratory muscles. Neither cure nor effective therapy is currently available. Several neurotrophic factors (NTFs) promote the survival of MNs *in vitro* and *in vivo*, thus being possible drug candidates for ALS. Among them, novel cerebral dopamine neurotrophic factor (CDNF) seems particularly promising, since it is highly expressed in muscle tissues, spreads better than other NTFs in the brain and rescues only degenerating neurons. Furthermore, CDNF is crucially involved in the regulation of the ER stress, which plays an important role in the pathophysiology of ALS. Here we show that intraventricular injection of human recombinant CDNF can significantly postpone appearance of clinical symptoms, improve motor coordination and increase lifespan in SOD1-G93A mouse model of ALS. CDNF treatment can prevent the death of MNs compared to controls and CDNF also preserves neuromuscular junctions (NMJs) in the studied gastrocnemius muscle. As mentioned above, ER stress is an

important pathway to cell death in sporadic ALS patients and in ALS rodent models. We found the upregulation of the mRNA level of unfolded protein response (UPR) genes GRP78, Xbp1, PERK, ATF6 and CHOP in SOD1-G93A model, whereas their levels were reduced in CDNF-treated animals. Therefore our results strongly suggest that CDNF has a protective effect in SOD1-G93A mouse model of ALS, promoting the survival of MNs and the preservation of NMJs. The decrease in UPR genes mRNA level after CDNF treatment also suggests the intriguing possibility that CDNF might rescue MNs by regulating the ER stress response.

Disclosures: F. De Lorenzo: None. **M.H. Voutilainen:** None. **E. Montonen:** None. **A. Saukkonen:** None. **M. Airavaara:** None. **R.K. Tuominen:** None. **D. Lindholm:** None. **M. Saarma:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.08/P17

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH

Target ALS

Robert Packard Center for ALS Research

MDA

Title: Mature oligodendroglia is not a contributor to amyotrophic lateral sclerosis

Authors: *Y. LI[1], D. BERGLES[2], J. D. ROTHSTEIN[2];
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Abstract: Amyotrophic lateral sclerosis is a fatal disease characterized by the death of upper and lower motor neurons. The etiology of the disease is largely unknown, although certain genes, such as superoxide dismutase 1 (SOD1), TDP43, FUS and C9orf72, have been found to be linked to disease development. Previous studies, by using a mutant SOD1 transgenic mouse model, showed that injury to motor neurons is the primary determinant of disease onset, while astroglial and microglial cells affect disease progression. Recently to determine the role of oligodendrocyte lineage in ALS pathogenesis, we genetically deleted mutant SOD1 in

oligodendrocyte progenitors and found disease onset was dramatically delayed. In addition, we found that gray matter oligodendrocytes degenerate not only in a SOD1 mouse model but also in both familial and sporadic ALS patient central nervous system motor areas. These findings together strongly suggest that oligodendrocyte lineage is a critical player in ALS pathogenesis. However, given that, in the OPC mutant SOD1 deletion study, the immature oligodendrocytes and mature oligodendrocytes generated from those OPCs did not have the mutant sod1 gene, it raised the question as to what is the role of mature/myelinating oligodendrocytes or immature/differentiating oligodendrocytes alone in ALS pathogenesis. Therefore, to further exam the role of mature/myelinating oligodendrocytes in ALS, we used a similar strategy to genetically delete mutant SOD1 selectively in mature myelinating oligodendrocytes. Surprisingly, we did not see any effects on disease onset, early disease and animal survival. In addition, no differences in gliosis were found. Together, this study showed that the mature oligodendrocyte is not a contributor to ALS pathogenesis and suggested immature oligodendrocytes are probably involved in the disease onset and development.

Disclosures: **Y. Li:** None. **D. Bergles:** None. **J.D. Rothstein:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Les Turner ALS Foundation

Title: Understanding the basis of CSMN vulnerability and degeneration using a proteomics approach

Authors: ***J. KLESSNER**[1], P. THOMAS[2,3], S. SANCHEZ[1], B. GENÇ[1], J. JARA[1], R. DAVIS[2], P. GOTTLIEB[2], N. KELLEHER[2,3], P. OZDINLER[1,3,4];
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Abstract: Ubiquitin carboxy-terminal hydrolase L1 (UCHL1) is a deubiquitinase that plays a critical role in maintaining free ubiquitin levels in neurons. Patients with mutations in their UCHL1 gene develop motor function defects, paralysis, and upper motor neuron defects. Mice that lack all UCHL1 function (UCHL1nm3419, UCHL1-/-) display motor neuron defects and

profound corticospinal motor neuron (CSMN) degeneration characterized by increased ER-stress, vacuolated apical dendrites, and spine loss. These findings show the importance of UCHL1 for CSMN health. Here, we used a bottom-up proteomics approach coupled with UCHL1-immunoprecipitation to reveal the proteins that interact with UCHL1. We then investigated if these interactions were specific to neurons in the motor cortex by including neurons in the spinal cord and trigeminal ganglia (TG). Our initial findings suggest that UCHL1 binds to and interacts with different proteins in the motor cortex, spinal cord, and TG, and this may in part explain why in its absence CSMN display the most prominent cell loss and neuronal vulnerability. CSMN vulnerability is a key component of disease pathology in a number of motor neuron diseases, such as primary lateral sclerosis, hereditary spastic paraplegia, and amyotrophic lateral sclerosis. Understanding the underlying causes of their vulnerability using a proteomics approach will have significant impact on revealing key cellular and molecular pathways responsible for their degeneration.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR Grant JNM-90963

Title: Isoform specific antibodies reveal distinct subcellular localizations of c9orf72 in amyotrophic lateral sclerosis

Authors: ***S. XIAO**[1], L. MACNAIR[2], P. MCGOLDRICK[2], P. MCKEEVER[2], J. MCLEAN[2], M. ZHANG[2], J. KEITH[3], L. ZINMAN[3], E. ROGAEVA[2], J. ROBERTSON[2];
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Abstract: Objective: A noncoding hexanucleotide repeat expansion in *C9orf72* is the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). It has been reported that the repeat expansion causes a downregulation of *C9orf72* transcripts, suggesting that haploinsufficiency may contribute to disease pathogenesis. Two protein isoforms are generated from three alternatively spliced transcripts of *C9orf72*; a long

form (C9-L) and a short form (C9-S) and their function(s) are largely unknown due to lack of specific antibodies. **Methods:** To investigate C9orf72 protein properties, we developed novel antibodies that recognize either C9-L or C9-S. Multiple techniques including western blot, immunohistochemistry and co-immunoprecipitation were used to determine the expression levels and subcellular localizations of C9-L and C9-S. **Results:** Investigation of expression of C9-L and C9-S demonstrated distinct biochemical profiles, region-specific changes and distinct subcellular localizations in ALS tissues. In particular, C9-L antibody exhibited a diffuse cytoplasmic staining in neurons, and labeled large speckles in cerebellar Purkinje cells. In contrast, C9-S antibody gave very specific labeling of the nuclear membrane in healthy neurons, with apparent re-localization to the plasma membrane of diseased motor neurons in ALS. Co-immunoprecipitation experiments revealed an interaction of the C9-isoforms with both Importin 1 and Ran-GTPase, components of the nuclear pore complex. **Interpretation:** Using these antibodies, we have shown that C9orf72 may be involved in nucleocytoplasmic shuttling and this may have relevance to pathophysiology of ALS/FTLD. Our antibodies have provided improved detection of C9orf72 protein isoforms, which will help elucidate its physiological function and role in ALS/FTLD.

Disclosures: S. Xiao: None. **L. MacNair:** None. **P. McGoldrick:** None. **P. McKeever:** None. **J. McLean:** None. **M. Zhang:** None. **J. Keith:** None. **L. Zinman:** None. **E. Rogaeva:** None. **J. Robertson:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.11/P20

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Ontaro Brain Institute

Title: Phosphorylated tau at threonine 175 and its role in ALS with frontotemporal degeneration

Authors: J. GOPAUL[1], A. MOSZCZYNSKI[2], P. MCCUNN[2], S. SCHMID[1], R. BARTHA[2], *M. J. STRONG[3];
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Abstract: Introduction: The majority of patients with amyotrophic lateral sclerosis (ALS) also have frontotemporal degeneration of the brain that can lead to severe personality and behavioural

dysfunctions. In ALS patients with frontotemporal degeneration, pathological tau aggregates are found in different brain regions. These tau proteins are aberrantly phosphorylated at Threonine residue 175 (p-Thr175-tau). **Hypothesis:** Our hypothesis is that the frontotemporal syndromes associated with ALS are a result of aberrant tau protein phosphorylation at Threonine residue 175. **Materials and Methods:** Rats are undergoing bilateral stereotactic hippocampal inoculations with one of four inoculums: a phosphorylated tau mimic (Thr175Asp-tau), a tau phosphorylation inhibitor (Thr175Ala-tau), wild-type human tau, or rAAV9-GFP (control). The specific aim is to characterize the behavioural effects of expressing Thr175Asp-tau *in vivo*, which is expected to lead to tau protein abnormalities in the brains of injected rats. Rats are undergoing behavioural testing (Morris Water Maze and Locomotor testing), as well as MRI neuroimaging at 1 month, 3 months, and 6 months post-injections. This will indicate changes in brain anatomy and determine if any cognitive deficits are observed. Groups of rats are to be euthanized at different time points to analyze pathology at a tissue level. There are 8 groups of 10 rats in this project. **Results:** Currently, 40 rats with hippocampal inoculations have undergone behavioural testing for the 1 and 3-month post-surgery time points. They have performed the Morris Water Maze (MWM) as well as Locomotor box testing. MWM data indicates that all groups of rats show normal cognitive abilities given this spatial learning task. In other words, there are no observed behavioural differences between the rats administered different tau constructs. The Locomotor data also displays normal motor activity and levels of anxiety in the rats. Behavioural experimentation is still to be carried out for the 6-month time-point. **Summary and Discussion:** Currently, there is neither a cure nor treatment for ALS or frontotemporal degeneration. People diagnosed with ALS die within 3-5 years, and the pathology is only enhanced by the associated dysfunction of the brain. This project investigates the mechanism behind frontotemporal degeneration associated with ALS. The effects of tau protein phosphorylated at Threonine residue 175 are being examined by inoculation of the Thr175Asp-tau construct in rat hippocampus. Understanding this mechanism offers potential for the development of a rational pharmacotherapy of ALS with frontotemporal degeneration.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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VAMC/BLRD 1I21BX001841

Michigan Alzheimer's Disease Center

CDC

A. Alfred Taubman Institute

Title: A screen for novel G4C2 hexanucleotide repeat expansions at ALS and FTD-associated loci

Authors: *F. HE[1], C. FIGUEROA-ROMERO[1], D. ZHANG[2], E. L. FELDMAN[1], P. K. TODD[1]; [1]Dept. of Neurol., Univ. of Michigan, Ann Arbor, MI; [2]Natl. Ctr. for Biotech. Information, NIH, Bethesda, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is a common untreatable neurodegenerative disease affecting both upper and lower motor neurons. A dominantly inherited hexanucleotide GGGGCC repeat expansion in the first intron of C9ORF72 was recently identified as the most prevalent known cause of familial and sporadic ALS and frontotemporal dementia (FTD). In normal individuals, this repeat is usually quite short (2 repeats in a majority of the population) but it becomes dramatically unstable after expansion to 30 or more repeats. We hypothesized that other genes harboring short GGGGCC repeats could also induce ALS/FTD phenotypes. However, thousands of these types of repeat exist within the genome, making screening for such loci challenging. To overcome this, we took a candidate gene based approach, selecting 25 GGGGCC repeat containing candidate genes that reside within 2 Mb of either known ALS loci or SNPs associated with ALS identified by GWAS studies. Gene specific primers were designed, and the GGGGCC repeat numbers were determined by repeat primed PCR followed by fragment analysis. In a sample of 200 of ALS cases and 138 of controls, we found that these candidate gene showed very narrow variation in repeat number, and none of them were expanded to a predicted pathogenic range (>30 times). In comparison, we identified 25 of C9orf72 repeat expansion cases in this same cohort. We conclude that expansion of GGGGCC repeats in C9ORF72 as the cause of ALS is exceptional and that similar repeat expansions at other ALS associated loci are unlikely to be common contributors to disease.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: FAPESP Grant 2013/26097-0

FAPESP Grant 2009/14027-2

CNPq Grant 467566/2014-3

Title: The expression and secretion of the co-chaperone STI1 in astrocytes from SOD1G93A ALS mouse model

Authors: *G. P. OLIVEIRA, T. G. SANTOS, V. R. MARTINS;
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is an adult-onset and fast progressive neurodegenerative disease caused by motor neuron loss, leading to muscle paralysis and death within two to five years of symptoms onset. About 20% of familial forms of ALS are linked to dominant mutations in the Cu/Zn superoxide dismutase gene (SOD1). Toxic signals from non-neuronal cells, mainly astrocytes and microglia, have been proposed to contribute to motor neuron death in ALS. Mutant SOD1 (mSOD1) is known to be prone to aggregation and a number of heat shock proteins have been found at mSOD1 aggregates in rodent models of ALS. In addition, the level of chaperones is decreased in the spinal cord of these mice. The co-chaperone STI1 (stress inducible protein 1) binds to Hsp70 and Hsp90 in a complex responsible for the correct folding of client proteins. STI1 could be actively secreted and, in extracellular milieu, interacts specifically with prion protein (PrPC). This interaction modulates several functions such as protection against cell death and neuritogenesis in hippocampal neurons. Herein, STI1 levels were evaluated in primary cultures of astrocytes derived from wild-type and transgenic SOD1G93A mice as well as in the conditioned medium of astrocytes submitted to 48 hours of serum deprivation. The levels of STI1 were 20% lower in SOD1G93A astrocytes when compared to wild-type cells. Otherwise, when cells were cultured for 48 hours in serum free medium, STI1 levels were increased to about 20% in SOD1G93A when compared to the wild-type controls at the same conditions. Furthermore, the secretion of STI1 by SOD1G93A astrocytes in serum deprived conditions were two-fold higher than in wild-type cells. It was hypothesized by others that astrocytes activate secretory pathways in order to eliminate mSOD1 and possibly other misfolded proteins that may cause intracellular toxicity. Furthermore, it was described that Hsp70 has a stronger affinity to mSOD1 than to the wild-type form. It remain to be determined if the increased levels of soluble STI1 detected in SOD1G93A astrocyte conditioned medium can be related to its sequestering in a complex with mSOD1/Hsp70, and also, if this might implicate in the impairment of its activity as a neuroprotective molecule.

Disclosures: **G.P. Oliveira:** None. **T.G. Santos:** None. **V.R. Martins:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

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Program#/Poster#: 159.14/P23

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Endothelin-1 induces degeneration of motor neurons: a possible pharmacological target for amyotrophic lateral sclerosis

Authors: S. D'ANTONI[1], E. RANNO[1], M. SPATUZZA[1], P. LA ROCCA[1,2], *M. CATANIA[1,3]; [1]Inst. of Neurolog. Sciences, Natl. Res. Council (CNR), Catania, Italy; [2]Univ. of Catania, Catania, Italy; [3]IRCSS Oasi Maria SS, Troina (EN), Italy

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of motor neurons (MNs) and astrogliosis. Factors secreted by activated astrocytes might contribute to MN degeneration. We have found that endothelin-1 (ET-1), a peptide strongly up-regulated in reactive astrocytes under pathological conditions, is abundantly expressed in reactive astrocytes in the spinal cord of SOD-1 G93A mice and ALS patients and exerts a toxic effect on cultured MNs through activation of astrocytic ET receptors (Ranno et al., Neurobiol Dis. 2014; 65:160-71). In this study, we investigated the mechanisms underlying ET-1 toxicity, focusing on cell survival pathways, such as phosphatidylinositide 3-kinase (PI3K) and JAK-STAT pathways, and/or inflammatory processes. To this aim, mixed spinal cord cultures were treated with ET-1 (100 nM for 48 h) with/without LPS (40 ng/ml) or inhibitors of PI3K (LY294002, 50 uM) and JAK-STAT (AG-490, 50mM). The number of surviving MNs was assessed after treatments by direct counting SMI32-positive neurons. We observed that the inhibition of PI3K pathway or JAK-STAT pathway caused MN death, as expected; however, exposure to ET-1 in the presence of LY294002 did not result in a further increase of MN death whereas a treatment with AG-490 in the presence of ET-1 increased the toxic effect of the peptide. We also observed that LPS induced MN death, but its effect was not additive with that of ET-1. Our results suggest that ET-1 may cause neuronal death by a mechanism that involves the PI3K pathway. An inflammatory component may account for the ET-1 toxicity, but this requires further investigation. Considering the toxic effect that ET-1 exerts on MNs, we can assume that the modulation of ET-1 mediated mechanisms might be envisaged as a new therapeutic approach for ALS.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Motor Neurone Disease Association

Title: Early dysfunction and non-cell autonomous disease mechanisms in a human iPSC- based model of ALS

Authors: A.-C. DEVLIN[1], C. ZHAO[2], B. SELVARAJ[2], S. BOROOAH[2], E. M. CLEARY[2], K. BURR[2], C. E. SHAW[3], S. CHANDRAN[2], *G. B. MILES[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease that remains largely untreatable and incurable due to our incomplete understanding of the key pathogenic mechanisms that underlie motor neuron (MN) loss in the disease. Through the use of induced pluripotent stem cells (iPSCs), we can now study the consequences of ALS-related mutation(s) in functional disease-relevant human cell types. This can be done at a range of time points, including those prior to overt pathology, in order to understand early causative events in ALS and hopefully reveal novel therapeutic targets. In this study, we report the use of human induced pluripotent stem cell (iPSC)-derived MNs and astrocytes to study the pathophysiology of ALS. Using whole-cell patch-clamp recording techniques we have recently shown that patient iPSC-derived MNs harbouring C9ORF72 or TARDBP mutations, display an initial hyperexcitability followed by progressive loss of action potential output due to a decrease in voltage-activated sodium and potassium currents which occurs in the absence of changes in cell viability (Devlin et al., 2015, Nat Commun, 6:5999). Given evidence supporting non-cell autonomous disease mechanisms in ALS, we are currently studying whether interactions between neurons and astrocytes are involved in the pathophysiological phenotype we have recently revealed. Data to date indicate that patient iPSC-derived astrocytes induce

pathophysiological changes in control human iPSC-derived MNs. These changes are similar to those we have revealed in patient iPSC-derived MNs and include progressive loss of action potential output, due to decreases in voltage-gate sodium and potassium currents, but no indication of early hyperexcitability. We have also observed a reduction in synaptic input to control iPSC-derived MNs co-cultured with patient iPSC-derived astrocytes. We are currently investigating if such non-cell autonomous disease mechanisms are common across patient iPSC-derived astrocytes harbouring C9ORF72 and TARDBP mutations and whether they rely on direct astrocyte-MN interactions. Overall, our data implicate MN dysfunction, potentially due to non-cell autonomous disease mechanisms, as an early contributor to downstream degenerative pathways that ultimately lead to MN loss in ALS.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Depletion of TDP-43 induces mitochondrial fragmentation

Authors: ***G. ITO**[1], A. KOYAMA[2], A. SHIGA[1], S. HIROKAWA[1], Y. TOYOSHIMA[1], A. KAKITA[1], M. NISHIZAWA[1], O. ONODERA[3];
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Abstract: Background Mitochondria are movable organelles and build up a large network called mitochondrial dynamics by its fusion and fission. Mitochondrial dysfunction has been reported as a molecular pathogenesis of neurodegenerative diseases including Amyotrophic lateral sclerosis (ALS). Abnormal mitochondrial morphology and fragmentation have been detected in motor neuron with ALS (Sasaki et al., 2007). However, the molecular background for these findings are still obscure. Dislocation of nuclear protein TDP-43 to cytoplasm and form inclusions is a pathological hallmark of ALS. Thus, a loss of function of TDP-43 is thought to be a key process for the molecular pathogenesis of ALS. Here, we investigated whether a loss of TDP-43 affects mitochondrial morphology. Method and Result We found that the depletion of TDP-43 by siRNA induced mitochondrial fragmentation in HEK293T cells. By Western

Blotting, we investigated the amounts of mitochondrial dynamics related proteins: DRP1, FIS1, OPA1, MFN2. Among them, OPA1 regulate the fusion of mitochondrial inner membrane and its 87 kDa protein significantly increased under the depletion of TDP-43. OPA1 has eight-variation of mRNA splicing variants, and each of them undergoes protein cleavage finally produces five peptides. We investigated the amounts of each splicing variant by qPCR and found that variant 8, which produces 87 kDa peptide, increased. Finally, we found overexpression of OPA1 variant 8 resulted in the mitochondrial fragmentation and increased expression of OPA1 87 kDa. Conclusion The depletion of TDP-43 induced mitochondrial fragmentation and increased the OPA1 splicing variant 8, which produces OPA1 87 kDa peptide. Mitochondrial fusion is regulated by OPA1 mRNA splicing, and OPA1 variant 8 doesn't have an ability of mitochondrial fusion (Song Z et al., 2007). The perturbation of OPA1 splicing variants induced by TDP-43 depletion may associate with the mitochondrial fragmentation.

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Poster

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Les Turner ALS Foundation

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Ride for Life

Foglia Family Foundation

Title: Analysis of polymorphisms in cbl-c gene for association with sporadic amyotrophic lateral sclerosis in African Americans

Authors: *F. L. NUNEZ SANTANA[1], M. SAEED[2], E. A. USACHEVA[3], D. ELLIOT, IV[1], N. A. SIDDIQUE[1], T. SIDDIQUE[1];
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Chicago, IL; [2]Mol. Pathology, Liaquat Natl. Hosp., Karachi, Pakistan; [3]Pathology and Lab. Med., NorthShore Univ. HealthSystem / Univ. of Chicago, Chicago, IL

Abstract: Sporadic amyotrophic lateral sclerosis (SALS) accounts for 90% of all cases of ALS. Etiology of SALS is largely unknown and it is not well studied in African Americans. SALS is hypothesized to be a complex disorder in which disease is modulated by variations in multiple genetic loci interacting with each other and perhaps environmental factors. We have shown that the ubiquitin proteasome system (UPS) may be directly involved in the pathology of ALS. Cbl proteins negatively regulate receptor and nonreceptor tyrosine kinases by functioning as ubiquitin protein ligases that mediate the ubiquitination of activated tyrosine kinases. Therefore, mutations in cbl-c gene may affect degradation of tyrosine kinases in motor neurons, thus leading to motor neuron diseases. In this study we investigated association of SALS in African-Americans to a coding region cytosine duplication (dupC) mutation in CBLC. This variation has very low frequency in people of European descent, but common in Africans. It leads to frame-shift and premature stop codon. We analyzed dupC variant in a case-control cohort of 356 African-Americans for association with SALS. DNA samples extracted from whole blood from our own collection and others obtained from Coriell Cell Repositories were screened using four primers PCR reaction for the presence of dupC mutation in cbl-c. We investigated the association between sporadic ALS and the mutation using $\chi 2$ analyses. We found no significant difference between polymorphism frequency in the disease samples as compared to controls ($p=0.34$). Additional studies are underway to further investigate the association between SALS and the presence of additional polymorphism in cbl-c.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS-RO1 NS051488

Farber Family Foundation

Title: Building an ipsc-derived astrocyte-motor neuron co-culture model to study non-cell autonomous toxicity in cases of fused in sarcoma (fus)-linked amyotrophic lateral sclerosis

Authors: *K. MCAVOY, K. KRISHNAMURTHY, S. SHAMAMANDRI MARKANDAIAH, D. TROTTI, P. PASINELLI;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: It is well established that astrocytes contribute to motor neuron degeneration and disease progression in Amyotrophic Lateral Sclerosis (ALS), an incurable neurodegenerative disease. Mutations in fused in sarcoma/translocated in liposarcoma (FUS/TLS, FUS) account for approximately 5% of familial (fALS) cases and FUS-positive pathological inclusions have been identified in sporadic ALS (sALS) cases. We have identified a pro-inflammatory phenotype in primary rodent astrocytes expressing human ALS-linked FUS mutations. Furthermore, astrocytes expressing mutations in FUS damage and kill co-cultured primary motor neurons and this toxicity is at least partially mediated by the cytokine tumor necrosis factor alpha (TNFα) triggering increased motor neuron susceptibility to excitotoxic damage. The aim of the current study is to validate and extend these findings from primary rodent cultures using a patient-derived induced pluripotent stem cell model (IPSC). IPSCs were fully differentiated into astrocytes expressing glutamate transporters EAAT1 and EAAT2 and well as other astrocytic markers S100β and GFAP. Their effect on the health of motor neurons was examined. This work was supported by NINDS-RO1 NS051488 and the Farber Family Foundation.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.19/P28

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01NS091440

Department of Defense

ALS Association

Wisconsin Alumni Research Foundation

Title: Activated inflammation and glial response in the skeletal muscle of a rat model of familial amyotrophic lateral sclerosis (ALS)

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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive motor dysfunction and loss of large motor neurons in the spinal cord and brain stem. While much research has focused on mechanisms of motor neuron cell death in the spinal cord, degenerative processes in skeletal muscle and neuromuscular junctions (NMJs) are also observed early in disease development. Relatively little is known about the role inflammation and glial responses in skeletal muscle at or near NMJs, and how these responses affect motor neuron survival or motor dysfunction in ALS. In this study, we evaluate inflammatory and glial responses in the skeletal muscle near NMJs in the limb muscle of familial ALS rat model (SOD1-G93A transgenic rats) as the disease progresses. Muscle samples were collected from pre-symptomatic, symptomatic, and endpoint stage animals and immunostained for inflammation markers (CD11b and CD68). The expression of these inflammatory markers increased significantly in the skeletal muscle during the symptomatic and end-stage SOD1-G93A rats. Interleukin 1 alpha (IL-1α) and tumor necrosis factor alpha (TNF-α) expression was measured in SOD1-G93A rat muscle homogenates. Both IL-1α and TNF-α were elevated in homogenates from symptomatic and end-stage rats. We next determined whether active glial responses occur in the muscle of SOD1-G93A rats near intramuscular axons and NMJs. Interestingly, strong expressions of glial fibrillary acidic protein (GFAP) and nestin, were observed in the areas adjacent to NMJs. The expression of these markers has been linked with the dissociation of NMJs and the activation of perisynaptic (terminal) Schwann cells. Together, these data suggest a significant increase in inflammation within the muscle tissue of ALS rats and that this increase occurs near degenerating NMJs. Understanding the inflammatory responses at the NMJ is important when investigating suitable cellular and molecular targets for treating ALS and other neuromuscular diseases.

Disclosures: J. Van Dyke: None. **I. Smit-Oistad:** None. **D. Krakora:** None. **C. Macrander:** None. **M. Meyer:** None. **M. Suzuki:** None.

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.20/P29

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: β-Sitosterol β-D-glucoside causes Parkinsonism in rats but ALS-like behaviours and motor neuron pathology in mice

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Abstract: Evidence suggests that amyotrophic lateral sclerosis-parkinsonism dementia complex of the Western Pacific (ALS-PDD) is caused at least in part by toxic sterols such as β-sitosterol β-D-glucoside (BSSG), which is present in cycad nuts. Rats fed chronically with cycad seed extracts or with BSSG progressively develop many if not all the symptoms of Parkinson's disease, including premotor olfactory deficits, neuroinflammation, motor deficits, aggregation of phospho-α-synuclein and cognitive decline. Some evidence suggests that mice and rats respond differently to cycad phytosterols. In contrast to rats, when mice were fed BSSG, motor behaviours suggested motor neuron deficits; similar results had previously been observed following feeding of cycad extract. Mice (CD1) were fed (1mg BSSG/mouse/day, 5 days/wk) daily for 15 weeks. At 28 and 35 weeks, there are significant locomotor deficits. BSSG administration significantly shortened stride length and significantly widened rear stance at week 28 and these deficits increased in magnitude by week 35. BSSG did not alter open field, rotarod, wire hang or splay reflex. JNX1001, a neurotrophic enhancer, improved motor behaviours in BSSG treated mice. Neuropathological changes were assessed by Nissl, ChAT, ILB4 and 3-NT staining. Total neuron loss in dorsal horn was approximately 50% and ChAT+ neurons are reduced by 60%. BSSG treatment led to a 66% increase in IL4+ cells in dorsal horn and a significant (140%) increase in 3-nitrotyrosine labeling. Locomotor deficits and histopathological changes in dorsal horn were all prevented by daily oral administration of JNX1001 (1 and 10 mg/kg/day in 0.5% HPMC containing 0.2% Tween 80). Currently, we have no adequate explanation for the difference in outcome between mice and rats. On a weight basis, the mice received a much higher dose of BSSG. It is however interesting that the human condition (ALS-PDD) also appears to exhibit a broad range of outcomes. BSSG treatment of mice may provide a progressive alternative to genetic models for ALS.

Disclosures: **J.M. Van Kampen:** A. Employment/Salary (full or part-time):; Neurodyn Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Inc. **D.G. Kay:** A. Employment/Salary (full or part-time):; Neurodyn Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Inc. **P.A. Howson:** A. Employment/Salary (full or part-time):; Atuka. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atuka. **J.M. Brotchie:** A. Employment/Salary (full or part-time):; Atuka. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atuka. **D.C. Baranowski:** A.

Employment/Salary (full or part-time):; Neurodyn Inc. **C.A. Shaw:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Inc. **H.A. Robertson:** A. Employment/Salary (full or part-time):; Neurodyn Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurodyn Inc..

Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 159.21/P30

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR MOP-137005

Title: MTHFSD and DDX58 are novel RNA-binding proteins abnormally regulated in ALS

Authors: *L. MACNAIR[1], S. XIAO[2], D. MILETIC[2], M. GHANI[2], E. ROGAEVA[2], J. KEITH[3], L. ZINMAN[3], J. ROBERTSON[2];
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Abstract: INTRODUCTION: Tar DNA-Binding Protein 43 (TDP-43) is an RNA-binding protein normally localized to the nucleus of cells, where it elicits function related to RNA metabolism such as transcriptional regulation and splicing. In Amyotrophic Lateral Sclerosis (ALS), TDP-43 is mislocalized from the nucleus to the cytoplasm of diseased motor neurons, forming ubiquitinated inclusions. Although mutations in the gene encoding TDP-43, *TARDBP*, are found in ALS, these are rare. However, TDP-43 pathology is common to over 95% of ALS cases, suggesting that abnormalities of TDP-43 play an active role in disease pathogenesis. HYPOTHESIS: Since there is a loss of TDP-43 from the nucleus of affected motor neurons in ALS this will lead to a change in mRNA expression, and identifying these changes will uncover molecular pathways that underpin motor neuron degeneration. METHODS AND RESULTS: Translating ribosome affinity purification (TRAP) was coupled with microarray analysis to identify changes in mRNAs being actively translated in spinal cord motor neurons of pre-symptomatic and symptomatic TDP-43^{A315T} mice when compared to age-matched non-transgenic (NTg) littermates. The translational profile for pre-symptomatic TDP-43^{A315T} mice was not significant, however the translational profile of symptomatic TDP-43^{A315T} mice revealed an overrepresentation of genes involved in RNA metabolic process, immune response, and regulation of cell cycle. Of the 28 differentially expressed genes, 7 had a $\geq$ 2-fold change; 4

correlated with microarray results as validated by immunofluorescence labeling of motor neurons in TDP-43^{A315T} mice, and 2 were confirmed by immunohistochemistry in ALS cases. Both of these identified genes, DEAD Asp-Glu-Ala-Asp box polypeptide 58 (*DDX58*) and methenyltetrahydrofolate synthetase domain containing protein (*MTHFSD*) are RNA-binding proteins that are directly regulated by TDP-43. CONCLUSIONS: Translational profiling of a TDP-43 transgenic mouse model identified gene ontologies related to ALS pathogenesis. More importantly, this method identified 2 genes, *DDX58* and *MTHFSD* that were differentially expressed in spinal cords of ALS patients. TDP-43 bound to both of these transcripts. This discovery-based approach has, for the first time, revealed translational changes in motor neurons of a TDP-43 mouse model, which correlate with changes in ALS patients, providing greater insight into how TDP-43 abnormalities contribute to ALS pathogenesis.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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the National Institute on Aging (AG20506)

the Les Turner ALS Foundation

Title: Dominant-negative effect" mechanism in OPTNE478G-linked amyotrophic lateral sclerosis

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Abstract: Mutations in the gene encoding optineurin (OPTN) cause amyotrophic lateral sclerosis (ALS) in both autosomal recessive and autosomal dominant fashions, an uncommon phenomenon in genetic diseases. A convergent mechanism to explain this phenomenon is lacking. In this study, we analyzed a well-established autosomal dominant ALS mutant,

OPTNE478G. We show that wild-type OPTN (OPTNWt), but not OPTNE478G, is recruited to the autophagosomes. Intriguingly, the OPTNE478G prevents OPTNWt from being recruited to the autophagosomes. These data, therefore, indicate that the OPTNE478G acts in a "dominant-negative effect" mechanism in the OPTNE478G-mediated dominant ALS. Our data also suggest that loss of functional OPTN is the convergent pathway leading to the disease in both OPTN-linked recessive and dominant ALS. Because OPTN primarily localizes to autophagosomes and is an autophagy receptor, our data also suggest that autophagic dysfunction is the key molecular event in OPTN-linked ALS. OPTN-linked dominant ALS appears to be the first neurodegenerative disorder showing a "dominant-negative effect' in its pathogenesis.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Motor neurons are impaired in Prune2 gene deficient mice

Authors: M. ITOH, E. NISHIDA, S. ISLAM, S. LI, M. OSAWA, D. LEE, H. CHEN, M. UEDA, M.-X. WANG, M. HAYAKAWA, G. IKENO, K. ICHIHASHI, K. OKAMOTO, J. TSUTSUMI, *T. NAKAGAWA;
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Abstract: Motor neurons were degenerated in the Prune homolog 2 (*Drosophila*) (*Prune2*) gene deficient mice generated by CRISPR/Cas9 system. PRUNE2 is one of Bcl-2/adenovirus E1B 19 kDa-interacting proteins (BNIPs), which plays critical roles in several cellular processes such as cellular transformation, apoptosis, neuronal differentiation, and synaptic function, mediated by the BNIP2 and Cdc42GAP homology (BCH) domain. *Prune2* and its isoforms—*C9orf65*, BCH motif-containing molecule at the carboxyl terminal region 1 (*BMCC1*), and BNIP2 Extra Long (*BNIPXL*)—have been shown to be a susceptibility gene for Alzheimer's disease, a biomarker for leiomyosarcomas, a proapoptotic protein in neuronal cells, and an antagonist of cellular transformation, respectively. And also the prostate cancer antigen 3 (*Pca3*) gene is a marker of prostate cancer, which is localized within the *Prune2* gene and is transcribed by responsiveness to androgen in the opposite orientation. *Prune2* mRNA is predominantly expressed in the neurons of the cranial nerve motor nuclei and the motor neurons of the spinal cord (Li et. al., *Neurosci. letters*, 503, 208-214, 2011; sfn 33.07, 2011). However, function and distribution of

PRUNE2 protein are largely unknown. We generated *Prune2* gene deficient mice (*Prune2*$^{Ex16-/-}$) by CRISPR/Cas9 system, which deleted exon16. The levels of *Prune2* mRNA in the spinal cord of *Prune2*$^{Ex16-/-}$ were significantly decreased. Microscopical analysis indicated that motor neurons in the spinal cord were not stained by anti-PRUNE2 antibody. By electron microscopy, we observed that morphology of mitochondria in motor neurons was changed, suggesting that PRUNE2 may play a crucial role of maintenance of motor neurons.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Support: NIH RO1 NS085207

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MDA

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Jean and Pape Adams Foundation

Title: Mechanisms of synaptic dysfunction of C9ORF72 neurons *in vitro* and *in vivo*

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Abstract: The most common genetic mutation in sporadic and familial ALS is found in the non-coding region of C9orf72 gene. An abnormal expansion of the hexanucleotide repeat GGGGCC (G4C2) is present in this region causing up to 10% of sporadic and up to 40% of the familial ALS as well as up to 10% of familial FTD. Our laboratory used induced pluripotent stem cells (iPSCs) as a valuable research approach to study C9ORF72 (C9) disease pathogenesis. It is known that iPSCs differentiated neurons (iPSNs) carrying the C9 mutation present intranuclear repeat RNA foci, changes in neuronal excitability, increased susceptibility to cellular stressors, including excitatory amino acid glutamate and RNA interacting proteins sequestered to the G4C2 repeat. Based on these previous studies, we hypothesize that the G4C2 repeat expansion can sequester proteins critical for synapse function. To test this hypothesis, we performed structural and functional analysis of spinal and cortical iPSNs (ALS and FTD patients). The cells were analyzed for dendritic morphology and expression of synaptic protein markers. Confocal microscopy and 3D image analysis revealed that spinal C9ORF72 iPSNs show altered dendritic tree morphology as well as aberrant spine morphology. Specifically, we discovered an overall decrease in spine density as well as a decreased ratio of mature mushroom-shaped spines to immature stubby spines per dendritic length in C9ORF72 iPSNs when compared to control iPSNs. We further determined that the expression pattern of the synaptic protein synapsin-1 is altered in aged C9ORF72 spinal cord iPSNs when compare to control. To confirm synaptic alterations *in vivo*, we injected AAV9 viral construct overexpressing G4C2 repeats of different lengths into wild-type and Thy-1 eGFP mice . In conclusion, our data suggest that synaptic dysfunction plays a role in C9ORF72 pathogenesis and may explain the changes in neuronal excitability, the increased susceptibility to cellular stressors as well as cognitive impairment, as observed in C9ORF72 FTD patients and ALS patients.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: TDP-43 regulates expression of Ulk1/Atg1 and autophagy

Authors: *K. MATSUKAWA, R. IHARA, T. MATSUMOTO, M. MIURA, T. CHIHARA, T. WAKABAYASHI, T. HASHIMOTO, T. IWATSUBO;
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Abstract: TDP-43 is an RNA-binding protein that was identified as a proteinaceous constituent of ubiquitin-positive inclusions that characterize the neuropathology of frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). Mutations in TDP-43 gene are found in familial ALS, supporting the causative roles of TDP-43 in neurodegeneration. We previously reported that transgenic *Drosophila* melanogaster (tg flies) overexpressing human TDP-43 showed a progressive retinal degeneration, whereas those expressing TDP-43 lacking the RNA recognition motif 1 (RRM1) did not exhibit any degenerative phenotypes, proposing the RNA-binding dependent neuronal toxicity of TDP-43. To investigate into the molecular mechanism whereby TDP-43 causes neuronal death, we utilized a microarray-based gene expression analysis and identified Atg1 (Autophagy-specific gene 1), a *Drosophila* homologue of human ULK1, as one of the genes whose expression is upregulated in the retina of TDP-43 tg flies. Quantitative RT-PCR revealed that the expression level of Atg1 in the retina of TDP-43 tg flies was increased by ~4.7 fold compared with that in lacZ tg flies, whereas the level was not changed in the TDP-43 ΔRRM1 tg flies. RNA-immunoprecipitation assay showed an interaction of TDP-43 with Atg1 mRNA in the retina of TDP-43 tg flies. To determine whether Atg1 is involved in the TDP-43-induced neuronal toxicity, we crossed TDP-43 tg flies with Atg1 RNAi line, and found that the suppression of Atg1 expression in the retina of TDP-43 tg flies mitigated the retinal degeneration. We also crossed TDP-43 tg flies with RNAi lines of other autophagy-related genes, i.e., Atg3, 6 or 13, and found a significant mitigation of the retinal degeneration induced by TDP-43. Moreover, we revealed an increase in phosphatidylethanolamine-conjugated form of GFP-Atg8a, suggesting the autophagic activation in the retina of TDP-43 tg flies. These data suggest that overexpression of TDP-43 in the *Drosophila* retina causes neurodegeneration through an excessive autophagic activation by an increase in Atg1 expression. To examine whether TDP-43 regulates the expression of ULK1 in mammalian cells, we silenced the expression of endogenous TDP-43 in Neuro-2a cells by siRNA. Silencing of TDP-43 decreased the expression of ULK1 by ~60%, and suppressed autophagy, suggesting that TDP-43 is involved in autophagy through regulating ULK1 expression in mammalian cells. Taken together, we propose that TDP-43 may play a role in autophagy through ULK1/Atg1, and that excessive autophagy induced by overexpression of TDP-43 may contribute to the neurodegeneration in *Drosophila*.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

Location: Hall A

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH/NINDS 1K08NS072233-01A1

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NIH/NINDS 2R01 NS083390

Target ALS

The Robert Packard Center for ALS Research

Roddenberry Stem Cell Program

Title: Amelioration of toxicity in neuronal models of amyotrophic lateral sclerosis by hUPF1

Authors: *S. BARMADA[1,2], S. JU[3], A. ARJUN[4], A. BATARSE[4], H. ARCHBOLD[1], D. PEISACH[1], Y. ZHANG[1], X. LI[1], E. TANK[1], H. QIU[8], E. HUANG[8], D. RINGE[10], G. PETSKO[11,12,13,10], S. FINKBEINER[4,5,6,9,7];
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Abstract: Over 30% of patients with amyotrophic lateral sclerosis (ALS) exhibit cognitive deficits indicative of frontotemporal dementia (FTD), suggesting a common pathogenesis for both diseases. Consistent with this hypothesis, neuronal and glial inclusions rich in TDP43, an essential RNA-binding protein, are found in the majority of those with ALS and FTD, and mutations in TDP43 and a related RNA-binding protein, FUS, cause familial ALS and FTD. TDP43 and FUS affect the splicing of thousands of transcripts, in some cases triggering nonsense-mediated mRNA decay (NMD), a highly-conserved RNA degradation pathway. Here, we take advantage of a faithful primary neuronal model of ALS and FTD to investigate and characterize the role of hUPF1, an RNA helicase and master regulator of NMD, in these

disorders. We show that hUPF1 significantly protects mammalian neurons from both TDP43- and FUS-related toxicity. Expression of hUPF2, another essential component of NMD, also improves survival, while inhibiting NMD prevents rescue by hUPF1, suggesting that hUPF1 acts through NMD to enhance survival. These studies emphasize the importance of RNA metabolism in ALS and FTD, and identify a novel and potentially effective therapeutic strategy for these disorders.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Department of Biotechnology, Govt. of India

CSIR-UGC, Govt. of India

Title: Neuron-glial interaction in pathogenesis of sporadic amyotrophic lateral sclerosis (als): inflammation mediated neurotoxicity

Authors: *P. S. MISHRA[1], D. K. DHULL[4], K. VIJAYALAKSHMI[2], T. N. SATHYAPRABHA[2], A. NALINI[3], P. A. ALLADI[2], T. R. RAJU[2];
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Abstract: The intricate glial-neuron interplay in neurodegeneration remains ambiguous. Investigations on Sporadic Amyotrophic Lateral Sclerosis (SALS) models from our laboratory reported reactive astrogliosis along with neurodegeneration. Further, proteomic analysis of cerebrospinal fluid of ALS patients (ALS-CSF) revealed up-regulation of glial proteins, viz. chitotriosidase-1 and osteopontin, the mediators of neuroinflammation. Thereby, investigating glial responses in ALS becomes physiologically and therapeutically relevant. Briefly, pure astroglial and microglial cultures derived from Wistar rats (P3) were propagated in DMEM+10% FBS alone or exposed to 10%v/v ALS-CSF and CSF from patients suffering from non-neurodegenerative diseases (NALS-CSF), respectively for 48 hrs. Parameters studied included

assay for glutamate levels and reactive oxygen species (ROS) levels; expression patterns of iNOS, PGE2, and COX-2 using confocal microscopy, m-RNA expression and/or ELISA. MTT assay was performed on NSC-34 cells to study the effect of the conditioned media from glial cultures exposed to ALS-CSF. We found significant up regulation of inflammatory markers like PGE-2 and COX-2 in both astroglial and microglial cultures exposed to ALS-CSF. A phase contrast study, revealed microglial activation in the form of predominantly amoeboid phenotype over ramified ones in cultures exposed to ALS-CSF. Simultaneously, the ROS and glutamate levels were also elevated. The conditioned media from glial cultures exposed to ALS-CSF induced death in NSC-34 motor neuron cell line, suggesting a degenerative effect of the glial inflammatory profiles on motor neurons. We propose accentuated neuroinflammation and excitotoxity mediated by glia as the major events in the disease pathogenesis. Further investigating the synergism of glial-neuronal interactions can lead to better understanding of the disease, and possible therapeutic targets.

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Poster

159. Motoneuron Disease: Cellular Mechanisms II

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Program#/Poster#: 159.28/P37

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: *In vitro* analysis of axolotl spinal cord in terms of regeneration

Authors: *E. KUBAT OKTEM, B. POLAT, C. B. AKAGUNDUZ, G. OZTURK; Regenerative and Restorative Med. Res. Ctr. (REMER), Istanbul Medipol Univ., Istanbul, Turkey

Abstract: Mammals have a limited central nervous system (CNS) regeneration capacity in contrast to the salamander *A. mexicanum* (Axolotl), which has continuing axon and cell differentiation throughout life. The aim of this study is to investigate the regenerative capacity of freshly isolated and injured axolotl spinal cord *in vitro* which would shed light to mammalian regeneration research. A complete spinal cord cut was executed at lumbosacral level of the spinal column of young adult wild type axolotls. Response to injury effect was tested between three groups as; uninjured control spinal cord cells, spinal cord cells plated on 7^{th} day post-injury (DPI7) and spinal cord cells plated on 14^{th} day post-injury (DPI14). The cells were dissociated and cultured around 300 cells / mm^2 to either laminin or fibronectin coated petri dishes. Cell

cultures were kept at 18 °C and 5% CO_2. After 24 h in culture, propidium iodide was added for viability assesment. The same coordinates were examined and recorded with dedicated software for 10 days *in vitro*. Immunofluorescence staining was performed with antibodies against neural, glial and stem cell markers and imaged with laser scanning confocal microscope. Survival percentage, spheroid percentage (SP), total sphere number, total neurite growth and average neurite growth were the investigated parameters. Control survival percentage was best wheraas DPI7 was worst for both coating agents and laminin coating induced survival to a higher extent than fibronectin coating for every day *in vitro* (laminin (%): control=91, DPI7=87, DPI14=90; fibronectin (%): control=88, DPI7=79, DPI14=81). Spheroid percentage and sphere number of DPI 7 was higher than the two groups for all days *in vitro* regardless of the coating effect (SP for laminin (%): control=48, DPI7=54, DPI14=34; for fibronectin (%): control=23, DPI7=45, DPI14=19). Injury had a negative effect on neurite outgrowth and delayed outgrowth onset. ICC studies also confirmed the existence of proliferating cells, neurons and glia. This work is the only axolotl mix sc culture study to our knowledge. Results with high survival rate for uninjured control group, even for over three weeks *in vitro* without additional serum or factors, indicate an intrinsic regeneration capacity. The injury model also worked but the time window assigned for healing was not sufficient. Although high spheroid percentage and low survival observed at DPI7 implies an acute phase in healing; further studies of groups with more post injury days will be executed in order to measure the boosting phase of the injury healing.

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Poster

160. Motor Unit Recruitment

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Topic: D.13. Motor Neurons and Muscle

Support: ERC DEMOVE 267888

Title: The relation between alpha motor neuron excitability and discharge rate depends on the membrane noise level

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Abstract: Simulation and experimental studies have individually shown that the amplitude (probability) of the reflex response of motor neurons (MNs) to composite excitatory post synaptic potentials is dependent on the background discharge frequency and the membrane noise. In the present study, we hypothesized that the discharge frequency and the membrane noise may be the factors that determine the reflex response regime of a MN population activated at a given force level. For this purpose, the mono-synaptic H-reflex and the bi-synaptic reciprocal inhibition of a relatively large population of motor units were recorded from the tibialis anterior muscle of 12 subjects (6 for excitation and 6 for inhibition) at 10% and 20% of maximum voluntary contraction using high density EMG electrodes. During the task, the subjects received electrical stimulation of tibial and peroneal nerves in different sessions. The hypothesis was additionally tested using a model of 100 MNs for a specific case as the membrane resistance of MNs changed gradually while the afferent input received was kept constant. The reflex amplitudes were measured using the cumulative sum of peri-stimulus frequencygram. For both simulated and experimentally recorded MNs, the correlation between the reflex amplitude and the pre-stimulus mean discharge rate were estimated. A significant positive correlation was found between the discharge rate and the reflex amplitude for 10 subjects at 10% MVC (4 in excitation: $p<0.05$; 6 for inhibition: $p< 0.05$) and for 8 subjects at 20% MVC (3 in excitation: $p<0.05$; 5 for inhibition: $p< 0.05$). Moreover the correlation coefficients (r) decreased with decreasing coefficient of variation for the inter-spike-intervals ($p < 0.05$), that was used as a measure of membrane noise. The simulations supported the experimental results. The results suggest that the MNs that fire with higher frequency in a population activated at moderate contraction forces have higher responses to inhibitor or excitatory postsynaptic potentials. However this is dependent on the membrane noise.

Disclosures: U. Yavuz: None. **F. Negro:** None. **O. Sebik:** None. **C. Frömmel:** None. **K.S. Türker:** None. **D. Farina:** None.

Poster

160. Motor Unit Recruitment

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 160.02/P39

Topic: D.13. Motor Neurons and Muscle

Support: European Research Council under the Advanced Grant DEMOVE (contract #267888) to DF

Title: Is the synaptic input common to the whole motor neuron pool?

Authors: *F. NEGRO, S. MUCELI, M. CASTRONOVO, D. FARINA;
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Abstract: During sustained contractions, motor neurons receive both common and independent synaptic inputs from presynaptic neurons and supraspinal centers. The common synaptic inputs generate a certain degree of correlation between the membrane potentials of the motor neurons that result in a significant level of synchronization between their spike trains [1]. Several studies have showed this phenomenon and quantified the amount of synchronization using different indexes [2]. Due to technical limitations, most of the studies have focused on the estimation of synchronization from pairs of motor neuron spike trains and not from larger populations. For this reason, it has not been proven experimentally if all motor neurons in a pool share a source of common input. Since the motor neurons discharge at relatively low rates, there is also a theoretical constraint in the identification of common inputs at high frequencies since the sample rate is too small [3,4]. For these reasons, we tested the existence of a shared synaptic input across relatively large motor neuron populations recorded from the tibialis anterior (TA) muscle. The presence of higher order correlations was tested using a novel approach based on the comparison between the binned superimposition of multiple spike trains (composite spike train, CST) and its shuffled version. The method was validated using simulated motor neuron spike trains and then applied to experimental recordings. For the experimental part, two subjects performed three isometric contractions of the TA muscle at 10, 20 and 30 % MVC for 30 s. Intramuscular EMG signals were recorded using two novel thin film electrodes with 16 contact points and decomposed manually with an advanced algorithm [5]. This technique provided the possibility to extract a relatively large population of motor neuron spike trains. A total of 231 (38 ± 20 per subject and contraction) motor unit spike trains were recorded. Using the proposed method, we detected a correlation degree greater than 9 (i.e., the maximum tested correlation degree) in all the recorded contractions. These results strongly point out that the synaptic input received by the motor neuron pool in the tibialis anterior muscle is common across the full motor neuron population in humans. This work was supported by European Research Council under the Advanced Grant DEMOVE (contract #267888) to DF. [1] Semmler J. (2002). Exerc Sport Sci Rev, 30(1):8-14 [2] Nordstrom M, et al. (1990). J Physiol, 426(1):409-21 [3] Negro F., et al. (2012). PLoS ONE, 7(9):e44894 [4] Farina D. & Negro F. (2015). Exerc Sport Sci Rev, 43.1:23-33 [5] McGill et al. (2005). J Neurosci Methods, 149.2:121-133

Disclosures: F. Negro: None. **S. Muceli:** None. **M. Castronovo:** None. **D. Farina:** None.

Poster

160. Motor Unit Recruitment

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 160.03/P40

Topic: D.13. Motor Neurons and Muscle

Title: The motor neuron pool as a linear system

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Abstract: Most voluntary movements occur as a result of a coordinated central drive to the spinal motor neurons whose activation triggers muscle contractions and thus movement. This drive is believed to be determined by internal forward models. These models need to be flexible to allow adaptation to changes in the requirement for the movement, such as different inertial loads [1]. We hypothesize that such flexibility is most easily achieved with a linear system. However, the nervous system at the spinal level consists of several non-linear elements, including the motor neurons [2] and sensory neurons that provide a non-linear sampling of their inputs [3]. In this study, we characterized the linearity of the motor neuron pool using a novel computational model. In the model, two populations of motor neurons received synaptic input from descending drive, spinal interneurons and afferent feedback. Muscle force, simulated based on motor neuron activity, determined limb movement which gave rise to afferent feedback from muscle spindles and Golgi tendon organs. A series of simulation was performed in which a sinusoidal current (1-12 Hz in steps of 1 Hz) or the sum of these 12 sine waves was imposed to the motor neuron pool to study their output. This series of simulations was repeated with different offsets of the neural drive implying different average contraction levels. Linearity was assessed based on two criteria: 1) the amount of power in the neural drive to the muscle at the imposed frequency with respect to the total, and 2) the degree to which the sum of the neural drives for the 12 simulations with single sine waves imposed was equal to the neural drive reflecting the simulation with the combined neural input of all 12 sine waves. The power at frequencies outside the imposed sine wave decreased gradually as the contraction level increased. For example, at 5% of the maximum voluntary contraction level (MVC), this power was on average 5.4±10.8% of the power at the frequency of the imposed sinusoid, but only 1.9±3.1% at 25% MVC. Similarly, the average relative error between the power of the sine waves when imposed individually or combined decreased from 59.9±31.6% (5% MVC) to 22.2±25.5% (25% MVC). In conclusion, the motor neuron pool integrates and transmits descending commands and afferent input in an approximately linear way, especially for high excitatory input. This observation has implications for our understanding of cost functions used

to estimate optimal patterns of muscle activation, as greater contraction levels may imply a more reliable control. References: [1] Wolpert et al. Nat Rev Neurosci 2011 [2] Negro & Farina. J Physiol 2011 [3] Mileusnic et al. J Neurophysiol 2006

Disclosures: J.L. Dideriksen: None. **F. Negro:** None. **D. Farina:** None.

Poster

160. Motor Unit Recruitment

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 160.04/P41

Topic: D.13. Motor Neurons and Muscle

Support: Neilsen Foundation 260215

NIH NS034382

NIH NS089313

NIH NS085331

Title: Decorrelated neural drive to muscle from highly consistent inputs of specific sensory pathways in the *in vivo* cat

Authors: *C. K. THOMPSON[1], F. NEGRO[2], M. D. JOHNSON[1], M. R. HOLMES[1], L. C. MILLER[1], D. FARINA[2], C. J. HECKMAN[1];
[1]Dept. of Physiol., Northwestern Univ., Chicago, IL; [2]Dept. of Neurorehabilitation Engin., Univ. Med. Center, Georg-August Univ., Göttingen, Germany

Abstract: The motor unit (MU) provides a 1:1 linkage between the discharge of a spinal motoneuron and the activation of muscle fibers. Recent hardware and computational advances have allowed for the accurate decomposition of a large number of concurrently active MU spike trains. Using these approaches, statistical tools, such as coherence, can be used to infer qualities of the synaptic input received by the spinal motor pool. For example, a significant amount of MU coherence a given frequency ranges indicates that the motor pool is receiving common synaptic input at those frequencies. Here we assess the degree of coherence induced by three forms of evoked synaptic drive using the unparalyzed, unanesthetized, decerebrate cat. With force and 64-channel EMG collected from isolated hind limb muscles, we first applied small amplitude (~80 um) trains of vibration at frequencies from 80 to 200 Hz to the soleus tendon and measured the evoked motor output from the homonymous soleus muscle. Second, we stimulated the caudal

cutaneous sural nerve at frequencies ranging from 2.5 to 200 Hz and measured the evoked motor output from the ipsilateral medial gastrocnemius and soleus muscles. Third, we stimulated the distal branch of the superficial peroneal nerve at frequencies from 2.5 to 200 Hz and measured the evoked motor output from the contralateral soleus muscle. Offline, the multichannel EMG activity was decomposed into corresponding MU spike times using automated algorithms, and coherence between composite MU spike trains was calculated. Tendon vibration produced strong MU coherence at the vibration frequency, ipsilateral cutaneous activation produced moderate amounts of coherence at the stimulation frequencies, and contralateral cutaneous activation produced an insignificant amount of coherence, except for the very lowest of stimulation frequencies. These data are consistent with the presumed anatomy of spinal pathways: Tendon vibration has strong monosynaptic connections, ipsilateral cutaneous pathways are internuncial with relatively few interneurons, while contralateral cutaneous cutaneous pathways may contain relatively larger number of interneurons. Though the anatomical substrate underlying these polysynaptic pathways is unresolved, it appears a putative increase in the number of synaptic relays can serve to decorrelate the neural drive to muscle, despite highly consistent inputs. These finding suggest the anatomical connections to spinal motoneurons may have an important role shaping the frequency content of muscle force generation.

Disclosures: C.K. Thompson: None. **F. Negro:** None. **M.D. Johnson:** None. **M.R. Holmes:** None. **L.C. Miller:** None. **D. Farina:** None. **C.J. Heckman:** None.

Poster

160. Motor Unit Recruitment

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 160.05/P42

Topic: D.13. Motor Neurons and Muscle

Title: Mapping the discharge of motor unit populations in the human lower extremity

Authors: *D. M. HURLEY[1], S. A. HRUBY[1], I. JOSHI[1], H. KANG[1], C. K. THOMPSON[2], L. C. MILLER[1], N. SÁNCHEZ[1,3], R. K. POWERS[4], F. NEGRO[5], D. FARINA[5], J. P. A. DEWALD[1,3], C. J. HECKMAN[1,2];
[1]Dept. of Physical Therapy and Human Movement Sci., [2]Dept. of Physiol., [3]Dept. of Biomed. Engin., Northwestern Univ., Chicago, IL; [4]Dept. of Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [5]Dept. of Neurorehabilitation Engin., Univ. Med. Center, Georg-August Univ., Göttingen, Germany

Abstract: Muscle force is regulated by the activation of motor units. Quantifying the discharge patterns across multiple motor pools can provide insight into the sources of synaptic drive underlying motor output. For example, the onset/offset hysteresis among high and low threshold motor units can provide a surrogate measure of intrinsic excitability of spinal motoneurons (delta-f). Recent data from ramp contractions in the upper extremity demonstrate a 2-fold increase the delta-f estimates of extensor (triceps) motoneurons versus flexor (biceps) motoneurons. Here we present our initial work assessing the onset/offset hysteresis of motor units from multiple lower extremity muscles. With the subjects secured to a custom isometric frame, 12 forces and moments were measured from 2 6-degree of freedom sensors to derive the torques generated at the hip, knee and ankle joint centers. The frame was positioned at a 30% angle from the horizontal so that the subject was semi-recumbent. From here, subjects performed 2-3 bouts of ramp contractions to 20% of maximum volitional torque with the assistance of auditory and visual feedback. Electromyographic (EMG) activity was collected from the soleus (Sol), medial gastrocnemius (MG), lateral gastrocnemius (LG), and tibialis anterior (TA) using 64-channel surface array electrodes. Offline, the multichannel EMG activity was decomposed into motor unit spike trains using an automated decomposition algorithm. Comparisons between the onset/offset hysteresis were made between 205, 33, 113, and 295 appropriate motor unit pairs for the Sol, MG, LG, and TA, respectively. In both participants, the Sol and LG demonstrated lower measure delta-f estimates than both the MG and TA. Such measures were consistent across repeated contractions and mode of contraction. Delta-f estimates for MG motor units were nearly identical during isolated plantarflexion and knee flexion contractions (3.57 versus 3.45 pps). Despite apparent stability in intrinsic excitability, unpaired t-tests revealed the average slope of the rate-rate plots among motor unit pairs was lower (0.82 versus 1.22) and the difference in recruitment time was greater (2.7 versus 1.3 s) during performance of knee flexion versus plantarflexion. These changes in the activation of the motor pool are consistent with alterations in the distribution of the effective synaptic drive towards high threshold MG motor units during knee flexion as compared to plantarflexion. These data provide initial hypotheses regarding the organization of the excitability of lumbar spinal motoneuron pools - intrinsic excitability varies across, but not within, lower extremity motor pools for a given task.

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Poster

160. Motor Unit Recruitment

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 160.06/Q1

Topic: D.13. Motor Neurons and Muscle

Support: R21HD080828-Strath

Title: Force fluctuations and motor unit activity during a handgrip task in individuals with chronic stroke and multiple sclerosis

Authors: ***M. JOSHI**[1], J. PETERSON[1], A. HYNGSTROM[2], S. STRATH[1], A. SWARTZ[1], F. NEGRO[3], D. FARINA[3], K. KEENAN[1];
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Abstract: Introduction Variability in force during steady voluntary contractions is most commonly reported to increase in individuals with chronic stroke and multiple sclerosis (MS) relative to healthy controls. Interestingly, studies have shown an increase in discharge rate variability in MS, though reduced discharge rate variability on the paretic side in chronic stroke. The finding of decreased motor unit discharge variability in chronic stroke is confounding as experimental and computational studies have established positive associations between force fluctuations and discharge rate variability. However, few studies have examined motor unit activity and force fluctuations concurrently in these two populations. The purpose of our study was to compare the performance of chronic stroke and MS subjects to neurologically intact subjects on a handgrip force steadiness task while recording motor unit activity in forearm muscles. **Methods** Handgrip force was measured in 8 stroke, 10 MS and 21 healthy subjects using a MSP 300 sensor (Measurement Specialties, Hampton, VA). The steadiness task consisted of 2 trials holding a force at 5% of maximal for 30 s with visual feedback. High-density surface EMG (128 channels) was recorded from wrist flexors and extensors using the EMG-USB2 system (OT Bioelettronica, Torino, Italy). EMG was decomposed using the convolution kernel compensation technique. Force steadiness and motor unit discharge rate variability were computed as the coefficient of variation (CV) of force and discharge rate, respectively. **Results** CV of force on the paretic side in stroke (7.5 ± 11.3%) was increased compared with the non-paretic side in stroke (1.4 ± 0.6%; $p = .002$), MS (1.0 ± 1.5%; $p < .007$), and healthy controls (0.9 ± 1.0%; $p < .002$). There were no differences in the CV of force between MS, healthy controls, and the non-paretic side in stroke ($p > .167$). Preliminary analysis of EMG data includes decompositions of action potential trains from 46 motor units in controls, 29 in the paretic and 25 in the non-paretic side in stroke, and 84 in MS. The CV of discharge rate on the paretic side in stroke (12.8 ± 6.9%) was similar compared with the non-paretic side (15.4 ± 9.8; $p = .088$), but decreased compared with MS (18.3 ± 6.6%; $p < .001$) and controls (19.9 ± 6.9%; $p < .001$). **Discussion** The increased force fluctuations observed on the paretic side in stroke were not

associated with increased discharge rate variability. This indicates other mechanisms likely contribute to increased force fluctuations for the handgrip task in stroke, possibly low-frequency modulation of motor unit discharge rates within and across muscles, and altered motor unit recruitment patterns.

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Poster

160. Motor Unit Recruitment

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 160.07/Q2

Topic: D.13. Motor Neurons and Muscle

Support: University of Wisconsin - Milwaukee College of Health Sciences Graduate Student Research Grant

Title: The effect of a visuospatial memory task on measures of postural control and motor unit activity in young and older adults

Authors: *J. J. PETERSON, K. G. KEENAN;
Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: Background: Choice step response time (CSRT), postural sway, and the ability to hold a steady force are predictors of postural control and falls risk in older adults. Performance of a secondary cognitive task has been reported to decrease postural control. However, most studies focus on the influence of a secondary task on postural sway during quiet standing, which may not sufficiently challenge postural control. Of the few studies that have studied the effect of a secondary cognitive task on stepping, only one has used a visuospatial (VS) memory task. The purpose of this study was to quantify the influence of a VS task on three measures of postural control. **Methods:** 16 young (26.2 ± 4.9 yrs) and 13 older (75 ± 6.9 yrs) adults performed the CSRT, postural sway, and a dorsiflexion force steadiness task under two conditions: with and without a VS task. The VS task involved visualizing and remembering the location of a star within a 2×2 grid. 20 trials of the CSRT were performed and response times were averaged across trials. Two 30 s postural sway trials were performed in three bilateral stances and mean velocity of the center of pressure was calculated. For the dorsiflexion steadiness task, a target force of 5% maximum voluntary contraction was held for two trials of 30 s and the coefficient of variation of force was quantified. High-density surface EMG was collected from tibialis anterior

using a 64-channel electrode (OTBioelettronica, Torino, IT) during the postural sway and steadiness tasks, then decomposed to obtain motor unit spike trains. **Results:** CSRT was slower when performed with the VS task compared to performance of the CSRT alone for young (1023 ± 234 ms and 815 ± 160 ms, respectively; $p < .001$) and older (1583 ± 262 ms and 1120 ± 176; $p < .001$) adults, with a greater increase in older adults ($p < .001$). Postural sway was not altered ($p = .85$) by the addition of the VS task. Force fluctuations were greater for older adults than young adults (7.1 ± 1.2% and 2.7 ± 1.1%, respectively; $p = .014$), and were increased in the older adults ($p < .001$) with the VS task (8.3 ± 6.9%) compared to without the VS task (6.0 ± 6.5%), with no change in young adults ($p = .472$). Preliminary analysis of EMG collected during the steadiness task from five young and five older adults found higher discharge rate variability ($p < .001$) for the older (16.2 ± 6%) than for the young (14.3 ± 6.3%) adults, but no change with VS task ($p = .319$). **Conclusions:** Practical implications are that tasks requiring VS memory may adversely influence postural control, though this effect is task specific and appears to disproportionately affect older adults. In addition, motor unit discharge behavior influences the age differences in postural control.

Disclosures: J.J. Peterson: None. **K.G. Keenan:** None.

Poster

160. Motor Unit Recruitment

Location: Hall A

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Program#/Poster#: 160.08/Q3

Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant HD-050111

Neuromuscular Research Foundation

Title: The notion of central fatigue: findings from a simulation study

Authors: *P. CONTESSA[1], A. PULEO[2], C. J. DE LUCA[1];
[1]Delsys Inc, Natick, MA; [2]Politecnico di Torino, Torino, Italy

Abstract: The sources of exercise-induced muscle fatigue remain subject to debate. There is general agreement on the influence of peripheral factors on muscle fatigue, i.e. factors within the muscle that impair the fiber contractile mechanism [1]. Central factors of muscle fatigue arising within the Central Nervous System (CNS) have also been hypothesized but have not yet found direct empirical evidence [2]. They are suggested to cause central fatigue by diminishing the

central drive to the motoneuron pool and limiting muscle performance. We developed a simulation model to investigate whether central factors of muscle fatigue are required to explain the motor unit and muscle force behavior observed during fatiguing isometric contractions in human subjects. The model simulates the firing behavior of motor units and the muscle force during isometric voluntary [3] and electrically elicited contractions [4] performed with the first dorsal interosseous muscle. Only peripheral factors of fatigue were included as a time-dependent decrease in the amplitude of the force twitches of the active motor units [3]. We replicated empirical protocols of fatiguing maximal [5] and sub-maximal contractions [6] during which central fatigue has been suggested to develop. Central fatigue was estimated based on the amplitude of the muscle force elicited by electrical stimulation during the voluntary efforts, a method known as the twitch-interpolation technique [2]. The simulations of maximal voluntary contractions indicated questionable assumptions associated to the notion of central fatigue. They showed that maximal muscle force evocable by supra-maximal electrical stimulation is always greater than maximal voluntary muscle force; and that sub-maximal voluntary force does not necessarily represent a failure of the CNS to drive motoneurons adequately (i.e. maximally), as implied by the notion of central fatigue. We also found that a model based exclusively on peripheral factors of muscle fatigue can reproduce the force behavior commonly attributed to central factors during fatiguing sub-maximal voluntary contractions. Our analysis does not directly refute the concept of central fatigue. However, it raises significant and fundamental concerns about the interpretation of the proposed causes of central fatigue. [1] Adam & De Luca. J Appl Physiol, 99: 268-280, 2005. [2] Gandevia. Physiol Rev, 81: 1725-1789, 2001. [3] Contessa & De Luca. J Neurophysiol, 109: 1548-1570, 2013. [4] Crago et al. J Neural Eng, 11(5), 2014. [5] Bigland-Ritchie et al. J Neurophysiol, 50: 313-324, 1983. [6] Eichelberger & Bilodeau. Clin Physiol Funct Imaging, 27: 298-304, 2007.

Disclosures: **P. Contessa:** A. Employment/Salary (full or part-time):; Delsys Inc.. **A. Puleo:** None. **C.J. De Luca:** Other; Delsys Inc..

Poster

160. Motor Unit Recruitment

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 160.09/Q4

Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant 1R43NS077526

Neuromuscular Research Foundation

Title: Evidence that synchronized motor unit firings are epiphenomena of the onion skin property not common inputs

Authors: *J. C. KLINE[1,2], C. J. DE LUCA[1,2];
[1]Delsys, Inc, Natick, MA; [2]Biomed. Engin., Boston Univ., Boston, MA

Abstract: The majority of the literature on the synchronization of motor unit firing attributes its occurrence to physical presynaptic inputs shared by motoneurons. Yet, there is a lack of empirical evidence confirming the notion that common inputs actually elicit synchronized firing instances under normal voluntary conditions. In our previous studies we provided statistical arguments questioning common inputs as the cause of synchronization. In our present work, we designed an experiment to explicitly test if, during voluntary contractions in human subjects, synchronized motor unit firing instances can be accounted for by the common input notion. Our experiment involved an isometric contraction paradigm consisting of two force plateaus; one greater than the other. Recorded surface electromyographic (EMG) signals were processed using our decomposition and error reduction algorithms to extract the firing instances of motor units active during voluntary contractions. We found that the average amount of synchronization measured between the same motor unit pairs active during both force levels decreased as the force level increased. The current documented framework of the common input notion provides no explanation that can account for these results. Therefore, we set out to identify a more probable explanation for synchronization. We compiled a data set of motor unit firings across different human subjects, muscles and force levels. Our analysis of 17,546 motor unit pairs - a data set more than an order of magnitude greater than any previously published - enabled a comprehensive study of synchronization across populations of concurrently active motor units with diverse firing properties. We found that the amount of synchronization between motor unit pairs varied depending on the sensitivity of the motor units to voluntary excitation, as indicated by the slope of the firing rate as a function of force. The inverse hierarchical arrangement of motor unit firing rates demonstrated by De Luca and Contessa (2012) classifies the synchronization-sensitivity relationship across different muscles and force levels. These findings substantiate the proposition that occasionally coincident firing instances, often referred to as synchronization, are merely naturally occurring epiphenomena of the Onion Skin property of motor units. Thus, it is not meaningful to attribute a physiological purpose to the incidence of synchronization.

Disclosures: J.C. Kline: A. Employment/Salary (full or part-time):; Delsys, Inc. **C.J. De Luca:** Other; Delsys, Inc.

Poster

160. Motor Unit Recruitment

Location: Hall A

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Program#/Poster#: 160.10/Q5

Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant 2-R44-NS077526-03

Title: Onion skin and common drive of motor units during voluntary dynamic contractions

Authors: *C. J. DELUCA[1,2], S. S. CHANG[1], S. H. ROY[1], J. C. KLINE[1], S. H. NAWAB[3]; [1]Delsys, Inc., Natick, MA; [2]Biomed. Engin., [3]Electrical and Computer Engin., Boston Univ., Boston, MA

Abstract: Over the past three decades various algorithms used to decompose the electromyographic (EMG) signal into its constituent motor unit action potentials (MUAPs) have been reported. The use of these algorithms has been vital to advancing the field of motor control demonstrating such properties as the onion skin of firing rates and the common drive of motor units. However, to date all algorithms have been limited to decomposing EMG signals from isometric contractions. In this report we describe a successful approach at decomposing the surface electromyographic (sEMG) signal collected from cyclic (repeated concentric and eccentric) dynamic contractions during flexion/extension of the elbow and during gait. The increased signal complexity introduced by the changing shapes of the MUAPs due to relative movement of the electrodes and the lengthening/shortening of muscle fibers was managed by an incremental approach to enhancing our established algorithm for decomposing sEMG signals obtained from isometric contractions. We used machine-learning algorithms and time-varying MUAP shape discrimination to decompose the sEMG signal from an increasingly challenging sequence of pseudo-static and dynamic contractions. The accuracy of the decomposition results was assessed by two verification methods that have been independently evaluated. The firing instances of the motor units had an accuracy of approximately 90%, with a MUAP train yield as high as 25. Preliminary observations from the performance of motor units during cyclic dynamic activities indicate that during repetitive concentric and eccentric contractions the control of motor units is governed by the same rules as those evidenced during isometric contractions. As the contraction increases from a relatively lower angle to a higher one, motor unit firing rates are regulated in a hierarchical manner according to the onion skin property: that is motor units first recruited to fire during relatively low contraction angles reach higher firing rates than other motor units recruited at greater contraction angles. This behavior is preserved across multiple cycles and is present in dynamic contractions of the elbow and during gait. Additionally, motor unit firing rates varied in unison with each other and were correlated with changes in the contraction angle corroborating evidence of a common drive to the motor units. In fact the relatively high degree of cross-correlation observed between firing rates of different motor units indicates that the common drive is a dominant motor unit control scheme during dynamic activities.

Disclosures: **C.J. DeLuca:** Other; Delsys, Inc. **S.S. Chang:** A. Employment/Salary (full or part-time):; Delsys, Inc. **S.H. Roy:** A. Employment/Salary (full or part-time):; Delsys, Inc. **J.C. Kline:** A. Employment/Salary (full or part-time):; Delsys, Inc. **S.H. Nawab:** None.

Poster

160. Motor Unit Recruitment

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Topic: D.13. Motor Neurons and Muscle

Support: NSERC Grant 112342

SFU Grant 711217

Title: Changes in motor unit recruitment threshold and firing behavior after pairing high frequency repetitive transcranial magnetic stimulation with robot-assisted movement practice following stroke: a pilot study

Authors: *T. D. IVANOVA[1], K. J. MILLER[1,3], A. GALLINA[1], N. J. SNOW[2], J. L. NEVA[2], N. M. H. LEDWELL[2], Z. G. XIAO[4], C. MENON[4], L. A. BOYD[2], S. J. GARLAND[1];
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Abstract: High-frequency repetitive transcranial magnetic stimulation (rTMS) can alter motor cortical excitability following stroke. Similarly, robotic-assisted intensive movement practice has been advanced as a therapy for stroke. However, the impact of combining these interventions on motor unit (MU) recruitment and firing behavior is not known. This study investigated the effects of simultaneous application of rTMS, over the ipsilesional primary motor cortex, with robotic-assisted active wrist extension practice (rTMS+RW). Four participants with chronic stroke (Fugl-Meyer upper extremity scores 8-30/66) completed 30 repetitions of 5Hz rTMS+RW. Muscle activity was recorded from the paretic extensor carpi radialis (ECR) and extensor carpi ulnaris (ECU) with two high-density surface electromyography (HDsEMG) 64-channel grids during isometric handgrip contractions. Two maximal voluntary contractions (MVC) and 3 ramp-and-hold contractions to 30% MVC were performed before and after rTMS+RW. HDsEMG was decomposed using MatLab-based decomposition software DEMUSE 4.0. Recruitment threshold (RT) as a percentage of MVC, MU firing frequency over the first 4

spikes (initial firing frequency, IFF) and over 2s during the holding phase of the ramp (mean firing frequency, MF) were calculated for each MU that was present both before and after rTMS+RW. The ability to modulate the firing rate was examined by subtracting the IFF from the MF. The participants demonstrated a 20% increase in MVC on average after rTMW+RW. Twenty-five wrist extensor MUs (16 ECR and 9 ECU) were identified with recruitment thresholds before rTMS+RW ranging from 0.2 – 30%MVC. Changes in MU RT after rTMS+RW were observed in 22/25 MUs despite comparable rate of force increase during the ramp contractions (3.4 vs 3.6%MVC/s, before and after rTMS+RW, respectively). RT decreased in 12 MUs by a mean of 14%MVC, increased in 9 MUs by 3%MVC and remained unchanged in 3 MUs. The MF increased by a mean of 2Hz (n=25), with larger MF increases (3.5Hz) being observed in the 12 MUs with a lower RT after rTMS+RW. The firing rates (n=25) showed larger modulation over the course of the ramp contraction after (8.3Hz; MF-IFF) than before rTMS+RW (4.2Hz). A single session of rTMS+RW was associated with changes in MU firing behaviour in the paretic ECR and ECU that suggest improved recruitment and modulation of MU firing. However, this was not consistent across participants. A full-scale crossover study is underway to determine the effects of combined rTMS+RW intervention compared to robotic-assisted active training alone. Future work should address which individuals respond to rTMS+RW after stroke.

Disclosures: **T.D. Ivanova:** None. **K.J. Miller:** None. **A. Gallina:** None. **N.J. Snow:** None. **J.L. Neva:** None. **N.M.H. Ledwell:** None. **Z.G. Xiao:** None. **C. Menon:** None. **L.A. Boyd:** None. **S.J. Garland:** None.

Poster

160. Motor Unit Recruitment

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 160.12/Q7

Topic: D.13. Motor Neurons and Muscle

Support: NIH R01NS080839

Memorial Hermann Foundation

Title: Assessing motor unit number and motor unit action potentials in hemiparetic hand muscles from F wave responses

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Abstract: The F-response was used in this study to assess changes in the first dorsal interosseous (FDI) muscle after a hemispheric stroke. The number of motor units and their sizes were estimated bilaterally in 12 stroke survivors by recording both the compound muscle action potential (CMAP), and F wave responses. These F waves were induced by applying a large number of electrical stimuli to the ulnar nerve. The amplitude distribution of repeater F waves or individual motor unit action potentials (MUAPs) were also compared between paretic and contralateral muscles. When averaged across all the subjects, a significantly lower motor unit number estimate was obtained for the paretic FDI muscle (88 ± 13) compared with the contralateral side (139 ± 11) ($p < 0.01$). Pooled surface MUAP amplitude analysis demonstrated a right-skewed distribution for both paretic (kurtosis 3.0) and contralateral (kurtosis 8.5) muscles. When normalized to each individual muscle's CMAP, the surface MUAP amplitude ranged from 0.22% to 4.94% (median 1.17%) of CMAP amplitude for the paretic muscle, and from 0.13% to 3.2% (median 0.62%) of CMAP amplitude for the contralateral muscle. A significant difference in MUAP outliers was also observed between the paretic and contralateral muscles. The findings of this study suggest that there is significant motor unit loss and muscle structural reorganization after stroke.

Disclosures: X. Li: None. **M. Fisher:** None. **W.Z. Rymer:** None. **P. Zhou:** None.

Poster

160. Motor Unit Recruitment

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 160.13/Q8

Topic: D.13. Motor Neurons and Muscle

Title: The effect of strength training on maximal motor unit discharge properties

Authors: *Z. K. POPE, G. M. HESTER, F. M. BENIK, J. SELLERS, J. M. DEFREITAS; Oklahoma State Univ., Stillwater, OK

Abstract: The physiological adaptations to strength training have been the focus of many research investigations. The general consensus suggests that changes occur not only within the muscle, but also within the nervous system structures which govern its' behavior. **PURPOSE:** To evaluate changes to maximal motor unit (MU) discharge properties in response to an 8 week

strength training intervention. **METHODS:** Surface electromyography (EMG) from 10 subjects was detected from the Vastus Lateralis muscle during maximal voluntary isometric knee extensions both before and following 8 weeks of dynamic strength training. The EMG signals were decomposed into their constituent MU action potential trains to determine the following properties for each MU: A.) recruitment threshold (RT – the relative force at the MU's first discharge), B.) mean discharge rate (MDR – obtained during peak force production), and C.) action potential size (AP_{SIZE} – calculated as the peak-to-peak amplitude). MU data from all the subjects were then pooled together and averaged into 5% intervals based on RT. Linear regression analyses were performed on both the pre- and post-training data to obtain regression coefficients for the following relationships: MDR vs. RT and AP_{SIZE} vs. RT. **RESULTS:** A total of 481 MUs were detected (209 pre + 272 post). Following training, maximal force production increased significantly by 11.0% ($p < 0.01$). Essentially no difference was observed for the relationship between MDR vs. RT following training ($y = -0.218x + 26.061$; $R^2 = 0.8937$ and $y = -0.2204x + 27.002$; $R^2 = 0.8727$ for pre- and post-training, respectively). However, the regression coefficients calculated for AP_{SIZE} vs. RT were affected as a result of strength training ($y = 3e^{-06}x + 7e^{-05}$; $R^2 = 0.8333$ and $y = 7e^{-06}x – 3e^{-05}$; $R^2 = 0.8727$ for pre- and post-training, respectively). Moreover, a qualitative, visual inspection of the regression lines suggests that AP_{SIZE} for MUs with high RTs (i.e. RT > 50%) were most affected by the strength training. **CONCLUSIONS:** Our findings indicate that the increased maximal force production following 8 weeks of strength training was not due to an increased MU discharge rate. These data do, however, suggest that the relationship between AP_{SIZE} vs. RT was positively affected by strength training. This may be interpreted as a training-related MU adaptation, particularly in high RT MUs, elicited by a repetitive, strong neural drive to resist heavy loads. Physiologically, the increased AP_{SIZE} of high RT MUs may suggest an enhanced excitability of the sarcolemma and/ or an increase in sarcolemma area due to muscle fiber hypertrophy.

Disclosures: **Z.K. Pope:** None. **G.M. Hester:** None. **F.M. Benik:** None. **J. Sellers:** None. **J.M. DeFreitas:** None.

Poster

160. Motor Unit Recruitment

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Topic: D.13. Motor Neurons and Muscle

Support: NICHD R15HD075207

Title: Motor neuron studies using multielectrode arrays

Authors: *A. THARANEETHARAN, M. HARRINGTON;
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Abstract: Motor neurons are one of the classic model systems in neuroscience. While it has been known for 60 years that the neurotransmitter released at neuromuscular junctions is acetylcholine, evidence has been accumulating that motor neurons release multiple neurotransmitters in a spatially segregated way - acetylcholine at neuromuscular junctions and both acetylcholine and glutamate at inter-neuronal synapses in the spinal cord. Recently published work from our lab and others has shown that in cultures of motor neurons grown in the absence of contact with muscle cells, excitatory neurotransmission in the culture is entirely through glutamate. These results indicate that the neurotransmitter phenotype of motor neurons is plastic. We have used transgenic mice expressing a yellow fluorescent protein and channelrhodopsin 2 fusion protein (mhChR2::YFP) under the control of the choline acetyltransferase (ChAT) promoter to assess synaptic function of primary motor neurons in culture. A second model used was a double floxed ChR2 lentivirus in spinal ventral horn cultures from a Chat-Cre mouse model. A third model used were motor neurons differentiated from iSPCs and transduced with a ChR2 lentivirus. We used the 64-electrode, MED64 multielectrode array to characterize network activity resulting from photostimulation of ChR2 expressing motor neurons. Using immunofluorescence we found changes in ChAT expression from early postnatal cultures as compared to embryonic cultures, while MED64 recordings also show changed bursting activity in postnatal as compared to embryonic. Our results highlight interesting variations between the synaptic activation and network properties of motor neurons established in culture during embryonic development as compared to cultures of motor neurons that have matured in the spinal cord. These findings pose interesting implications upon the use of primary motor neuron cultures and differentiated stem cells as model systems and potential therapeutics for motor related neuropathologies.

Disclosures: A. Tharaneetharan: None. **M. Harrington:** None.

Poster

160. Motor Unit Recruitment

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Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant 8KL2TR000056

Title: Modulation of motor units during repeated contractions post-stroke

Authors: *S. A. MURPHY[1], R. BERRIOS[1], S. HUNTER[1], F. NEGRO[2], D. FARINA[2], A. NELSON[3], K. KEENAN[4], B. SCHMIT[1], A. HYNGSTROM[1];
[1]Marquette Univ., Milwaukee, WI; [2]Gottingen Univ., Gottingen, Germany; [3]Med. Col. of Wisconsin, Milwaukee, WI; [4]Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: The purpose of this study was to use high density surface EMG (sEMG) recordings to quantify stroke-related effects of a serotonin antagonist (cyproheptadine) on motor unit firing behavior during repeated sub-maximal knee extensor contractions. A high density (64 channel) sEMG was used to record and extract single motor unit firing behavior in the vastus lateralis muscle of 6 individuals with chronic stroke and 8 controls during repeated ramp and hold isometric knee extension contractions. Each subject performed the protocol under two different conditions on different days: a single dose of cyproheptadine (8 mg) and a placebo (double blind, crossover design). During the torque decline phase of the ramp and hold cycles for the placebo condition, paretic motor unit firing rates were increased with subsequent contractions (6.19±0.35 pps vs 7.89±0.66 pps, $P < 0.05$) compared to controls (6.95±0.40 pps vs 6.68±0.41 pps). This corresponded with decreasing rates of torque decline for the paretic leg with successive contractions as compared to the control leg. In contrast, in the cyproheptadine condition, there was no significant difference in firing rates between the first and last repetitions for the paretic (7.79±0.86 pps vs 7.65±0.91 pps) or control groups (7.45±0.45 pps vs 7.32±0.41 pps). This also corresponded to increasing rates of torque decline for both the paretic and control legs with subsequent ramp and hold cycles for the cyproheptadine condition. These results suggest that regulation of declining forces may be impaired post stroke due to increased firing rates of paretic motor units. Ingestion of a serotonin antagonist may decrease motor unit firing rates and help regulate the rate of force decline with subsequent contractions post-stroke. Moreover, these abnormal firing behaviors may be due to changes in the intrinsic electrical properties of paretic motoneurons and can be modulated with serotonergic drugs.

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Poster

161. Social Behavior: Aggression

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 161.01/Q11

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant DA10547

Title: Anabolic steroid exposure during adolescence alters the response and the physiological parameters of neurons in the lateral anterior hypothalamus of the male hamster

Authors: *T. R. MORRISON[1], R. SIKES[2], R. H. MELLONI, Jr.[1];
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Abstract: Syrian hamsters exposed to anabolic/androgenic steroids (AAS) during adolescence consistently show increased aggressive behavior when tested during early adulthood. Previous data from our lab have shown that the activity of neurons in the latero-anterior hypothalamus (LAH) is sensitive to vasopressin (AVP), serotonin (5HT), and dopamine (D2-receptor selective) ligands, i.e., three neurochemical systems known to modulate aggression. These data are important in understanding how substances that affect AAS-induced aggression influence the circuitry of aggressive behavior. Despite these data, little is known about how AAS exposure alters the basal neurophysiology of cells in the LAH. In female adolescent mice, AAS exposure has been shown to alter the distribution of neuron response types (i.e., bursting, regular, and irregular firing neurons) within the central amygdala that regulate cells within the bed nucleus of the stria terminalis (BNST) (Oberlander, 2012). Interestingly, the LAH is the center of the AAS-induced aggression circuit in adolescent hamsters, and has been shown to share reciprocal connections with various regions of the brain associated with both aggression and anxiety including the BNST and amygdala. In the present study, we used extra-cellular single neuron recording techniques to record the electrophysiological activity of cells within the LAH of the aggressive, adolescent AAS-treated male hamsters. Preliminary data indicate that AAS exposure increases the mean and median interspike interval of regular firing neurons and also alters the proportion of neuronal types within this region, most notably by decreasing the number of bursting neurons. These data shed further light on how developmental exposure to AAS affects the physiology of neural substrates controlling aggression adding further detail to our model circuit(s) that underlies adolescent AAS-induced aggressive behavior.

Disclosures: T.R. Morrison: None. **R. Sikes:** None. **R.H. Melloni:** None.

Poster

161. Social Behavior: Aggression

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant R01 MH104603

EU COST Action CM1103

Tourette Syndrome Association

Kansas Strategic Initiative Grant

Title: Early postnatal blockade of corticotropin-releasing hormone 1 receptor prevents aggressive behaviors in the low MAOA activity x early stress interaction model of aggression

Authors: *M. BORTOLATO, S. C. GODAR, L. J. MOSHER, H. STRATHMAN; Pharmacol. and Toxicology, Univ. of Kansas, Lawrence, KS

Abstract: Ample evidence has shown that monoamine oxidase (MAO) A, the major enzyme for the metabolism of serotonin and norepinephrine, plays a key role in pathological aggression. MAO A-deficiency in humans and rodents is associated with high levels of brain monoamines and a greater severity of aggressive and antisocial traits, however, complete gene deficiency is very rare and may not present the optimal conditions to model aggression. A host of studies have reported that individuals carrying low MAO A activity polymorphic variants who were subjected to early maltreatment have a significantly more susceptible to develop aggression and antisocial behavior. These findings underscore the importance of gene (low levels of monoamine oxidase A) x environment (early neglect or abuse) interactions in the pathogenesis of aggression. We recently generated a novel model mouse based on this gene x environment interaction by subjecting non-aggressive MAO A hypomorphic mutant mice (MAO ANeo) to early stress (ES) from postnatal day 1 through 7. In particular, early stress consisted of daily maternal separation (~3h) and a saline (or vehicle) injection. ES markedly increased aggression in MAO ANeo, but not in WT mice. To investigate the neurobiological underpinnings of this interaction, we examined the role of the corticotropin-releasing hormone 1 (CRH1) receptors, in view of their importance in the regulation of stress mechanisms in the brain. We found that selective CRH1 receptor blockade during the first postnatal week significantly reduced aggression in MS-MAO ANeo mice. Taken together, these data provide a potential molecular mechanism to explain how low MAO A activity and early childhood maltreatment interact in the pathogenesis of aggression.

Disclosures: M. Bortolato: None. **S.C. Godar:** None. **L.J. Mosher:** None. **H. Strathman:** None.

Poster

161. Social Behavior: Aggression

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Topic: E.03. Behavioral Neuroendocrinology

Support: Sydney Bridging Grant to JArnold

Bosch Translational Grant to JArnold

NHMRC to MKassiou

Title: P2X7 knockout mice display less aggression which is associated with alterations in microglial number and morphology

Authors: *S. TODD[1,3,2], A. BOUCHER[3,2], F. WALKER[6,7], I. MCGREGOR[4], M. BENNETT[2], M. KASSIOU[5], J. ARNOLD[2,3];
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Abstract: The P2X7 receptor is an ATP-binding ligand gated ion channel found predominantly on microglia and to a lesser extent on neurons. This receptor has been associated with increased susceptibility for depression, and P2X7 knockout mice show resistance to behavioral despair. This might be explained by the role of P2X7 receptors on microglia which when activated release inflammatory cytokines such as interleukin-1 beta. Central cytokines also increase aggressive behavior, implying that P2X7 may also alter aggression. Here we aim to observe whether P2X7 knockout mice display altered aggressive behavior and if this is associated with changes in microglial number and morphology. WT and P2X7 knockout animals underwent the resident-intruder behavioural test and were scored for aggressive behaviours. Iba-1 immunohistochemistry was also performed to examine the influence of P2X7 genotype on aggression-induced microglial density and morphology by comparing highly aggressive (high numbers of aggressive behaviours and biting) to minimally aggressive mice (low instances of aggressive behavior with no biting). Microglia were imaged and analyzed for number and average pixel density per microglia as an approximation of complexity. P2X7 mice were significantly less aggressive than WT mice. A significant effect of P2X7 genotype was found in the 4 regions - the nucleus accumbens shell, medial posteroventral amygdala, dorsomedial and lateral part of the PAG. In all cases the P2X7 mice showed higher numbers of microglia compared to WT mice regardless of behavior exhibited. However in a further 8 regions - the ventrolateral septum, caudate putamen, nucleus accumbens core, paraventricular thalamic nucleus, lateral hypothalamus, CA3 of the hippocampus, medial posterolateral amygdala and dorsolateral PAG - a significant interaction effect was found between P2X7 and aggression. While in WT mice higher aggression was associated with a decrease in microglial number

compared to low aggressors, highly aggressive P2X7 knockout mice either had no change or higher numbers of microglia compared to low aggressors. When examining the average pixel density per microglia as an indication of morphology, a significant interaction effect was found in the caudate putamen, and nucleus accumbens shell and core. WT aggressive mice tended to have enlarged microglia compared to low aggressive mice, but the opposite was observed in P2X7 mice. These results show that the P2X7 receptor plays a role in aggressive behavior associated with alterations in microglial density and morphology.

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Poster

161. Social Behavior: Aggression

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Topic: E.03. Behavioral Neuroendocrinology

Support: Macao Science and Technology Development Fund, FDCT, Project 011/2014/A1

Title: A Thai tale: artificial selection for aggression in the siamese fighting fish Betta splendens

Authors: *D. GONÇALVES[1], C. HUANG[2], F. LAM[1], M. PODLESNA[1], L. S. W. LEI[2], S. M. Y. LEE[2];
[1]Univ. of St. Joseph, Macau, Macao; [2]State Key Lab. of Quality Res. in Chinese Med. and Inst. of Chinese Med. Sci., Univ. of Macau, Macau, Macao

Abstract: The Siamese fighting fish Betta splendens has been selected for fighting contests across more than two centuries in Thailand and other Asian countries. This has produced domesticated strains of B. splendens notoriously different from wildtypes. This artificial selection procedure has presumably favored genetic variations that promote the phenotypic expression of traits that increase aggression and the probability of winning fights. Because original wildtype phenotypes still occur in nature, the comparison of fighting strains with wildtypes offers the opportunity to identify the genetic, behavioral, endocrine and neuronal mechanisms that have been selected for promoting aggressive phenotypes in the domesticated strains. Males from a fighting strain were obtained from a commercial breeder and compared with males captured in the wild. As predicted, males of the fighting strain were more aggressive than wildtypes in a mirror challenge, both in the frequency of aggressive displays and in the persistence of aggressive behaviors towards the mirror image. Males of both the fighting strain

and wild types markedly increased the production of testosterone (T) and 11-ketotestosterone (11KT) in response to the mirror image. There was a trend for fighters to have higher levels of 11KT, but not of T, when compared with wildtypes. Finally, the gene expression profile in whole brains of wildtypes subject to the mirror test was compared with controls using Ion Torrent sequencing and differential gene expression analyses. In brief, around 9 Gb base-pairs were yielded and, 94,163 transcripts were de novo assembled using Trinity, with 54.3% of the transcripts annotated to known proteins. Parallel to the results obtained for androgens, the mirror challenge produced a significantly altered brain gene expression profile with over 8,269 unique transcripts being differentially expressed. KEGG analysis revealed differences in numerous important pathways between males expressing aggression and controls, including cytochrome P450, gluconeogenesis and immune-related pathways. The study shows that artificial selection of wildtype B. splendens was successful in generating an aggressive phenotype, suggests that selection acted at the level of the androgen response and provides data on genetic pathways regulating aggressive behavior in this species. In addition, the study provides the first annotated transcriptome for B. splendens.

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Poster

161. Social Behavior: Aggression

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS-0923301

Title: Sex differences in the effects of dominance status on serotonin (5-HT) and vasopressin (AVP) cell activity in Syrian hamsters (Mesocricetus auratus)

Authors: *J. I. TERRANOVA[1,2], Z. E. SONG[1,2], T. E. LARKIN[1,2], N. S. HARDCASTLE[1,2], A. NORVELLE[1,2], H. E. ALBERS[1,2];
[1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Ctr. for Behavioral Neurosci., Atlanta, GA

Abstract: 5-HT and AVP containing neurons are essential components of the circuitry that controls aggression. In male hamsters, microinjection of 8-OH-DPAT, a 5-HT1a receptor agonist, into the anterior hypothalamus (AH) profoundly *inhibits* aggression, whereas microinjection of AVP *stimulates* aggression. Conversely, in females, microinjection of 8-OH-

DPAT into the AH profoundly *stimulates* aggression, whereas microinjection of AVP *inhibits* aggression. These data suggest that 5-HT and AVP are critical components that underlie the neural control of aggression in both sexes. However, how endogenous AVP and 5-HT circuits are recruited during an aggressive encounter is unknown. Moreover, no published studies on hamsters have considered contributions of sex differences or dominance relationships to 5-HT and AVP activity during an agonistic encounter. Thus, the goal of this study is to investigate endogenous 5-HT and AVP cell activity in neural regions that project to the AH with regards to sex and dominance status. We predicted that 5-HT cell activity would be reduced in dominant males and increased in dominant females, whereas AVP cell activity would be increased in dominant males and reduced in dominant females. Hamsters were isolated for 2 weeks and handled daily for 1 week. Females were monitored for estrous stage and tested during diestrus. Hamsters were paired with a partner of the same sex and weight in a resident-intruder paradigm for 15 minutes or moved to an empty, dirty cage as a control. Dominance status was determined by observing at least 3 consecutive submissive behaviors by one animal during the encounter. Pairs were excluded if a clear dominance hierarchy was not established. Tissue was processed with immunofluorescence for 5-HT, AVP, or c-Fos, a marker for neural activity, and analyzed using confocal microscopy to ensure accurate colocalization of 5-HT and AVP with c-Fos. We measured AVP cell activity in the medial supraoptic nucleus (mSON), nucleus circularis (NC), and paraventricular nucleus and 5-HT cell activity in the anterior dorsal raphe (DRNa), posterior dorsal raphe (DRNp), and median raphe nuclei. 5-HT activity was higher in the DRNa and DRNp in dominant females compared to controls and subordinates (2.3% vs. 0.70% & 0.63% 5-HT activation, respectively). AVP activity was higher in the mSON and the NC in dominant males compared to controls and subordinates (mSON: 21.72% vs. 13.72 % & 17.37% AVP activation, respectively; NC: 71.94% vs 33.84 % & 24.72% AVP activation, respectively). Our data suggest endogenous 5-HT and AVP circuits are crucial for aggression and are modulated by both sex and dominance status. (This work was supported by NSF IOS-0923301)

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Poster

161. Social Behavior: Aggression

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 161.06/Q16

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF-IOS-0923391

Title: The role of sex differences and social experience in the regulation of arginine-vasopressin (AVP) V1a receptors and aggressive behavior in Syrian hamsters (Mesocricetus auratus)

Authors: *A. P. ROSS, T. LARKIN, E. Z. SONG, H. E. ALBERS;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Social isolation increases aggression in both male and female hamsters. In males, AVP increases aggression by acting on V1a AVP receptors in the anterior hypothalamus (AH). In females, however, AVP decreases aggression in the AH. The following experiment investigated the effects of social isolation on V1a receptor number in the AH. Male and female Syrian hamsters were housed individually or with 2 other hamsters for 4 weeks. To measure aggressive behavior, each hamster was paired with a same-sex nonaggressive intruder in a neutral arena for 5 min. Brains were collected immediately and processed for receptor binding using autoradiography. Social isolation increased aggression in both males and females. V1a receptor binding in the AH was greater in socially isolated males compared to those housed in groups. In contrast, there was no difference in V1a receptor binding in the AH between females that were socially isolated and those that were group-housed. The data support the hypothesis that social isolation increases aggression in males by increasing the number of V1a receptors in the AH. In contrast, the effects of social isolation on aggression in females are not mediated by the effects of social isolation on the number of V1a receptors. In a second experiment we investigated the effects of social isolation on the dose-response properties of AVP. For this experiment, male and female Syrian hamsters were again housed individually or with 2 other hamsters for 4 weeks. During the 4th week, all hamsters were implanted with guide cannula aimed at the AH, and estrous cycle was determined in female hamsters. Each hamster was then injected with 0.009, 0.09, 0.9, or 9 μM AVP. All hamsters were given each dose in a counterbalanced order 4 days apart. To measure aggressive behavior, each hamster was paired with a same-sex nonaggressive intruder in a neutral arena for 5 min immediately after each injection. Aggressive behavior and flank marking were scored for each hamster. Preliminary results indicate that social isolation increased aggression, but had no effect on flank marking. These results indicate that sex and social experience play important roles in the vasopressinergic regulation of aggression and social communication. This work was supported by NSF-IOS-0923391.

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Poster

161. Social Behavior: Aggression

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS-0923301

Title: Social experience modulates the ability of GABAA receptor activation to induce aggression in male Syrian hamsters (Mesocricetus auratus)

Authors: *J. BORLAND[1,2], T. E. LARKIN[1,2], A. NORVELLE[1,2], H. E. ALBERS[1,2];
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Abstract: Social isolation can produce dramatic dysfunctions in social behavior such as enhancing responsivity to social stress and increasing aggression. The mechanisms underlying these effects of social isolation are not well understood. The lateral septum is well known to have an inhibitory effect on aggression and we have recently demonstrated that GABAA activation induces aggression by acting within the lateral septum. As a result, in the following experiment we tested the hypothesis that social isolation modulates the ability of GABAA receptors within the lateral septum to induce aggression. Male hamsters (N=54) were implanted with guide cannula aimed at the lateral septum. Half of the subjects were socially isolated, and half were group-housed (4 per cage) for 4 weeks before behavioral testing. Hamsters were injected with the GABAA agonist muscimol (10mM/200 nl) or saline into the lateral septum and then tested 10 minutes later in a neutral arena with a smaller sex matched non-aggressive intruder for 5-min. As expected, the duration of aggression was significantly larger in the socially isolated hamsters compared to those housed in social groups. The duration of aggression was significantly ($p=.018$) greater in the socially isolated hamsters injected with muscimol (93.6 ±17.3 sec) than in the socially isolated hamsters injected with saline (41.9 ±14.4 sec). In contrast, there were no significant differences ($p=.998$) in the duration of aggression between socially housed hamsters injected with muscimol (1.2 ±1.2 sec), versus those injected with saline (6.0 ±5.8 sec). These data demonstrate that social experience can dramatically alter the ability of GABAA receptors in the lateral septum to increase aggression.

Disclosures: J. Borland: None. **T.E. Larkin:** None. **A. Norvelle:** None. **H.E. Albers:** None.

Poster

161. Social Behavior: Aggression

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 161.08/Q18

Topic: E.03. Behavioral Neuroendocrinology

Title: Behavioral studies of basic emotions

Authors: *F. WANG, Mr.[1,2], S. GU[3];
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Abstract: Emotional mental disorders are a leading cause of disabilities worldwide. Despite the obvious importance of emotion for human existence, the controversy still abounds over the definition of emotion, the number of basic emotions that exit, whether different basic emotions have different physiological signatures. The controversy prevents the diagnosis and treatment of affective disorders. In this paper we proposed some theories about basic emotions and proposed neuromodulators as the neural substrate for the basic emotions. First, we propose five basic emotions: happiness, sadness, anger and fear, and missing. They depend on whether the things or situations are fit for our needs, and how they fit for our needs, which are presented in the emotional circumplex model. Second, we propose that neuromodulators are the neural substrates for the basic: dopamine-happiness; serotonin-sadness; norepinephrine-arousal (anger and fear); Acetyl choline-missing. Third, we found that fear and anger are twin emotions, which result from the same neuromodulator NE, and have similar behavioral and somatic reactions. And they are interchangeable, fear lead to anger, and anger is vent of fear. Therefore, fear and anger are the two sides of a coin; or double edges of a sword. Last, we speculate a new psychotherapy method, using emotion to cure emotional diseases. For example, induce anger to cure phobia. In all, I think this paper helps clarify many things about the basic emotions, and can help us understand our everyday emotional changes.

Disclosures: **F. Wang:** A. Employment/Salary (full or part-time):; Nanjing University of Traditional Chinese Medicine. 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.; Nanjing Normal University. **S. Gu:** None.

Poster

161. Social Behavior: Aggression

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 161.09/Q19

Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS-1353859 to H.K.C.

Title: Behavioral effects of prenatal oxytocin manipulation in mice

Authors: *T. V. MILLER[1], S. TAMBORSKI[2], H. K. CALDWELL[1,2];
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Abstract: Male oxytocin knockout (Oxt-/-) mice have increased intermale aggression when born to Oxt-/- dams, but not when born to heterozygous (Oxt+/-) dams. Moreover, oxytocin receptor knockout (Oxtr-/-) mice also have increased intermale aggression, whereas mice with a conditional oxytocin receptor knockout (OxtrFB/FB), in which the gene is not excised until approximately three weeks postnatal, show normal levels of intermale aggression. These observations have led us to hypothesize that oxytocin during fetal development acts to organize the neural circuitry that underlies aggressive behavior. Based on previous work in which we mapped the developing oxytocin system, we hypothesize that blocking oxytocin's ability to bind to the oxytocin receptor at embryonic day (ED) 14 and/or ED16 will result in increases in intermale aggression in adulthood. To test this hypothesis, we injected an oxytocin receptor antagonist (OTA) into the lateral ventricles of fetal C57/BL6 mice at ED14 and ED16; after birth, and upon reaching adulthood, these mice underwent three resident-intruder tests 1 day apart to test for aggressive behavior. Our preliminary results suggest that that treatment with an OTA can affect adult aggressive behavior.

Disclosures: T.V. Miller: None. **S. Tamborski:** None. **H.K. Caldwell:** None.

Poster

161. Social Behavior: Aggression

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 161.10/Q20

Topic: E.03. Behavioral Neuroendocrinology

Title: Pre-weaning behavioral manners in prenatal bisphenol A treated rats

Authors: T. FUJIMOTO, *S.-I. HIRANO, Y. NISHIKAWA;
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Abstract: Bisphenol A (BPA) is well known as an environmental endocrine disrupter. Our previous studies showed that pre- and postnatal administration of low-level BPA impaired gender differences in the open-field behavior of rats. BPA also induced depression-like behavior and enhanced the response to predator odor after maturity. In this study, we focused on behavior

in the pre-weaning period. We administered low-level BPA (1.5 mg/kg/day) to prenatal rats, and examined their behavior at 8 and 20 days of age using the predator fox odor. We first examined the spontaneous behavior without fox odor, and then tested the same parameters in the presence of the odor. At 8 days of age, twitching, pivoting, head movement, crawling and immobility were observed for 2 minute. In the presence of the fox odor, decreases in head movement and increases in immobility were seen in female rats in both the control and BPA groups. However, the effect was pronounced in the BPA rats. The number of animals that displayed the crawling response was decreased by the fox odor only in the BPA rats. At 20 days of age, rearing, locomotor activity, grooming and immobility were observed for 3 minute. Among the spontaneous behaviors, gender differences were observed in locomotor activity of the controls but not in the BPA rats. Under the fox odor, the rearing and the locomotor activity were decreased, while immobility was increased in females in both the control and BPA groups. Grooming scores were not significantly affected by gender, treatment or fox odor. At 24 days of age, each of the four groups (control-male, control-female, BPA-male and BPA-female) was divided into two groups, one in which the blood was collected immediately, and the other in which it was collected after 20 min exposure to the fox odor. Corticosterone levels were higher in the odor-exposure group than in the non-exposure group in both the control and BPA rats. Behavioral changes in the susceptibility to predator odor were observed at both 8 and 20 days of age. We found that the BPA rats and female rats were more susceptible to odor. As in the previous study, we found that the gender difference in locomotor activity was impaired by BPA. This study showed that pre-weaning small size rats could be used for several neurotoxicologic studies.

Disclosures: **T. Fujimoto:** None. **S. Hirano:** None. **Y. Nishikawa:** None.

Poster

161. Social Behavior: Aggression

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 161.11/R1

Topic: F.03. Motivation and Emotion

Support: JSPS KAKENHI Grant Number 26430020

JSPS KAKENHI Grant Number 26860851

Title: Early period of development is critical for the effect of monosodium glutamate on decreased aggressive behavior in ADHD model rat

Authors: S. MISUMI, R. MARUMOTO, Y. YOKOYAMA, Y. SHIMIZU, R. NISHIGAKI, H. NAGAI, *H. HIDA;
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Abstract: Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopmental disease, in which dysfunction of mesocorticolimbic dopaminergic system is related to emotional regulation. External stimulus during the period of development such as environment and food might affect emotional formation strongly. We previously reported that oral intake monosodium L-glutamate (MSG), a taste substance for umami, for 5 weeks from postnatal day 25 (P25) to P60 decreased aggressive behavior in ADHD model rat (SHR) and this MSG effect was mediated by nevus vagus from gut, indicating involvement of brain-gut communication in emotional formation. However, the mechanism of the effect of MSG intake during the period of development on decreased aggressiveness in adulthood is still unknown. To investigate the critical period in MSG effect on social behavior, SHR rats (P25) were housed in a isolated condition (one rat per cage) treated with 0.6% MSG for various period until P60: early-treated group (P25-P40), late-treated group (P40-P60), all-period group (P25-P60) and non-treated group. Early-treated group decreased the number of riding (parameter of aggression to unfamiliar rat) compare with control group, which is the same level as all-period group. However, no significant difference was found between late-treated group and non-treated group. Sniffing time (parameter of exploration) failed to change among the each group, indicating that the interest to unfamiliar rat was not effect by MSG treatment. We next investigated plasma oxytocin by ELISA that is recently reported to improve social communication. No significant change of plasma oxytocin level was shown in all groups, and no correlation was also detected between aggressive behavior and the plasma level. Data suggest that early period of development post is critical for the effect of MSG on aggressive behavior in adulthood, although plasma oxytocin fails to explain MSG effect on aggressiveness.

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Poster

161. Social Behavior: Aggression

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 161.12/R2

Topic: F.01. Human Cognition and Behavior

Support: FAPESP Processo 11/08575-7

FAPESP Processo 13/20602-5

Title: Insights about amygdala and aggression

Authors: *F. V. GOUVEIA[1], C. C. OLIVEIRA[1], L. T. C. SANTOS[1], M. D. J. SENO[1], E. T. FONOFF[2], H. BRENTANI[3], A. MARTINS[3], L. C. D. AMORIM[3], E. ALHO[2], S. P. RIGONATTI[3], M. J. TEIXEIRA[2], R. C. R. MARTINEZ[1];
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Abstract: Aggressive behavior is fundamental to compete for food, territory and females that will ultimately results in the maintenance of the animal and the species. It is a heterogeneous behavior that has multiple ways and, in humans, is related to violence, impulsiveness, irritability and hostility. Drug treatment with antipsychotic and anticonvulsant drugs are most commonly used for the control of aggressiveness, nevertheless, there is a small population of patients that displays extremely high levels of aggression and has drug-refractory symptoms. The amygdala is an important structure in the neurocircuitry of aggressive behavior and previous studies using lesions of the amygdala showed improvement in patient condition. However, those studies had many limitations as follow-up, criteria for selecting patients and the surgery techniques were obsolete. As an attempt to reintegrate extremely aggressive patients into society, a judicial law proposed amygdala lesion in three adult men. In order of that and to have a better understanding of the neurobiology of human aggression, we analyzed the levels of different hormones - thyroid-stimulating hormone (TSH), T4, T3, Cortisol, Luteinizing Hormone (LH), Estradiol, Prolactin, Progesterone, testosterone, and sex hormone-binding globulin (SHBG) - before, 1, 6 and 12 months after surgery. Our results showed an average lesion of 59.68% of the amygdala nuclei. There was a positive correlation between the ratio testosterone/cortisol and aggressive behavior ($r2=0.13$, $F(1,7)=1.04$, $p>0.05$) until the 6 months follow-up. There were no statistical differences in all other hormones studied TSH ($F(6,14)=0.96$, $p>0.05$), T4 livre ($F(6,14)=0,022$ $p>0.05$), T3 ($F(6,14)=0.12$, $p>0.05$), LH ($F(6,14)=0.46$, $p>0.05$), FSH ($F(6,14)=0.48$, $p>0.05$), Estradiol ($F(6,14)=1.05$, $p>0.05$), Prolactin ($F(6,14)=0.14$, $p>0.05$), Progesterone ($F(6,14)=0.56$, $p>0.05$), SHBG ($F(6,14)=0,97$, $p>0.05$). Our results showed that partial amygdala lesion reduced aggression one month after surgery and this decreased was accompanied by a reduction in the ratio testosterone/cortisol until the 6 month of follow-up with no alteration in other hormones. Nevertheless, our results pointed out for a transitory effect that last until 6 months of follow-up, and by 12 months those effects were lost.

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Poster

161. Social Behavior: Aggression

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 161.13/R3

Topic: F.03. Motivation and Emotion

Support: MOST (Taiwan) 102-2410-H-194-030

NSC (Taiwan) 102-2410-H-008-021-MY3

MOST (Taiwan) 104-2420-H-008-001-MY2

Title: Understanding adolescent aggressive behavior: clues from the brain's response to laboratory-induced aggression

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Abstract: Violent offenses often cause both serious problems and danger in society. This type of behavior is often ascribed to problems with the processes involved in inhibitory control. In this study, a Taylor Aggression Task was used, along with event related potentials, to examine the effects of aggressive situations and emotions on inhibitory control. The experiment manipulated the proportion of win and loses on the task and subjects gave and received punishment following successes and failures in order to mimic a social context in the laboratory. The Reactive Proactive Aggression Questionnaire (RPAQ) and Barratt Impulsiveness Scale (BIS) were used to measure the participants' aggression types and their degree of impulsive behavior. The participants included impulsive violent adolescent offenders (experimental group), non-violent adolescent offenders (control group 1) and normal adolescents (control group 2). The RPAQ and BIS showed that the scores of the experimental group were significantly higher than those of both control groups. The behavioral data from the Taylor Aggression Task showed that the experimental group, when performing the task, gave more punishment than did control groups 1 and 2. The punishment scores given by subjects for the first trial, which involved no provocation (i.e. proactive aggression), was higher for the experimental group than for the controls. The percentage of subsequent high punishment scores (scores in the top half of the range) given by the experimental group was also larger than in the two control groups. The N2 ERP component, which can index inhibition, had a significantly lower amplitude for the experimental group and the feedback related negativity amplitude for this group was also significant higher than that of control group 2. The pattern of results suggests that violent adolescents may have a deficit in solving cognitive and emotional conflict and in evaluating social context for the modification of inhibitory control.

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Poster

161. Social Behavior: Aggression

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Topic: F.03. Motivation and Emotion

Support: NIMH (R01 MH58354)

Adrian Raine was supported by Independent Scientist Award K02 MH01114-08

Title: Autonomic correlates of reactive and proactive aggression

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Abstract: Heterogeneity in the construct of aggression has motivated researchers to identify sub forms of aggressive behaviors. Two functional forms of aggressive behaviors have been identified: reactive (RA) and proactive aggression (PA). It has been suggested that "hot-tempered" RA may be characterized by an over-active autonomic nervous system and an under-active autonomic nervous system may underlie "cold-tempered" PA (Scarpa &Raine, 2000). However, results from previous studies are both sparse and mixed. The current study investigated the autonomic correlates of these two sub forms of aggression in a community sample of 338 twins between ages of 16-18 years old, based on self-reported RA and PA using the Reactive Proactive Questionnaire (RPQ). Skin conductance and heart rate were measured during a 3 minute rest period as indices of autonomic arousal. Skin conductance responses to auditory orienting stimuli were collected as measures of autonomic reactivity. Multiple regressions were run to assess the associations between RA, PA and the autonomic measures of arousal and reactivity. PA was significantly and inversely related to both resting heart rate skin conductance levels, indicating a decreased baseline arousal for proactively aggressive individuals. PA was also associated with lower average skin conductance orienting response, suggesting hyporeactivity of the autonomic nervous system. RA was significantly and positively associated with resting skin conductance level, suggesting over arousal of the sympathetic nervous system for individuals with higher tendencies for reactive aggression. Results support a distinction between these two sub forms of aggression, and lend support to the theory that

proactive aggression is specifically characterized by autonomic under arousal and hyporeactivity. Evidence for reactive aggression to be characterized as a "hot-tempered" aggression was also found, as indicated by its association with increased skin conductance level.

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Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 162.01/R5

Topic: E.05. Stress and the Brain

Support: Science Without Borders (SWB)

Science Foundation of Ireland (SFI)

Alimentary Pharmabiotic Centre

Title: Postnatal oxytocin reverses selective behavioural and physiological effects of birth delivery by caesarean-section in mice

Authors: *L. H. MORAIS[1,2], Y. E. BORRE[2], A. GOLUBEVA[2], A. P. R. COSTA[2], K. SCOTT[2], G. MOLONEY[2], A. M. PEREZ[2], T. DINAN[2], J. F. CRYAN[2];
[1]Neurosci. and Anat., Univ. Col. Cork, Cork, Ireland; [2]Alimentary Pharmabiotic Centre, Univ. Col. Cork, Cork, Ireland

Abstract: Recent evidences points to an important role for the microbiome in regulating brain function and behaviour in health and disease. At birth, the infant microbiome is determined by maternal-offspring exchanges of microbiota. Simultaneously, the neuropeptide oxytocin (OXT) activates the newborn immune system responses and may be crucial for the establishment of a lifelong host-microbiome interaction. However, birth by Caesarean section (C-section) results in a different pattern of colonization. Interestingly, children delivered by C-section are more likely to develop immune and metabolic disorders, and more recently a small but significant increase in the propensity to develop autism spectrum disorder has been reported in this population. However,mechanistic insights into the long lasting consequences of C-section on brain-gut axis and behavior remain largely unexplored. The aims of the present study were to assess the temporal effects of C-section delivery on brain gut axis and behaviour in mice throughout their lifespan, and whether early postnatal subchronic subcutaneous treatment with OXT could prevent it. We applied a multi-disciplinary approach using a mouse model of C section, followed

by several behavioural assays and subsequent brain, microbiota and immune analyses. During postnatal days, animals born by C-section exhibited maternal attachment deficits. In adulthood, C-section born animals exhibited stereotyped behaviour, anxiety-like behaviour, deficiencies in social memory, disrupted hypothalamic-pituitary-adrenal axis responsivity, disrupted gastrointestinal motility and permeability, and an exacerbated immune-response. In early-life, OXT administration reversed C-section-mediated maternal attachment impairments. Remarkably, postnatal OXT administration prevented the social deficits, anxiety-like effects and stereotyped behaviour in adulthood, disrupted gastrointestinal motility and gut permeability in the C-section offspring. Taken together, these results demonstrate that C-section induces long-term changes across the brain-gut axis and behaviour. Moreover, our findings indicate that there is an early developmental window sensitive to manipulations of the OXT system that can prevent lifelong impairments induced by the mode of C-section.

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Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 162.02/R6

Topic: E.05. Stress and the Brain

Support: SFI/12/RC/2273

Title: Gut microbiota depletion during adulthood in rats: Implications for brain and behavior

Authors: *R. D. MOLONEY[1], A. E. HOBAN[2], G. CLARKE[3], T. G. DINAN[3,4], J. F. CRYAN[2,4];
[2]Anat. & Neurosci., [3]Psychiatry, [4]Lab. of NeuroGastroenterology, Alimentary Pharmabiotic Ctr., [1]Univ. Col. Cork, Cork, Ireland

Abstract: Background: There is growing appreciation for the importance of the gut microbiota in shaping brain function and behavior. Proof-of-principle studies in microbiota-deficient germ-free (GF) animals have played an important role in revealing the alterations reported in stress-related behaviors and neurochemistry. However, the maturation of multiple aspects of host physiology is contingent on normal patterns of gut microbiome assembly and development in early life. In order to more precisely parse the role of the gut microbiota outside of this critical

time window on brain and behavior, we set out to establish a rat model of microbiota depletion via chronic administration of antibiotics in adulthood. In particular, we assess the effects of gut microbiota depletion in adulthood on visceral sensitivity, cognitive and emotional behaviors and biological markers of microbiome-gut-brain axis dysfunction. Methods: Adult male Sprague Dawley rats (n=10/group) were treated with vehicle or a combination of antibiotics for 6 weeks in adulthood and throughout behavioral testing. Behavioral tests commenced after the 6 week antibiotic treatment period and were performed in the following order; elevated plus maze (EPM), open field (OF), novel object recognition (NOR), colorectal distension (CRD), fear conditioning (FC), forced swim test (FST), Morris water maze (MWM). HPLC and qRT-PCR was used to assess changes in key gut-brain axis neuromodulators (tryptophan, monoamines, and neuropeptides) in adulthood. Results: Antibiotic treatment reduced visceral sensitivity to CRD concomitant with decreased corticotrophin releasing hormone receptor 1 (CRHR1) and glucocorticoid receptor (GR) and in the amygdala, a key brain region involved in visceral perception. Moreover, antibiotic-treatment induced a depressive-like phenotype in the FST and induced cognitive deficits in the MWM in parallel with altered tryptophan metabolism and serotonin synthesis (5-HT) in the hippocampus, a critical brain region in emotional processing and cognitive functioning. Conclusion: Microbiota depletion in adulthood by means of chronic treatment with antibiotics impacts visceral sensitivity and depressive and cognitive behaviors as well as key neuromodulators of microbiome-gut-brain axis communication in a manner that is similar to that reported in GF animals. This model may represent an additional strategy for the assessment of the continuous role of the gut microbiota in the modulation of brain and behavior. Finally, these data confirm that the gut microbiota is a significant driving force of behavior and a key neuromodulator of gut-brain axis signaling during adulthood.

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Poster

162. Microbiota and Stress

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Program#/Poster#: 162.03/R7

Topic: E.05. Stress and the Brain

Support: Sckience Foundation Ireland Centre Grant 12/RC/2273

Title: Targeting the microbiota-gut-brain axis with prebiotics in mice: A novel strategy for stress-related disorders

Authors: A. BUROKAS[1], R. D. MOLONEY[1], S. ARBOLEYA[2], C. STANTON[2], T. G. DINAN[1], *J. CRYAN[1];
[1]Univ. Col. Cork, Cork, Ireland; [2]Teagasc, Moorepark, Cork, Ireland

Abstract: There is growing evidence for a critical role of the microbiota-gut-brain axis in health and disease. Indeed, modulation of the gut microbiome via probiotic treatment has been postulated to be a novel adjunctive strategy for stress-related psychiatric disorders such as depression and anxiety. The role of prebiotics is less investigated. Prebiotic fibres such as short-chain fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) are known to selectively modulate the composition of the intestinal microbiota, and to stimulate proliferation of lactobacilli and bifidobacteria in the gut. Prebiotics are commonly used in infant milk formula to alter the gut microbiota increasing concentrations of beneficial microbes in the gut and also short chain fatty acids which have been implicated as key signalling microbial metabolites. This study's objective was to test whether FOS and GOS alone or in combination altered behaviour in animal tests of anxiety, depression, cognition, stress response and social behaviour. 4 groups (n=10) of adult C57/Bl6/J male mice were administered with FOS, GOS, a combination of FOS and GOS (FOS/GOS), or water for the duration of the 10-week study; 3 weeks prior to - and 5 weeks during behavioural testing. Two weeks after the last behavioural test brains were harvested and gene expression of plasticity and stress-related genes quantified in hippocampus and hypothalamus. Administration of FOS/GOS decreased depressive-like behaviour in a number of behavioural models including the tail suspension and forced swim tests. Similarly, the administration of GOS and the combination of FOS/GOS decreased anxiety in the open field test and elevated plus maze and blunted stress-induced corticosterone concentrations. Moreover, FOS/GOS selectively increased hippocampal levels of brain-derived neurotrophic factor (BDNF) and GABAB1 receptor mRNA whilst decreasing the concentrations of corticotropin releasing factor mRNA (CRF). Interestingly, these prebiotic-induced behavioural changes were associated with increased caecal concentrations of the short-chain fatty acids acetate and propionate and reduced i-butyrate concentrations. These data show that these two prebiotics were actively influencing gut physiology, blunting the stress hormone response and positively modulating anxiety and antidepressant-related behaviours in healthy mice. It is tempting to speculate that these effects maybe mediated through changes in short chain fatty acid concentrations. Together these findings strengthen the role of gut microbiota supplementation as psychobiotic-based strategies for stress-related brain-gut axis disorders.

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Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: E.05. Stress and the Brain

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Title: Towards psychobiotics for stress & cognition: Bifidobacterium Longum blocks stress-induced behavioural and physiology changes and modulates brain activity and neurocognitive performance in healthy human subjects

Authors: *A. P. ALLEN[1,2,3], W. HUTCH[4], Y. BORRE[3], P. J. KENNEDY[2,3], A. TEMKO[5], G. BOYLAN[6], B. KIELY[8], G. CLARKE[2,3], J. F. CRYAN[3,7], T. G. DINAN[2,3];
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Abstract: Introduction: The emerging concept of the gut microbiome as a key regulator of brain and behaviour represents a paradigm shift in neuroscience. Precise targeting of the microbiome-gut-brain axis with psychobiotics - live microorganisms with a potential mental health benefit -is a novel approach for the management of stress-related conditions. Preclinical studies have identified a strain of Bifidobacterium, B. longum NCIMB 41676, as a putative psychobiotic with an impact on stress-related behaviours, physiology and cognitive performance. This study thus investigated whether these preclinical effects could be translated to healthy human volunteers. Methods: Healthy male volunteers (N = 22, Mean age = 25.5 +/- 1.2 years) completed the study.

Participants ingested (2.16 +/-0.20) X 109 CFU of B. longum NCIMB 41676 or placebo daily for four weeks each in a repeated-measures design. Participants completed study visits at baseline, post-placebo and post-probiotic. Acute stress (subjective and cortisol output) was assessed using the socially evaluated cold pressor test, and daily stress was assessed online via a validated questionnaire (Cohen Perceived Stress Scale). Cognitive performance was assessed using the CANTAB platform and neurological activity via resting electroencephelography (EEG). Results: In response to acute stress, B. longum NCIMB 41676 led to a reduction in cumulative cortisol output as well as a blunted increase in subjective anxiety. Self-reported daily stress was also lowered during daily consumption of the probiotic. There was a subtle improvement over placebo in visuospatial memory performance in paired associate learning (PAL) in the B. longum NCIMB 41676 group. Central EEG theta power was lower following B. longum NCIMB 41676 consumption compared to placebo. Conclusions: B. longum NCIMB 41676 is associated with attenuated responses to psychological and physiological stress and a modest improvement in cognitive performance, as well as with altered EEG output in healthy volunteers. These clear but subtle benefits are in line with the predicted impact from preclinical screening platforms and highlight the promise of precision-microbiome manipulation strategies. Further studies are warranted to evaluate the benefits of this putative psychobiotic in relevant stress-related conditions and to unravel the mechanisms underlying such effects.

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Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 162.05/R9

Topic: E.05. Stress and the Brain

Support: Science Foundation Ireland SFI/12/RC/2273

Brain and Behaviour Research Foundation NARSAD 20771

Title: Regulation of microRNAs in the amygdala by the gut microbiota: implications for brain and behaviour

Authors: ***G. CLARKE**[1,2], A. E. HOBAN[3,4], R. M. STILLING[3,4], F. SHANAHAN[4], T. G. DINAN[2,4], J. F. CRYAN[5,4];

[2]Psychiatry, [3]Anat. and Neurosci., [4]Alimentary Pharmabiotic Ctr., [5]Neurosci. and Anat., [1]Univ. Col. Cork, Cork, Ireland

Abstract: Background: According to the World Health Organisation, an estimated 16% of the population are affected by anxiety disorders which represent some of the most common illnesses experienced today. Anxiety disorders are extremely difficult to successfully treat, which may in part be due to failure to completely understand the aetiology of these disorders. While it is well known that a variety of genetic and environmental factors contribute to the development and severity of anxiety disorders, the ability of the gut microbiota to influence brain and behaviour is a relatively new area of research. One of the most consistent findings is in relation to anxiety-like behaviours and the stress response. Though the molecular mechanisms underpinning these effects remain poorly understood, they may well be due to microbiota-driven alterations in gene expression at the level of the CNS. It is unknown if the gut microbiota also recruits microRNA machinery to wield this influence. The aim of this experiment was to establish if germ-free animals have altered microRNA expression patterns in the amygdala, a key brain region for acquisition and expression of fear and anxiety. Methods: Using Illumina Next Generation Sequencing (NGS), we assessed alterations in microRNA expression in the amygdala of conventional, germ-free and colonized germ-free mice. Relevant alterations in microRNA expression levels were verified by quantitative real-time PCR (qRT-PCR) Results: The microbiota-deficient germ-free animals displayed altered expression of 103 miRNAs in the amygdala compared to conventional animals. However, colonisation of the germ-free animals post weaning normalised the expression of 6 miRNAs, suggesting partial reversibility of the cumulative molecular changes. Within these, miR-182 and mir-183 have been previously linked to amygdala-dependent stress-related outputs in preclinical models. Validation of these alterations by qRT-PCR verified changes in micrRNA expression indicated by NGS. Conclusion: This is, to our knowledge, the first demonstration that the gut microbiota can regulate miRNA expression in the amygdala. Moreover, our results confirm that the microbiota can be successfully targeted later in life to modulate miRNA expression. The analysis of mRNA targets may reveal important molecular pathways for microbiota-gut-brain axis signalling and will be essential in increasing our understanding of the molecular mechanisms underpinning this bidirectional communication. Additional studies are required to verify the exact contribution of these miRNAs to amygdala-dependent anxiety-related behaviours.

Disclosures: **G. Clarke:** A. Employment/Salary (full or part-time):; University College Cork. 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.; Science Foundation Ireland, Health Research Board, Brain and Behaviour Research Foundation. **A.E. Hoban:** A. Employment/Salary (full or part-time):; University College Cork. **R.M. Stilling:** A. Employment/Salary (full or part-time):; University College Cork. **F. Shanahan:** A. Employment/Salary (full or part-time):; University College Cork. 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.; Science Foundation Ireland. **T.G. Dinan:** A. Employment/Salary (full or part-time):; University College Cork. 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.; Science Foundation Ireland, Health Research Board. **J.F. Cryan:** A. Employment/Salary (full or part-time):; University College Cork. 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.; Science Foundation Ireland, Health Research Board.

Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 162.06/R10

Topic: E.05. Stress and the Brain

Support: IRC Grant GOIPD/2014/355

SFI Grant 07/CE/B1368

SFI Grant 12/RC/2273

NARSAD YI Grant 20771

Title: Social microbes - the microbiota regulates sociability behaviour through stimulus-induced RNA-based pathways in the amygdala

Authors: *R. M. STILLING[1,2,3], A. E. HOBAN[2,3,1], F. J. RYAN[2,4,1], G. M. MOLONEY[2,3,1], F. SHANAHAN[2,1], G. CLARKE[2,1,5], M. J. CLAESSON[2,4,1], T. G. DINAN[2,5,1], J. F. CRYAN[2,3,1]; [2]Alimentary Pharmabiotic Ctr., [3]Dept. Anat. and Neurosci., [4]Dept. Microbiology, [5]Dept. Psychiatry, [1]Univ. Col. Cork, Cork, Ireland

Abstract: The tight association of the human body with trillions of colonizing microbes that we observe today is the result of a long evolutionary history. Only very recently have we started to understand how this symbiosis also affects brain function and behaviour. We could demonstrate that mice raised without any microbiota (germ-free, GF) throughout development show sociability impairments and altered anxiety along with an amygdalar transcriptional profile

reminiscent of increased neuronal activity. However, it is currently unclear if changes in neuronal gene expression are underlying the observed behavioural alterations. To further elucidate the molecular underpinnings of microbe-brain interaction on the behavioural level, we therefore determined the dynamic transcriptional regulation of GF and GF mice colonised at postnatal day 21 in the amygdala in response to a social interaction experience. Using ribodepleted, stranded, and paired-end RNA-sequencing libraries combined with comprehensive downstream analysis pipeline we determined the amygdalar stimulus-dependent coding and non-coding transcriptome. As expected, conventional control animals show strong upregulation of the well-known stimulus-responsive MAP-kinase pathway in the amygdala in response to social behavioural stimulation. In contrast, in GF mice dynamic was attenuated and, surprisingly, we noticed a marked increase in splicing factors and splicosomal ncRNAs instead. Indeed, as a consequence of enhanced splicing factor production, we find increased alternative exon usage in GF mice upon stimulation. In conclusion, our data suggest that the observed altered behavioural response to a novel social situation is correlated to a differential gene-expression response in the amygdala of germ-free animals, which is likely to be established during early life as a result of absent critical host-microbe interactions. This finding may have far reaching implications for understanding social behavioural dysregulation in neurodevelopmental disorders such as autism and schizophrenia.

Disclosures: **R.M. Stilling:** None. **A.E. Hoban:** None. **F.J. Ryan:** None. **G.M. Moloney:** None. **F. Shanahan:** None. **G. Clarke:** None. **M.J. Claesson:** None. **T.G. Dinan:** None. **J.F. Cryan:** None.

Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 162.07/R11

Topic: E.05. Stress and the Brain

Support: SFI/12/RC/2273

Title: The microbiota regulates amygdaloid volume and dendritic length

Authors: *G. MOLONEY[1], P. LUCZYNSKI[2], F. SHANAHAN[2], G. CLARKE[3], T. G. DINAN[3], J. F. CRYAN[4];
[2]Alimentary Pharmabiotic Ctr., [3]Psychiatry, [4]Anat. and Neurosci., [1]Univ. Col. Cork, Cork, Ireland

Abstract: Increasing evidence points to a role of the microbiota in the regulation of brain and behaviour. Germ-free (GF) mice are an important tool to assess the role of the microbiota in brain function. We have shown that GF mice exhibit an exaggerated hypothalamic-pituitary-adrenal (HPA) axis response to an acute stressor and reduced anxiety-like behaviour but the mechanisms behind this phenotype remains unclear. The amygdala is an important structure known to regulate fear and anxiety in the brain and to activate the HPA axis. This implicates the amygdala as a possible target in the microbiome-gut-brain axis but it remains an understudied brain region in this field. The aim of this study was to determine if the volume and dendritic morphology of the amygdala differ in GF compared to conventionally colonized (CC) mice. To study amygdaloid volume, mice were perfused and brains were fixed, sectioned coronally. Dendritic morphology and spinal density was assessed using Golgi-Cox staining. Stereological measures of regions of the amygdala revealed significant expansions of the basolateral (BLA), lateral (LA) and central (CeA) nuclei in GF v CC mice. We also investigated the effect of GF status at the level of single excitatory and inhibitory neurons in the BLA by measuring the length and branching of Golgi-stained. In GF mice, the total dendritic length of stellate neurons was significantly increased while the number of branching points was also increased. Sholl analysis revealed a significant increase in dendritic material in stellate neurons localised to proximal and intermediate dendrites. In BLA pyramidal neurons, total, apical and basilar dendritic length is increased in GF mice. Sholl analysis indicates that dendritic elongation was localized to intermediate dendrites. Spine density was calculated by counting spines on 2-3 50 μm apical and basilar dendritic segments per pyramidal-like neuron. In GF mice, spine density was increased on both apical and basilar dendrites. These findings suggest that the presence of microbiota is critical for normal neurodevelopment of the amygdala and its nuclei and that neural remodelling along the microbiome-gut-brain axis could contribute to the altered behavioural and physiological phenotype observed in GF animals.

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Poster

162. Microbiota and Stress

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Program#/Poster#: 162.08/R12

Topic: E.05. Stress and the Brain

Support: NARSAD Young Investigator Grant 20771

Science Foundation Ireland SFI/12/RC/2273

Title: Regulation of myelination in the prefrontal cortex by the gut microbiota

Authors: *A. E. HOBAN[1], R. STILLING[1], F. SHANAHAN[2], T. DINAN[3], G. CLARKE[3], J. CRYAN[4];
[1]Anat. and Neuroscience, Univ. Col. Cork, Ireland, [3]Dept. of Psychiatry, [4]Dept. of Anat. and Neurosci., [2]Alimentary Pharmabiotic Ctr., Cork, Ireland

Abstract: Background: Myelination is a critical but vulnerable dynamic feature of normal brain development with implications for mental health and neurodegrenative disease In particular, normal cognition and social functioning appear to be contingent on intact myelination in the prefrontal cortex (PFC), a brain region implicated in multiple psychiatric disorders. Interestingly, social isolation stress results in a decreased myelination in the (PFC). Growing evidence points to a role for the gut microbiome in regulating brain function and behaviour. Studies in microbiota-deficient germ-free (GF) animals, have highlighted the impact the microbiota can have on neurodevelopment. GF animals demonstrate altered anxiety-related behaviours and decreased sociability. Given these overlaps between the microbiota, social, cognitive and emotional behaviour and myelination in the PFC, we aimed to investigate whether GF mice displayed altered myelination in adulthood in this region. Methods: We assessed both at the ultrastructural and transcriptional level changes in myelin sheath thickness and functional myelin sheath components which have been implicated in formation, stabilization, maintenance and remyelination. Using transmission electron microscopy we examined changes in myelin thickness via g-ratio assessment (quantification of myelin thickness relative to axonal diameter). Using qRT-PCR, we assessed expression levels of 6 myelin component genes in the PFC and other relevant brain in conventionally raised, germ-free and germ-free colonised mice Results: Ultrastructural analysis revealed hypermyelination within the PFC of GF mice as indicated by decreased g-ratio compared to conventionally colonised (CON) mice. These mice also displayed increased expression of six different myelin genes only within the PFC. However, colonisation of the germ-free animals immediately post-weaning normalised the expression of these myelin component genes. Coinciding with this increased expression, GF mice displayed altered expression of key myelin and oligodendrocyte regulating genes within the PFC. Conclusion: This is, to our knowledge, the first demonstration that the gut microbiota can regulate myelination. This effect is brain-region specific and occurs only in the prefrontal cortex. Moreover, our results suggest that the microbiota can be successfully targeted later in life to modulate myelination patterns, at least at the transcriptional level. This raises the possibility that targeting the gut microbiota during critical time windows could be a viable approach for treating disorders associated with aberrant myelination patterns.

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Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: E.05. Stress and the Brain

Support: Alimentary Pharmabiotic Centre Grant Number SFI/12/RC/2273

HRA_POR/2012/32; JFC, TGD

HRA-POR-2-14-647: GC, TGD

GC is supported by a NARSAD Young Investigator Grant from the Brain and Behavior Research Foundation (Grant Number 20771)

Title: Gut microbiome alterations in major depressive disorder: relevance to pathophysiology

Authors: *J. R. KELLY[1], Y. BORRE[3], C. O' BRIEN[4], J. DEANE[4], P. KENNEDY[3], S. EL AIDY[3], G. MOLONEY[3], L. SCOTT[1], E. PATTERSON[4], P. ROSS[4], C. STANTON[4], J. CRYAN[2], G. CLARKE[3], T. DINAN[1];
[1]Psychiatry, [2]Anat. & Neurosci., Univ. Col. Cork, Cork, Ireland; [3]Lab. of Neurogastroenterology, Alimentary Pharmabiotic Ctr., Cork, Ireland; [4]Teagasc Food Res. Ctr., Cork, Ireland

Abstract: Background: The biological mechanisms underlying the pathophysiology of MDD involve immune, endocrine and neurotransmitter dysregulation. Preclinical findings suggest that the gut microbiota can modulate brain development, function and behaviour by recruiting the same neuroimmune, neuroendocrine and neural pathways of the brain-gut-microbiome-axis which are considered dysfunctional in MDD. However, the extent to which these preclinical findings translate to clinical populations is currently unknown. The aim of this study was to determine the composition of the gut microbiota and its relationship to immune activity (plasma cytokines), hypothalamic-pituitary-adrenal axis (HPA-axis) function and tryptophan metabolism in patients with MDD compared to healthy control participants. Methods Thirty four patients with DSM IV major depression were recruited, together with 33 healthy subjects matched for gender, age and ethnicity. CRP and a panel of cytokines were measured using MSD. Salivary cortisol levels for assessment of the cortisol awakening response (CAR) were determined by ELISA. Plasma tryptophan and kynurenine were determined by HPLC. Faecal samples were collected for 16s rRNA gene sequencing to determine bacterial community structure and diversity. Results Preliminary analysis demonstrated significant differences in the gut microbiota in the MDD group with a reduced abundance of short-chain fatty acid-producing bacteria. In

parallel patients with MDD showed significantly higher plasma levels of IFN-y, IL-8 , IL-6 , IL-1β , TNF-α , and CRP . In addition, there was a significantly higher CAR (p=0.03) and an elevated kynurenine: tryptophan ratio (p=0.05) in patients with MDD. Conclusion Alterations in the gut microbiota in patients with MDD are pronounced and may drive the prominent pathophysiological features of this disorder. The mechanisms underpinning these effects require further investigation but may be related to an altered production of microbial metabolites such as short chain fatty acids and/or perturbations to intestinal barrier function. Ultimately, these findings may pave the way for therapeutic targeting of the gut microbiome as a viable strategy for novel antidepressant development.

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Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 162.10/R14

Topic: E.05. Stress and the Brain

Title: Disruption of the gut microbiota with oral antibiotic reduces core body temperature and disrupts diurnal rhythms of core body temperature and locomotor activity, but not sleep, in adult rats

Authors: S. J. BOWERS[1], R. S. THOMPSON[2], A. MIKA[2], B. N. GREENWOOD[3], *M. R. FLESHNER[2];
[1]Med., Northwestern Med. Sch., Chicago, IL; [2]Univ. Colorado, Boulder, CO; [3]Psychology, Univ. of Colorado at Denver, Denver, CO

Abstract: The bidirectional communication between the gut microbiota and the brain (i.e., the gut-brain axis) can modulate behavior and neurochemical systems. Recent evidence suggests that the absence of gut microbiota throughout development (i.e., gnotobiotic rodents) can affect clock genes in peripheral tissues and serotonin in the brain. Given the important role of clock genes in

regulating diurnal rhythms and serotonin's contribution to sleep and thermoregulation, we hypothesized that depletion of the gut microbiota, using oral antibiotics, may disrupt diurnal rhythms, thermoregulation and sleep. Diurnal rhythms of locomotor activity (LA), activity-independent core body temperature (CBT_{ind}), and sleep (%REM, %SWS, %WAKE, bout frequency/duration) were recorded using *in vivo* biotelemetry in freely moving adult male F344 rats. Rats were given oral antibiotics (4.0 mg/ml streptomycin and 2.0 mg/ml penicillin g) in their drinking water and efficacy was assessed by measuring total aerobic, anaerobic, *Lactobacillus* spp. and *Bifidobacterium* spp. per g of feces using selective culture. After seven days of antibiotic treatment, animals had depleted gut microbiota, reduced overall CBT_{ind} along with increased amplitude of CBT_{ind} diurnal rhythm, and a flattened diurnal rhythm of LA when compared to baseline, while sleep architecture remained unchanged. Circadian rhythms of CBT_{ind} and LA were not phase shifted and did not change in period length after antibiotic treatment. Interestingly, five days after cessation of antibiotic, only *Lactobacillus* spp. remained depleted. Reduced CBT_{ind} and altered diurnal rhythms of CBT_{ind} and LA also persisted. These results support a link between the gut microbiota, temperature regulation and diurnal physiology. Furthermore, they suggest that disruptions to the gut microbiota in adulthood, and any resultant physiological consequences, may be long lasting even if antibiotic administration is transient.

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Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 162.11/R15

Topic: E.05. Stress and the Brain

Support: Mead Johnson Nutrition

Title: Early life test diet improves NREM sleep and facilitates REM sleep rebound following an acute stressor: The potential role of the gut microbiota, metabolome, and hypothalamic orexin

Authors: *R. S. THOMPSON[1,2], A. MIKA[1,2], R. ROLLER[1], M. GAFFNEY[1], B. N. GREENWOOD[3], P. C. DORRESTEIN[4], R. KNIGHT[5], M. FLESHNER[1,2];
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Abstract: Stressor exposure produces maladaptive and adaptive disruptions in the sleep cycle. Stress can produce sleep fragmentation, which is associated with negative health consequences, and REM rebound, which is associated with better psychological recovery after trauma. Prebiotics, a form of dietary fiber, promote the expansion of specific microbial species in the mammalian gut that support stress robustness. Thus, we tested whether a prebiotic test diet would modulate the gut commensal bacteria and their metabolites, and that these changes would be associated with the protection of the sleep cycle following stress. Male F344 rats, postnatal day 24 (P24), were placed on a test diet or a control diet ad-libitum. Biotelemetry devices were implanted on P59, to examine real-time differences in the sleep cycle across rodent development due to test diet. Rats were exposed to an acute stressor on P87 to examine the potential protective effects of test diet on stress-induced disruptions of the sleep cycle. In a separate experiment, male F344 rats were placed on either test or control diet for 4 weeks and brains were collected for *in situ* hybridization analyses. Rats fed the test diet had greater NREM sleep consolidation in early adulthood (P71, P72) compared to control diet. Stepwise multiple regression analysis revealed that greater NREM sleep at P71, P72 was negatively correlated with early life Deferribacteres (P35) in the test diet only ($r = 0.627$). Rats fed the test diet also had enhanced REM rebound following stress (P87) compared to rats fed the control diet. Intriguingly, results from a stepwise multiple regression analysis showed a significant correlation ($r = 0.41$) between increased early life Proteobacteria (P35) and increased REM sleep in the dark cycle after stress (P87), which may be dependent on the interaction between diet and stress ($p < 0.05$) enhancing REM rebound following stress. Ongoing metabolomics analysis may reveal metabolites that could have contributed to the sleep effects observed. Finally, given previous reports of a linear relationship between higher levels of NREM sleep and lower levels of prepro-orexin mRNA, *in situ* hybridization for hypothalamic prepro-orexin mRNA may reveal additional contributions of the test diet on the observed sleep effects. These results demonstrate that test diet consolidated NREM sleep potentially through reduced early life Deferribacteres and conferred stress-protective effects on REM sleep following stress. Our results suggest modulation of the gut microbiota with test diet improves sleep and may reduce stress-induced disruptions to the sleep cycle and could, in part, depend on changes in hypothalamic orexin.

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Poster

162. Microbiota and Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 162.12/R16

Topic: E.05. Stress and the Brain

Title: Gut microbiota and blood analyses underlying reduced sickness-like behavior in serotonin transporter knockout mice following inflammatory challenge

Authors: *B. M. KILLE[1], D. J. DAVIS[2], K. S. CROWSON[2], K. E. TROTT[2], A. C. ERICSON[2,3,4], C. E. WIEDMEYER[2,5], J. N. ROUDER[1], D. Q. BEVERSDORF[1,6], C. E. HAGAN[2];
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Abstract: Serotonin transporter (SERT) knockout mice have been used extensively in neuropsychiatric research to better understand the role of serotonin signaling in affective disorders. Additionally, the model has illuminated a role for SERT in gut function and metabolic disorders. Given the recognized role of the gut microbiota in host metabolic function and the increasing interest of the microbiome in the gut-brain axis, we sought to characterize how the SERT model genetically constrains the enteric ecological niche in the gut, particularly given the abundance and importance of serotonin in gut physiology and homeostasis. Here we tested the hypothesis that littermates of different genotypes would have differing microbiota which could be perturbed acutely (within 4 hours) by an inflammatory challenge, an injection of lipopolysaccharide. Our experimental approach involved next-generation sequencing, complete blood counts, serum chemistry panels, serum cytokines, serum corticosterone, and behavioral testing. The three major results of this study are that 1) wild-type and SERT knockout littermates have different gut microbiota; 2) the microbiota is highly responsive to inflammatory events occurring the host; and 3) SERT knockout mice have reduced sickness-like behavior in response to an inflammatory challenge. These data underscore the multi-systemic complexity in coordinating behavioral responses to stimuli.

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Poster

162. Microbiota and Stress

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 162.13/R17

Topic: E.05. Stress and the Brain

Support: IOER Funds

Title: Longitudinal effects of chronic stress on the murine gut microbiota

Authors: A. SHOSKES[1], A. PROCTOR[2], K. BATTANI[1], M. CARDER[1], L. SEMKE[1], V. DURIC[1], G. PHILLIPS[2], *L.-L. YUAN[1];
[1]Dept. of Physiol. and Pharmacol., Des Moines Univ., Des Moines, IA; [2]Iowa State Univ., Ames, IA

Abstract: Emerging evidence supports a bidirectional communication axis between the brain, the gastrointestinal tract, and the microbiota colonizing the gut. Various physical and psychological stressors have been shown to influence the function of the GI tract and its resident microbiota. Furthermore, dysbiosis of GI tract colonization has been associated with different diseases, including depression. We investigated the relationship between chronic stress and the murine gut microbiota by comparing the taxonomic composition before and after chronic stress. Mice were subjected to chronic unpredictable stress (CUS) for six weeks. Fecal samples were collected at different time points before and after CUS. Bacterial genomic DNA was extracted and amplicons representing the V4 region of 16s rRNA genes were amplified and sequenced. We observed phylum level differences: mean abundance of *Bacteroidetes* increased and *Firmicutes* decreased over the chronic stress period. Decreases in abundance of *Bacilli* (a class within *Firmicutes*) and *Lactobacillus* (a genus in *Bacilli*) appeared to contribute to the abundance decrease at the phylum level. No significant difference in alpha diversity was noted, indicating CUS induced abundance changes are unlikely due to differences in the amount of diversity. Changes in the ratio of the phyla *Bacteroidetes* to *Firmicutes* have been found to be associated with metabolic disease and obesity. Our results suggest that genera within *Bacilli* may be targets of prebiotic or probiotic therapies to restore a microbiota associated with well-being. Further studies are currently underway to address whether other forms of chronic stress such as chronic pain, result in similar taxonomic changes in GI resident microbiota.

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Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.01/R18

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant MH84970

Title: Social isolation reduces the excitability of neurons in the medial nucleus of the amygdala

Authors: *J. ROSENKRANZ;
Cell. and Mol. Pharmacol., The Chicago Med. School/RFUMS, North Chicago, IL

Abstract: A robust capacity for social interaction can enrich lives and provide buffering from psychiatric disorders. Several psychiatric disorders display features of social withdrawal and impaired social behavior. While social abilities develop across life, there is evidence for critical periods, wherein disruptions of social development lead to long term impairments. In rodents, social isolation after weaning can lead to abnormalities in aggression, interactions with novel rodents, sexual behavior and social learning. One shared commonality between these behaviors is the involvement of the medial nucleus of the amygdala (MeA). The purpose of this study was to test whether post-weaning social isolation (~P21-22) leads to abnormal activity of the MeA. *In vivo* and *in vitro* recordings of MeA neurons from group-reared and isolation-reared rats were compared. Social isolation significantly decreased the firing rate of MeA neurons and decreased afferent drive. Neuronal membrane responsiveness and excitability were decreased in the isolation-reared rats, along with evidence of decreased glutamatergic drive. To test whether these changes were due to stunted maturation of the MeA, neurons were recorded at the initiation of social isolation or at intervals over the course of social isolation. Group housed rats did not display a significant change of excitability between post-weaning and adulthood. In contrast, isolated rats displayed decreased excitability across time. These data indicate that post-weaning social isolation impairs the function of the MeA, but it is not due to a stunting of maturation, rather, it is due to a progressive emergence of impairment. These results are a first step towards understanding the pathophysiology of the medial amygdala in maldevelopment of social behaviors.

Disclosures: J. Rosenkranz: None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.02/R19

Topic: F.03. Motivation and Emotion

Title: Early life stress as a model for sexually dimorphic development of negative symptoms in mice

Authors: *H. GOODWILL, P. LACHANCE, S. TERAMOTO, K. G. BATH;
Brown Univ., Providence, RI

Abstract: Converging evidence from both clinical and experimental studies reveals that females are at increased risk for developing stress-associated pathology, including anxiety and depression. Negative symptoms are some of the most pervasive and treatment resistant across these disorders, in part, because their etiology is unspecified. Recent work has led to the hypotheses that 1) negative symptoms result from altered reward processing, suggestive of abnormal dopamine (DA) signaling in mesocortico/limbic pathways and 2) that midbrain dompaminergic systems are sexually dimorphic in their response to developmental stress. Here, we leverage an early life stress (ELS) paradigm in mice (maternal bedding restriction from P4-P11) to induce negative symptom development exclusively in females, allowing for sex-specific investigation of the molecular substrates of negative symptom development and stress vulnerability. Specifically, female mice exposed to ELS exhibit learned helplessness, measured in the forced swim test; enhanced risk assessment in a novelty induced hypophagia task and decreased exploration of novel contexts in the elevated plus maze in comparison with control animals. These behaviors were not observed in ELS exposed male mice. Additionally, using a newly developed, 24-hour, automated computer-vison home cage monitoring system, we found that ELS exposed females show a reduction in grooming behavior (avolition), walking (lethargy) and increased sleeping (hypersomnia) relative to control-reared animals. Taken together, female mice exposed to ELS develop behavioral profiles that parallel negative symptoms of depression in humans. In these same groups of mice, we carried out extensive realtime qPCR analysis of dopamine signaling molecules in the striatum, VTA and frontal cortex. We found a significant increase in DA receptor D4R mRNA expression exclusively in the frontal cortex of ELS females throughout development and into adulthood. There were no observed differences in mRNA levels of DA transporter, DA beta-hydroxylase, COMT, Darpp-32, or DA receptors D1R, D2R, D3R or D5R between groups. This suggests D4R regulation in the mesocortical DA pathway as a mechanism for sexually dimorphic vulnerability to stress and negative symptom development. Such work offers a possible target for sex-specific treatment of stress-associated disorders and negative symptoms.

Disclosures: **H. Goodwill:** None. **P. LaChance:** None. **S. Teramoto:** None. **K.G. Bath:** None.

Poster

163. Social Stress

Location: Hall A

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Program#/Poster#: 163.03/R20

Topic: F.03. Motivation and Emotion

Support: 1F31AA022824

2U01AA016647

6P60 AA10760

NS066159

Title: Social transfer of pain in the mouse

Authors: *M. L. SMITH, M. M. HEINRICHER, A. E. RYABININ;
OHSU, Portland, OR

Abstract: Clinically significant chronic pain often manifests in the absence of a noxious trigger or tissue damage, and is greatly impacted by social and cognitive factors. Without the ability to trace the stimuli that cause the onset of pain, our understanding of such chronic pain disorders is lacking. Because chronic pain and alcohol dependence are often co-morbid, we examined pain behavior during alcohol withdrawal following a period of voluntary drinking in C57BL/6J mice. Our experiments identify, for the first time, increased sensitivity to mechanical and inflammatory pain stimuli in mice during withdrawal from voluntary alcohol consumption. Remarkably, our studies also reveal a similar pain state in "bystander" mice drinking water and housed in the same room. These bystander mice exhibit hypersensitivity that lacks a nociceptive trigger, and relies solely upon social and environmental cues. Interestingly, bystander mice show no overt signs of generalized anxiety, but demonstrate activation of the anterior cingulate and insula, which are cortical areas that contribute to pain and empathy in humans. Additional experiments reveal that olfactory cues related to hypersensitivity during alcohol withdrawal, but not consumption or intoxication, are sufficient for transfer of this pain state. Finally, hypersensitivity is also induced in bystander mice housed near mice undergoing morphine withdrawal or chronic inflammatory pain. These data expand on the current understanding of the multidimensional nature of social information transfer between animals. Ultimately, these studies demonstrate that withdrawal from voluntary alcohol consumption in mice leads to increased pain sensitivity and provide the first evidence of pain in laboratory rodents that is induced solely by a social stimulus that is independent of injury, stress, anxiety, or emotional contagion.

Disclosures: **M.L. Smith:** None. **M.M. Heinricher:** None. **A.E. Ryabinin:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.04/S1

Topic: F.03. Motivation and Emotion

Support: NIMH K99/R00 MH085859

NINDS 5T32NS061788-07

NIGMS 5T32GM008361-20

Title: 12 things you didn't know about high responder/low responder rats, stress coping, and the dorsal raphe. Number 5 will blow your mind!

Authors: *J. COHEN[1], A. E. ATA[2], N. L. JACKSON[2], S. M. CLINTON[2];
[1]Univ. of Alabama at Birmingham Med. Scientist Training Program, Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Stress is one of the most widely studied environmental risk factors for the development of a variety of mental illnesses. Stress coping styles encompass a range of physiological, psychological, and behavioral responses aimed to avoid or tolerate distress, and are broadly characterized as “proactive” (a fight-or-flight response to defeat/escape a stressor) or “reactive” (a withdrawal response to avoid or outlast the stressor). In humans, proactive vs. reactive coping styles convey risk or resilience to psychopathology depending on the type of stress, since each coping style can be adaptive in some circumstances but maladaptive in others. To investigate the neurocircuit and molecular mechanisms underlying different stress coping styles, we utilized selectively bred high-responder (HR) and low-responder (LR) rats. HR/LR rats were selected for on the basis of high and low novelty-induced locomotion, and are a well-developed model for studying individual differences in emotionality and behavior. Here we show that HR rats display a proactive coping style while LRs exhibit a reactive coping style in the defensive burying test. HR rats spend more time burying the probe following a single electric shock (proactive coping), while LR rats spend more time immobile (reactive coping). Dual-labelling immunocytochemistry for c-Fos, a marker of neuronal activity, and tryptophan hydroxylase 2 (TPH2), a marker of serotonergic neurons, revealed that HR rats have higher basal c-Fos activation in TPH2 cells of the dorsal raphe. Following defensive burying, HR rats display an increase in c-Fos/TPH2 dual-labelled cells in the dorsomedial dorsal raphe and ventral dorsal raphe, while LR rats displayed a decrease in the dorsomedial dorsal raphe. Sequencing of microRNAs in the dorsal raphe of HR/LR rats identified 14 differentially expressed miRNAs, including miR-101a. miR-101a was one of the most abundant miRNAs in the raphe and is known to regulate multiple subunits of the polycomb repressor complex, which mediates gene silencing via histone tail methylation. This points to a role for the dorsal raphe and miR-101a in

regulating stress coping style. Future work will further characterize serotonin circuit differences in proactive and reactive coping animals by testing the effects of miR-101a on coping style.

Disclosures: **J. Cohen:** None. **A.E. Ata:** None. **N.L. Jackson:** None. **S.M. Clinton:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.05/S2

Topic: E.03. Behavioral Neuroendocrinology

Title: Social disruption produces lasting behavioral adaptations by reducing adult neurogenesis

Authors: *M. OPENDAK[1], L. OFFIT[1], T. J. SCHOENFELD[2], H. CAMERON[2], E. GOULD[1]; [1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Research on social instability has focused on its detrimental consequences, but most people are resilient and respond by invoking various coping strategies. To investigate cellular processes underlying such strategies, a dominance hierarchy of rats was formed and then de-stabilized. Regardless of social position, rats from disrupted hierarchies had fewer new neurons in the hippocampus compared to rats from control cages and those from stable hierarchies. Despite this suppression of adult neurogenesis, no behavioral impairments were observed. Social disruption produced preference for familiar over novel conspecifics, a change that was not linked to impaired memory or increased anxiety. Using the neuropeptide oxytocin as a tool to increase neurogenesis in the hippocampus of disrupted rats restored preference for novel conspecifics to pre-disruption levels. Conversely, reducing the number of new neurons by ablation of adult neurogenesis in naïve transgenic GFAP-TK rats resulted in social behavior similar to disrupted rats. Taken together, these results provide novel mechanistic evidence that social disruption shapes behavior in a potentially adaptive way by reducing adult neurogenesis in the hippocampus.

Disclosures: **M. Opendak:** None. **L. Offit:** None. **T.J. Schoenfeld:** None. **H. Cameron:** None. **E. Gould:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.06/S3

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH F32 MH102983

Title: Early life experience mobilizes DNA transposable elements

Authors: *T. A. BEDROSIAN, C. QUAYLE, F. H. GAGE;
Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: The brain exhibits remarkable plasticity in response to environmental experience, particularly during early life. Variation at the DNA level accounts for at least some of this plasticity. For example, in rodents, rapid and permanent epigenetic modifications occur as a result of natural variations in maternal care, which in turn shape the offspring's neuronal and behavioral phenotypes. We hypothesize that structural variation of the genome, particularly through the mobilization of active DNA transposable elements, may be another mechanism through which early life experience contributes to brain plasticity. LINE-1 (L1) retrotransposons are autonomous mobile elements that comprise ~20% of the genome. Most copies are truncated or otherwise mutated in such a way that mobilization is no longer possible; however, there are about 120 active copies in humans and at least 3000 in mice that are capable of mobilizing. It has been suggested that active transposable elements in the genome may allow for germline or somatic DNA rearrangement to occur in a fashion similar to that observed in the immune system. DNA recombination could cause permanent changes in gene expression and neuronal function. We used natural variations in murine maternal behavior as a model to study the effect of early life experience on L1 copy number variation in the offspring. Specifically, we developed an assay using droplet digital PCR to address the hypothesis that differences in maternal care influence L1 copy number in the brain. In addition, we examined L1 gene expression levels at different post-natal time points and DNA methylation levels of the L1 promoter to determine the effect of experience on mobile element regulation. Our results suggest that early life experience mobilizes L1 retrotransposons in the brain. This finding hints at a potential functional role for mobile DNA elements in brain plasticity and in shaping neuronal and behavioral phenotypes.

Disclosures: **T.A. Bedrosian:** None. **C. Quayle:** None. **F.H. Gage:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.07/S4

Topic: F.03. Motivation and Emotion

Support: W911NF1010093

Title: Sphingosine-1-phosphate receptor dysregulation contributes to depressive-like behavior in stress-susceptible rats

Authors: *B. CORBETT[1,2], S. BELTRAMI[2], S. LUZ[2], N. SUTOYO[2], S. BHATNAGAR[2]; [1]Abramson Res. Ctr., [2]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Chronic stress promotes depressive-like behaviors in humans. However, not all stressed individuals go on to develop stress related psychiatric disorders. That is, some individuals are resilient to the effects of stress and others are vulnerable to its effects. In a paradigm of chronic social defeat in rats, we have identified subpopulations that are resilient or vulnerable to the neuroendocrine, prodepressive and anxiogenic effects of defeat. However, the mechanisms underlying stress susceptibility and stress induced depressive-like behaviors remain unclear. In order to identify novel neural substrates underlying resilience and vulnerability, we used a targeted PCR array approach. We found that that the expression of sphingosine-1-phosphate receptor 1 (S1PR1) was decreased in the ventral hippocampus of stress-susceptible rats. The expression of S1PR3 was increased in the ventral hippocampus of stress-susceptible rats and the medial prefrontal cortex of stress-resilient rats. We then assessed whether modulation of these receptors regulates behavior in the Porsolt forced swim test using FTY720 (fingolimod), a non-selective modulator of S1P receptors. We injected rats with either vehicle or FTY720 (2.5 mg/kg) immediately following a 15 minute training swim. On the following day, we injected rats both 5 hours and 1 hour prior to a 5 minute test swim. FTY720 decreased immobility and increased climbing durations in the test swim, suggesting an anti-depressant effect of this drug. Adrenalectomy did not alter immobility, swimming, or climbing behaviors in the Porsolt forced swim test in FTY720-treated rats. Furthermore, we found that repeated treatment with FTY720 (2.5mg/kg for 5 days) increased the expression of BDNF in the dorsal and ventral hippocampus as assessed by ELISA. Current studies are assessing whether increasing the expression of S1PR1 and S1PR3 in the hippocampus affects depressive-like behaviors. These results suggest that altered expression of S1P receptors may serve as a regulator of stress susceptibility and that modulating their activity may improve stress induced depressive-like behavior.

Disclosures: B. Corbett: None. **S. Beltrami:** None. **S. Luz:** None. **N. Sutoyo:** None. **S. Bhatnagar:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.08/S5

Topic: F.01. Human Cognition and Behavior

Support: CONACyT grants 138663 and129303

Title: Measurement of markers lipid peroxidation in blood serum in response to stressful conditions

Authors: *S. GONZALEZ CANO[1], A. GONZÁLEZ[1], E. BALTAZAR[1], P. AGUILAR-ALONSO[2], G. FLORES[1];
[1]Inst. De Fisiologia BUAP, Puebla, Mexico; [2]Facultad de Ciencias Químicas, BUAP, Puebla, Mexico

Abstract: Recent research shows that academic stress occurs in students at various levels, including UNIVERSITY STUDENTS. It has been shown that this kind of stress plays an important role in the evolution of mental disorders as anxiety, depression and, in severe cases, psychosis schizophrenia type. One theory to analyze is the relationship between psychological stress, biological stress and the development of mental disorders since is known that if the psychological stress is added a system malfunction antioxidant then generate oxidative stress it contributes to the risk of trigger mental illness. The aim of this study was analyze two products of lipid peroxidation, which form part of the process of oxidative stress, these metabolites were Malondialdehyde (MDA) and 4-hydroxyalkenals (4-HDA) which were obtained of blood serum of students of the Faculty of Chemistry of the Autonomous University of Puebla, Mexico. Students of 3 different groups were asked for their consent to participate in this work and were applied surveys of anxiety and depression as well as tests of different types of knowledge as stressful event. The biological sample was obtained by venipuncture blood serum at different stages of the test: before, during and after, assessing lipid peroxidation products. The results obtained from the surveys showed that in the university population exists a high prevalence of anxiety (93% of the surveyed population have mild to severe anxiety) and depression (100% of the surveyed population has some type of depression). As for the results of the biochemical tests, showed that the levels of MDA in one and two groups were elevated during the stressful event declining in the later stage, while for group three were found before the test increased, decreasing in steps during and after. In the case of 4-HDA levels in group one showed a slight increase after the test, to group two an increase was observed during the test and a decrease in the later stage and group three were elevated before examination, decreased during the test and increased after the test. Thereby, in the university population analyzed, the model of

psychological stress tests generated leads to increased production of free radicals and reactive species generating lipid peroxidation. This work is the first step in trying to address the relationship between psychological stress and oxidative stress to identify early markers associated with schizophrenia. . (Supported by: CONACyT grants No. 138663 and129303 to G Flores).

Disclosures: **S. Gonzalez Cano:** None. **A. González:** None. **E. Baltazar:** None. **P. Aguilar-Alonso:** None. **G. Flores:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.09/S6

Topic: F.03. Motivation and Emotion

Support: NIDA Grant #431495

Title: Reminiscing about positive memories buffers acute stress responses and engages the prefrontal cortex

Authors: *M. E. SPEER, H. MANGLANI, M. R. DELGADO;
Psychology Dept., Rutgers Univ., Newark, NJ

Abstract: Recalling happy memories from the past can elicit positive feelings and enhance an individual's wellbeing (Young et al., 2013). One potential adaptive function in reminiscing about the positive past may be the ability to cope with stressors in daily life, such as reducing stress hormone levels (e.g., cortisol). Indeed, behavioral data from our lab lends support to this idea. Individuals who recalled positive memories (N = 33) showed a sharper decrease in cortisol rise 20 min after stress exposure compared to individuals who recalled neutral memories (N = 34). This highlights the beneficial effects of internally generated positive emotion to one's wellbeing. In the present study, we examined neural changes associated with the recall of positive memories after stress, and how it may aid coping with negative affect. To test this, participants described and gave subjective emotion ratings for specific episodic memories of positive (e.g., family vacation) and neutral (e.g., commuting to work) content. Three days later, they returned for the fMRI scanning session. Participants were first exposed to an acute stressor (i.e., socially evaluative cold-pressor task), and then asked to retrieve a subset of the same episodic memories triggered by visual word cues (e.g., family vacation) while in the scanner. The memories were of only positive valence or only neutral valence, creating 2 experimental groups (Positive and

Neutral). To measure changes in cortisol levels over time, salivary cortisol samples were collected at baseline (before stressor), immediately after the stressor (2 min), peak (after recalling autobiographical memories, 20 min), and recovery (50 min). As expected, participants who recalled positive memories rated their memories with greater positive affect and stronger emotional intensity than participants who recalled neutral memories. Preliminary neural analyses comparing high feeling vs. low feeling memories during autobiographical memory retrieval showed enhanced activity in the ventrolateral prefrontal cortex (vlPFC) in individuals who recalled positive memories, but not in individuals who recalled neutral memories. This potentially suggests that the engagement of prefrontal regions previously implicated in cognitive control and regulation may be essential for enhancing positive emotion in response to acute stress. Future analyses will look at these results in conjunction with cortisol and physiological data. Taken together, these findings have implications for using something naturally-occurring like recalling the past as a strategy for buffering negative affect and reinforcing positive wellbeing.

Disclosures: **M.E. Speer:** None. **H. Manglani:** None. **M.R. Delgado:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.10/S7

Topic: F.03. Motivation and Emotion

Title: Social isolation reveals a dopamine-independent rewarding motivational response to acute nicotine that is not observed in group-housed mice

Authors: ***T. E. GRIEDER**[1], D. VAN DER KOOY[2];
[1]Inst. Med. Sci., [2]Mol. Genet., Univ. Toronto, Toronto, ON, Canada

Abstract: Drug abuse, specifically nicotine addiction, is a worldwide epidemic that has led to many deaths. Recent research has suggested that social factors play an important role in both the initial response to abused drugs and the transition to addiction. In order to investigate such factors, we used a place conditioning paradigm to examine the effect of single- versus group-housing on the acute conditioned response to nicotine in nondependent C57Bl/6J mice and compared these nicotinic motivational effects with those elicited after dopamine receptor antagonism. Mice that were single-housed (and therefore socially isolated) for two weeks prior to conditioning and pretreated with the dopamine receptor antagonist α-flupenthixol prior to acute nicotine found nicotine rewarding. However, mice that were group housed or were single-housed

only during conditioning did not show this conditioned rewarding response. We also observed that individually housed mice with genetic deletion of the α5 nicotinic acetylcholine receptor (nAChR) subunit, which is important for nicotine's acute aversive effects, behave differently than group-housed litter mates. These results suggest that the stressful experience of social isolation makes single-housed nondependent mice behave as if they are nicotine-dependent and shifts the balance of acute nicotine's aversive and rewarding effects, such that socially isolated mice are more likely to experience the acute rewarding effects of nicotine after blockade of dopaminergic receptors. These results further highlight the important role that social factors play in the motivational response to acute nicotine.

Disclosures: T.E. Grieder: None. **D. van der Kooy:** None.

Poster

163. Social Stress

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Topic: F.03. Motivation and Emotion

Support: Ibaraki University Cooperation between Agriculture and Medical Science (IUCAM) (The MEXT, Japan)

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Council for Science, Technology and Innovation (CSTI), Cross-ministerial Strategic Innovation Promotion Program (SIP) (Bio-oriented Technology Research Advancement Institution, NARO) (The Cabinet Office, Japan)

Title: Effects of diet quality on stress-vulnerability and metabolomic profiles in chronic mild social defeat stress model of mice

Authors: *T. GOTO[1,2], S. TOMONAGA[3], Y. KUBOTA[1], A. TOYODA[1,2,4];
[1]Ibaraki Univ., Ibaraki, Japan; [2]Ibaraki Univ. Coop between Agri and Med. Sci. (IUCAM), Ibaraki, Japan; [3]Kyoto Univ., Kyoto, Japan; [4]Tokyo Univ. of Agr. and Technol., Tokyo, Japan

Abstract: Recently, the relation between diet quality and behavior has been discussed. In order to elucidate the effects of diet quality in animal models of depression, we made and analyzed a subchronic and mild social defeat stress (sCSDS) model mice (Goto et al., Behav Brain Res 2014) in two conditions feeding purified diet (AIN-93G) and non-purified diet (MF; standard

laboratory diet). C57BL/6J (B6) male mice were exposed to sCSDS using aggressors of ICR male mice as previously reported. Body weight, food intake, and water intake of B6 were monitored for the experimental periods consisting of 10 days of sCSDS. After the sCSDS, social behavior (stress-vulnerability) and metabolomic profiles of plasma, liver, and cecum contents of B6 were analyzed. Body weight gain, food intake, and water intake of the defeated mice were significantly higher than those of non-stressed control mice in both diet groups. Although the defeated mice showed significantly higher social avoidance behavior to unfamiliar ICR mice in the social interaction test compared with those of control mice, defeated mice fed purified diet showed significantly higher rates of stress-vulnerability than those of defeated mice fed non-purified diet (Goto et al., Nutr Neurosci 2015). These results suggest that diet quality affects the vulnerability to social defeat stress in mice. In addition, metabolomic analysis using CE-TOFMS revealed that levels of four metabolites in the liver of the defeated mice were significantly higher than those of control mice under the feeding condition of purified diet (Goto et al., J Proteome Res 2015). Also, metabolomic analysis using GC/MS revealed that some candidates of molecular markers for stress-vulnerability in the model mice.

Disclosures: **T. Goto:** None. **S. Tomonaga:** None. **Y. Kubota:** None. **A. Toyoda:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.12/S9

Topic: F.03. Motivation and Emotion

Support: Master project money

Title: Can behavioral inhibition in fish help endure a risky social environment?

Authors: *S. RIISE[1,2], M. VINDAS[2], G. NILSSON[2], Ø. ØVERLI[3];
[2]Dept. of biosciences, [1]Univ. of Oslo, Oslo, Norway; [3]Dept. of Animal and Aquacultural Sci., Norwegian Univ. of Life Sci., Ås, Norway

Abstract: Social subordination is a common model for chronic stress which is often used to study pathologies, such as depression. Subordinates are often characterized by high serotonin (5-HT) signaling and behavioral inhibition. Behavioral inhibition, in turn, is associated with reduced activity levels, suppressed aggression and suppressed neuronal plasticity/neurogenesis. We tested in a teleost model the hypothesis that behavioral inhibition could be advantageous in a risky We conducted three different experiments: (a) Dose-response experiment, in order to

characterize behavioral effects of ketamine in fish, (b) studying the neuroendocrine effects of ketamine with special reference to the possible involvement of the brain 5-HT system, and markers of neuronal plasticity. (c) Testing the consequences of ketamine-induced reversal of behavioral inhibition in a novel social contest. Methods used included microdissection of telencaphalic brain areas, q-PCR and HPLC. Results were as follows: a) the 5 mg/kg dose of ketamine significantly increased locomotion in socially isolated rainbow trout and did not cause any undesired side-effects b) Reduced 5-HT concentrations were evident in the suggested amygdala equivalent dorsomedial pallium (Dm) for ketamine-treated animals. There was also an increase in the proliferating cell nuclear antigen (PCNA) expression in the ventral part of the ventral telencephalon (Vv, suggested homologous to septal areas associated with lateral septum in mammals). c) Ketamine-mediated increased locomotion in subordinate fish resulted in more received aggression in a novel social contest. We here demonstrate that behavioral inhibition under socially risky environments (*i.e.* high aggression) confers and advantage for subordinate animals and is associated with 5-HT signaling in the Dm. We propose that our results are in agreement with the hypothesis that depressive-like behavior may have evolved as a life-history strategy in subordinate animals under highly socially unpredictable environments.

Disclosures: **S. Riise:** None. **M. Vindas:** None. **G. Nilsson:** None. **Ø. Øverli:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.13/S10

Topic: E.03. Behavioral Neuroendocrinology

Support: EXCL/BIA-ANM/0549/2012

SFRH/BD/44848/2008

Title: Neuroendocrine responses to social challenges in zebrafish

Authors: ***M. TELES**[1,2], M. GOZDOWSKA[3], E. KULCZYKOWSKA[3], R. F. OLIVEIRA[1,2]; [1]Inst. Gulbenkian de Ciência, Oeiras, Portugal; [2]Neurosciences, Champalimaud Neurosci. Programme, Champalimaud Ctr. for the Unknown, Lisbon, Portugal; [3]Genet. and Marine Biotechnology, Inst. of Oceanology of the Polish Acad. of Sci., Sopot, Poland

Abstract: The nonapeptides arginine-vasotocin (AVT) and isotocin (IT) which are the teleost homologues of arginine-vasopressin and oxytocin in mammals have been implicated in a variety

of social and stress related behaviours across vertebrate taxa. The actions of these peptides can vary depending on the species, sexes, and social context. To investigate the response of AVT and IT to social challenges we studied agonistic interactions and the establishment of dominance hierarchies in zebrafish. We used an agonistic paradigm where three different behavioural phenotypes emerge: animals could win the interaction (winners), lose the interaction (losers) or have an unsolved interaction by fighting their own mirror image (mirror-fighters). We quantified the levels of both nonapeptides in macrodissected brain areas (i.e. olfactory bulbs, telencephalon, diencephalon, optic tectum, cerebellum, and brainstem) of these three behavioural phenotypes. Our results show that AVT levels increased in the telencephalon, and diencephalon of winners, and in the telencephalon, diencephalon, optic tectum and brain stem of losers. For mirror-fighters differences were only detected in the telencephalon. Isotocin levels were also distinct between the three behavioural phenotypes: in winners only the cerebellum levels increased, in the losers there was an IT increase in the diencephalon and a decrease in the cerebellum, and mirror-fighters showed higher levels in the olfactory bulbs. Thus, one can conclude that AVT and IT are differentially regulated in different brain regions by social interactions. We have also measured cortisol and androgen (Testosterone and 11-Ketotestosterone) levels in response to the social challenge. Our data indicate that real opponent fighters (winners and losers) increased cortisol and 11-KT levels in response to the social challenge, while mirror fighters had no hormonal response, stressing out the pivotal role of the perception of the fight outcome in triggering an hormonal response. In summary, this study illustrates that a highly social model organism also displays a hormonal response to social challenges and that AVT and IT may play a role in socially driven behavioural changes.

Disclosures: **M. Teles:** None. **M. Gozdowska:** None. **E. Kulczykowska:** None. **R.F. Oliveira:** None.

Poster

163. Social Stress

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Topic: F.03. Motivation and Emotion

Support: Pew Grant

NSF 1117388

NSF 1010172

Title: A new rodent model to study empathy

Authors: *M. CONTRERAS, C. MEDINA, K. CRUZ, J.-M. FELLOUS;
Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Empathy is as an affective response stemming from the understanding of another individual's emotional state which allows one to respond to social stimuli with appropriate pro-social behaviors. Accumulating evidence indicates that emotional contagion (a basic form of empathy) exists in rodents. Mice are capable of sharing states of fear and pain. In this study we investigated whether rats can display empathy-like behavior in response to a distressed conspecific and if this display may differ depending on whether the conspecific is a cagemate or an unknown rat. We developed a new model of empathy in which animals were trained to obtain food pellets by pressing either of two cued levers in an operant chamber. During the empathy test, one of the levers was programmed to also administer a footshock (0.5 mA, 0.5 sec) to a conspecific animal (cagemate or stranger) which was placed in full view, in an adjacent chamber. Single lever (forced-choice trial) or both levers (free-choice trial) were cued throughout the course of testing. We observed that rats behaved differently depending on the social context. Some rats chose the non-shock lever more often when they were exposed to their cagemate as compared to a stranger, indicating that the social affiliation may have played a role in guiding decision making. We also found that some animals showed a decrease in the rate of bar pressing for both levers regardless of social context, suggesting that they were responsive to the affective state of both the cagemate and the stranger. Finally, other rats did not show any change in the rate of lever pressing during the test period. Our results are consistent with previous findings that rodents are capable of exhibiting empathy-like behaviors. Furthermore, the model captures some of the natural individual differences observed in human empathy. The successful implementation of an operant rodent empathy model will allow for a wide range of new studies that could be useful for advancing our understanding of the neural basis of empathy. We currently are investigating whether the insular cortex, a region that has been associated with interoceptive and emotional processing, is involved in the expression of empathy. The results of this research could be used to guide the treatment of mental disorders in which empathy is deficient.

Disclosures: **M. Contreras:** None. **C. Medina:** None. **K. Cruz:** None. **J. Fellous:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.15/S12

Topic: F.03. Motivation and Emotion

Support: ANR-10-LABX-0087 IEC

ANR-10-IDEX-0001-02 PSL*

ANR-11-EMCO-00902

Title: Effects of social threat on attention and action-related decisions in a realistic social context

Authors: *E. VILAREM[1], J. L. ARMONY[2], J. GRÈZES[1];
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Abstract: Evolutionary theories suggest that emotional displays serve a communicative function by providing information about the individuals expressing them, or the surrounding environment. Consequently, individuals who more efficiently detect and respond to these signals have a survival advantage. This evolutionary framework implies that 1) emotional signals have co-evolved with recipient's behavioral responses, and 2) that the recipient's response should reflect the social function of the perceived expression. Here, we set out to experimentally address these assumptions. In two experiments, participants faced stimuli reproducing a social environment, i.e. a waiting room with four seats, where the two middle seats are occupied by two individuals, one displaying a neutral expression and the other expressing either anger or fear of varying intensity. We selected these emotions as they both signal the presence of potential threat in the environment, but they differ in terms of their social functions and were suggested to be associated with different approach-avoidance reactions (Marsh *et al.,* 2005). In our first experiment, participants were requested to detect a "T" appearing on either of the two outer seats as we recorded their reaction times. This experiment capitalized on the knowledge that observers' current potential actions impact the appraisal of their environment, notably by enhancing the perception of relevant spatial information (Kirsch, 2015). Thus, if emotional displays are perceived as opportunities for action, and if fearful and angry expressions prompt different motor actions by conveying different signals, the observers' appraisal of space should be differently impacted. Moreover, the observer's choice of action should also be differently impacted, as tested in our second experiment, wherein participants were asked to freely decide where they want to sit by moving their cursor on the screen; here, movement kinematics and pupil dilation were recorded. Results from both experiments indicate that fearful and angry expressions induced opposite effects: anger, by signaling a potential direct threat, resulted in a better "T" discrimination in the opposite side of the scene and avoidance behaviors; in contrast, fear, by warning the observer about a danger in the environment, elicited better "T" discrimination in the same side of the scene and affiliative approach behaviors. Movement kinematics and pupil dilation further support the governance of these behavioral tendencies by distinct mechanisms. Overall, our data suggest that emotional displays promote elaboration of adapted decisions and emotion-specific motor actions.

Disclosures: **E. Vilarem:** None. **J.L. Armony:** None. **J. Grèzes:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.16/S13

Topic: F.03. Motivation and Emotion

Title: Surface deformities of right caudate nucleus are associated with verbal abuse in adolescence

Authors: ***D.-W. SHIN**, B. JEONG, S. LEE, J. YOO, T. YOON;
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Abstract: Introduction The specific brain structures which conduct linguistic and emotional functions are connected systematically by structural and functional circuits. Our research team will measure the thickness, surface, volume, shape, etc of specific cerebral cortex and subcortex areas in the peer verbal abuse group and the control group. Through these way, we will find the structural change, related brain networks, and clinical signification. Method We completed a Verbal Abuse Questionnaire targeting 31 high school students. Scores greater than 40 was defined as the peer verbal abuse group and scores less than 40 was defined as the control group. Thickness, surface, volume, shape, etc of cerebral cortex and subcortex were measured using Freesurfer software and FMRIB Software Library(FSL) FIRST tool form brain MRI. Collected data in peer verbal abuse group was compared with those of the control group, then we checked the specific location of significant difference between them. Additionally, we made an attempt to identify the relationship between Verbal Abuse Questionnaire scores and change of cortex / subcortex. Result The analysis of cerebral cortex area expressed that right lateral occipital surface showed a significantly positive correlation between the Verbal Abuse Questionnaire scores and cortical thickness in the peer verbal abuse group. Analysis of subcortex area showed surface deformities and reduced volume of lateral and medial right caudate nucleus in the peer verbal abuse group compared to the control group. Discussion The right lateral occipital surface functions as recognizing subjects from visual information. The thickness of the right lateral occipital surface decreases under conditions of posttraumatic stress disorder associated with strong visual stimulus. However, in the peer verbal abuse group, the thickness of the right lateral occipital surface could increase because of the compensative mechanism searching and avoiding the verbal abusing assailant. The right caudate nucleus in which surface deformities were detected in the peer verbal abuse group is the area which has functional connection between the

frontal and parietal lobe. Deformities of this area suggest dysfunction of front-striatal circuit charging of attention. Because the volume / shape of the caudate nucleus and the Verbal Abuse Questionnaire scores have a significant correlation, the caudate nucleus was also considered as a biomarker related to verbal abuse. We suggest that the verbal abuse could change brain network connected to the right lateral occipital cortex processing visual stimulus and caudate nucleus charging attention.

Disclosures: **D. Shin:** None. **B. Jeong:** None. **S. Lee:** None. **J. Yoo:** None. **T. Yoon:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 163.17/S14

Topic: F.03. Motivation and Emotion

Support: CNPq-INCT

FAPESP

Title: Repeated and brief social defeat stress during adolescence on behavior and MAX/MYC network

Authors: ***S. CHIAVEGATTO**[1,2], C. E. AMARAL[1,2], R. B. S. SOARES[2], A. S. ALVES[1], L. ALVES-DOS-SANTOS[1,2], L. R. G. BRITTO[1], L. S. RESENDE[1,2];
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Abstract: The MAX network comprises a group of transcription factors whose functions profoundly affect cell behavior, being mostly expressed during neurodevelopment. Inspired by its higher expression in brain regions strongly involved in emotional behaviors, our hypothesis is that this protein might be involved in the cerebral changes caused by stress early in life. Here we used the social defeat, a valuable animal model for bullying in humans to study the influence of social stress during adolescence on behavior and on the transcription factors MAX and MYC. C57BL/6 male mice aged 30 days were subjected to daily social defeat by an adult aggressor in a 30-min session for 21 days. After the first attack (up to 5 min of physical interaction), animals were separated in the cage for the remaining period (threat). At the end, adolescents were relocated to their original cages (group-housed). Behavioral tests were conducted and brains were processed for MAX and MYC expression. Following episodes of social stress, but

maintaining mice in social groups after each defeat, adolescent mice exhibit depressive-like phenotype as shown by a reduced intake towards a 2% sucrose and prolonged immobility time in the forced swim test ($p<0.05$). Molecularly, we show that repeated social defeat causes 30% increase of the transcription factor MAX in the hippocampus (HC). Higher MAX levels are found in the nucleus of most hippocampal neuronal cells. Conversely, MAX is reduced (20%; $p<0.05$) in dorsal striatum (ST), and is not differently expressed in the prefrontal cortex (PFC) of defeated mice. There is a positive correlation between MAX and MYC levels in the three brain areas of non-defeated adolescents ($p<0.05$), which is disrupted in the HC and ST by social stress ($p>0.05$). These results show that this protocol of repeated brief social defeat in adolescent male mice, associated to group-housing, may serve as an interesting animal model to study a subtype of depression dissociated from generalized (non-social) anxiety. Importantly, we show for the first time a dysregulation of MAX network of transcription factors in specific brain areas associated to social stress during adolescence.

Disclosures: S. Chiavegatto: None. **C.E. Amaral:** None. **R.B.S. Soares:** None. **A.S. Alves:** None. **L. Alves-dos-santos:** None. **L.R.G. Britto:** None. **L.S. Resende:** None.

Poster

163. Social Stress

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.03. Motivation and Emotion

Support: EU Seventh Framework Program (FP7/2010–2014) under Grant Agreement No. 265957 – COPEWELL

Title: Should I stay or should I go? Telencephalic neural correlates for individual behavioral differences in fish

Authors: *M. A. VINDAS[1,2], P.-O. THÖRNQVIST[3], E. HÖGLUND[4], M. GORISSEN[5], B. DAMSGÅRD[6], T. O. NILSEN[1], S. WINBERG[3], G. FLIK[5], Ø. ØVERLI[7], L. O. E. EBBESSON[1]; [1]Uni Res. AS, Bergen, Norway; [2]Dept. of Biosci., Univ. of Oslo, Oslo, Norway; [3]Dept. of Neurosci., Uppsala Univ., Uppsala, Sweden; [4]Section for Aquaculture, Tech. Univ. of Denmark, Hirtshals, Denmark; [5]Dept. of Organismal Animal Physiol., Radboud Univ., Nijmegen, Netherlands; [6]Fac. of Biosciences, Fisheries and Economy, Univ. of Tromsø, Tromsø, Norway; [7]Dept. of Animal and Aquacultural Sci., Norwegian Univ. of Life Sci., Ås, Norway

Abstract: Consistent and correlated behavioral and physiological traits affect how individuals perceive and react to their environment. Perception and processing of salient stimuli is mainly under forebrain control. A comparison of telencephalic areas in differing individual coping styles is still lacking in fish. We selected individuals from a population by their response to increasing hypoxia levels. Fish either left to an adjacent tank (Leavers) or stayed (Stayers). We measured basal and post-stress plasma cortisol levels and conducted *in situ* hybridization for the early activity gene cFOS and brain derived neurotrophic factor (BDNF) expression. We then microdissected activated telencephalic areas: the dorsolateral (Dl; proposed hippocampus homolog), dorsomedial (Dm; proposed amygdala homolog) pallium, and the ventral part of the ventral telencephalon (Vv, proposed lateral septum homolog). We measured basal and post-stress monoamine neurochemistry and gene expression of target genes for the serotonergic and corticotropin releasing hormone (CRH) systems, as well as indicators of neural plasticity/proliferation. We also measured the same target molecules in the whole telencephalon. We found that Leavers reacted with a potentiated cortisol response to acute stress, which together with the previously characterized passive behavioral response to hypoxia, suggests a reactive coping style. Whole telencephalon analysis was limiting and sometimes misleading since possible opposite and/or specific regulation of brain areas are most often masked when pooled together in whole-brain area analysis. Leavers were characterized by higher post-stress serotonergic activity and elevated serotonin receptors and CRH binding protein (CRHBP) expression in the Dm. In the Dl, Leavers had post-stress elevated BDNF and CRH receptor 1 expression and increased BDNF expression in the Vv. Stayers displayed higher dopaminergic activity and elevated basal proliferating nuclear antigen (PCNA) expression in the Dl. Taken together our results suggest that the neurological profile of Leavers is associated with increased behavioral flexibility (active coping) to acute stress, while Stayers express a higher degree of neural plasticity under basal conditions. Elucidating individual differences in neural plasticity and behavior is an important tool in understanding vulnerability to disease and may help elucidate target systems in neuropsychological disorders, such as anxiety and depression. This study was funded by the EU Seventh Framework Program (FP7/2010-2014) under Grant Agreement No. 265957 - COPEWELL

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Poster

164. Thirst and Water Balance

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.01/S16

Topic: E.06. Thirst and Water Balance

Title: Brain loci relating to the thirst reception in an amphibious teleost, mudskipper

Authors: *S. HAMASAKI[1], Y. FURUKAWA[1], K. UEMATSU[1], M. YOSHIDA[1], T. MUKUDA[1,2];
[1]Hiroshima Univ., Higashi-Hiroshima, Japan; [2]Tottori Univ., Yonago, Japan

Abstract: Terrestrial vertebrates including human perceive thirst and then follow water drinking, to avoid dehydration. Thirst is induced when dipsogenic hormones in systemic circulation act on some circumventricular organs (CVOs), which lack the blood-brain barrier (BBB) in the brain. In teleosts, meanwhile, water intake is thought to be completed only by swallowing reflex in response to dehydration without thirst reception. Thus, thirst reception seems to be a novel and essential sense acquired during an adaptation to land from water in the vertebrate evolution. Mudskippers, amphibious teleosts in brackish water, prefer land to water, showing a long-hour staying on land during the day. However, they go back to water transiently to supply water and electrolytes which have been gradually lost on lands. Because this would be voluntary behavior induced by thirst reception, mudskippers are suitable models to examine functional acquisition of thirst reception in the vertebrate evolution. In this study we searched for the thirst-related brain loci in the mudskippers. To identify the CVOs in the forebrain, mudskippers were intraperitoneally injected with Evans blue dye or sulfo-NHS-biotin, which immediately bind to systemic proteins such as albumin. We found an extravasation of the bound complexes, which cannot pass through the BBB, in the anteroventral third ventricular walls (AV3V) in the preoptic area. Further, an immunohistochemistry for tyrosine hydroxylase and a low vacuum scanning electron microscopic observation revealed that the AV3V contains neurons, which are identified as the anterior part of the parvicellular preoptic nucleus (PPa). To clarify whether the PPa is activated following to osmotic change, salinity of the habitable water was increased from 11 PSU (isotonic) up to 35 PSU (hypertonic). Three hours after exposing in the hypertonic water, water content of mudskipper body has been decreased significantly compared to controls (76.9% in hypertonic, 78.0% in isotonic, $P < 0.01$), indicating that the mudskippers dehydrated in this condition. The number of activated cells in the PPa of the dehydrated mudskippers, identified by c-Fos immunohistochemically, have shown ca. 1.5-fold increase compared to controls ($P < 0.01$). These suggest that the PPa of the mudskippers are activated following to dehydration induced by hyperosmotic exposure. Taken together, the AV3V including the PPa is likely to be a candidate of the ‘thirst center' in the mudskipper brain.

Disclosures: **S. Hamasaki:** None. **Y. Furukawa:** None. **K. Uematsu:** None. **M. Yoshida:** None. **T. Mukuda:** None.

Poster

164. Thirst and Water Balance

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.02/S17

Topic: E.06. Thirst and Water Balance

Title: Intracerebroventricular administration of thyrotropin-releasing hormone (TRH) suppresses water intake without affecting feed consumption in the neonatal chicks

Authors: *S.-I. KAWAKAMI, Y. HAYASHI;
Hiroshima Univ. Grad. Sch. of Biosphere Sci., Hiroshima, Japan

Abstract: Exposure to high environmental temperature is known to negatively affect feeding behavior of animals, the brain mechanisms regulating heat stress-induced feeding suppression remain unknown. Thyrotropin-releasing hormone (TRH) is a neuroendocrine tripeptide which is mainly synthesized in the hypothalamus and regulates thermogenesis in the hypothalamus-pituitary-thyroid axis, but it has not been known whether TRH affect feed and/or water intake in the avian brain. Therefore, the aim of the present study was to examine the effect of intracerebroventricular (ICV) administration of TRH or its antagonist (chlordiazepoxide) on feed and water intake of neonatal chicks. The mail layer chicks (5-day-old) were fixed in a headholder that has a fitting pinhole for ICV injection and the solutions (10 μl) were administered free-hand through the pinhole using a microsyringe, according to the procedure of Davis et al. (1979). Feed and water intake were measured at 30, 60 and 120 min after the treatment. In the first trial, chicks with free access to feed and water were ICV injected with one of four doses (0, 12.5, 25 or 50 nmol) of TRH. In the second trial, with free access to water but being deprived of feed for 3 h, chicks were ICV injected with TRH and refed. In the third trial, after being deprived of both feed and water for 3 h, chicks were ICV injected with TRH and rehydrated. In the fourth trial, chicks with free access to feed and water were ICV injected with one of four doses (0, 0.15, 1.5 or 15 nmol) of chlordiazepoxide. In the first, second and third trials, ICV administration of TRH significantly inhibited cumulative water consumption at all doses in layer chicks when compared with vehicle ($P<0.05$). Cumulative feed consumption was unaffected by TRH administration in feed-deprived chicks, but significantly increased in chicks fed ad libitum only at the dose of 50 nmol after 120 min of TRH administration. In the fourth trial, TRH antagonist, chlordiazepoxide, increased cumulative water consumption at the dose of 15 nmol after 60 min of ICV administration, but did not affect cumulative feed consumption in layer chicks. These data suggest that TRH mainly plays an essential role in the control of water intake, not of feed intake, in the brain of neonatal chicks.

Disclosures: S. Kawakami: None. **Y. Hayashi:** None.

Poster

164. Thirst and Water Balance

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.03/S18

Topic: E.06. Thirst and Water Balance

Support: FAPESP

CNPq

CAPES

Title: CO (carbon monoxide) blockade has different effects on 24h and 48h dehydration-induced hormone secretion and progressive dehydration induces distinct HO-1 (hemeoxygenase type 1) and nNOS (neuronal nitric oxide synthase) transcription patterns

Authors: *J. B. LIMA[1], F. LUCIO-OLIVEIRA[2], R. COLETTI[1], F. M. V. VECHIATO[1], L. L. K. ELIAS[1], J. ANTUNES-RODRIGUES[1];
[1]Physiol., Univ. of São Paulo, Ribeirao Preto, Brazil; [2]Federal Inst. of Education, Sci. and Technol. of Southern Minas Gerais, Muzambinho, Brazil

Abstract: Recently it has been reported evidences of the gaseous molecule carbon monoxide modulation on neuroendocrine and other homeostatic systems. Data from literature have indicated CO as a fine modulator of neuroendocrine response to challenges which disrupt hydrosaline equilibrium such as water deprivation (WD) and salt loading and besides CO system could interact with NO system in these situations. The aim of this present study is to evaluate whether CO blockage in progressive WD leads to different patterns of hormonal secretion and if different times of WD induce distinct HO-1, HO-2 and nNOS transcription patterns. To that, 250g Wistar male rats were submitted to 24h, 48h and 72h of dehydration with free access to chow, then were euthanized and the hypothalamic supraoptic nuclei collected; or, after dehydration time (24h or 48h), they were icv injected with HO inhibitor (ZnDPBG, 200 nmol) or vehicle (Na2CO3, 50 mM), were euthanized after 30 min and, then, the trunk blood collected. We observed that in 24h dehydration only nNOS mRNA level was increased while in 48h WD both HO-1 and nNOS were raised ($F(3, 15) = 7.7$, $p<0.01$; $F(3, 15) = 18.9$, $p<0.0001$, respectively), in 72h the same pattern of 48h WD was observed, the HO-2 mRNA level was not affected by these conditions. Also, we demonstrated that in basal condition (euhydrated animals) the inhibition of central CO formation increased only corticosterone (CORT) plasma concentration with no effect on vasopressin (AVP) and oxytocin (OT) plasma levels, while ZnDPBG injection reverted the 24h WD-induced AVP and OT secretion increase with no effect

on CORT level. Whereas the inhibition of hemeoxigenase reversed 48h WD-induced AVP, OT and CORT secretion increase ($F(2, 33) = 5.0$, $p<0.05$; $F(2, 37) = 3.7$, $p<0.05$; $F(2, 33) = 19.7$, $p<0.0001$, respectively). Together these data suggest that the same manipulation of central CO content can affect differently the neuroendocrine system depending on the hydration state of the animal, so the CO effects could act in time-dependent manner in this experimental protocol.

Disclosures: **J.B. Lima:** None. **F. Lucio-Oliveira:** None. **R. Coletti:** None. **F.M.V. Vechiato:** None. **L.L.K. Elias:** None. **J. Antunes-Rodrigues:** None.

Poster

164. Thirst and Water Balance

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Topic: E.06. Thirst and Water Balance

Support: FAPESP

CNPq

CAPES

Title: Activities of hypothalamic nitric oxide synthase (NOS) and cystathionine beta-synthase/3-mercaptopyruvate sulfurtransferase (CBS/3MST) are decreased by NO and H2S in hyperosmolality *in vitro*

Authors: *R. COLETTI, J. B. M. LIMA, F. M. V. VECHIATO, G. ALMEIDA-PEREIRA, F. LUCIO-OLIVEIRA, L. L. K. ELIAS, J. ANTUNES-RODRIGUES;
Physiol., Sch. of Med. of Ribeirao Preto/Usp, Ribeirao Preto, Brazil

Abstract: NO and H2S are gaseous molecules produced in the central nervous system by NOS and CBS/3MST systems respectively. It is already known that water deprivation increases NO and H2S production in the hypothalamus, which, in turn, act inhibiting and increasing vasopressin (AVP) and oxytocin (OT) secretion respectively; additionally, it has already been demonstrated *in vivo* exogenous H2S decreases hypothalamic nitrate content, a NO metabolite, which is typically found increased in response to extracellular hyperosmolality. Nevertheless, little has been shown about NO and H2S actions on their own enzymatic systems. Due to it, we aimed to evaluate *in vitro* NOS and CBS/3MST activities in extracellular hyper- or isotonicity, in the presence or absence of NO and H2S donors. To that, male Wistar rats' (270-300g) medial basal hypothalami (MBH) were collected and kept in isotonic (280 mOsm/Kg H2O) oxygenated

Krebs-Ringer bicarbonate buffer (KRBG) for pre-incubation (37°C, 1L/min carbogenic mixture, 60 min). Then, medium was replaced by new hypertonic (340 mOsm/Kg H2O) or isotonic KRBG, with or without donors of NO (sodium nitroprusside, SNP; 150, 300, 600 μM) or H2S (sodium sulfide, Na2S; 0.1, 1, 10 mM), from which samples were collected for AVP and OT radioimmunoassay. Afterwards, MBH were submitted to specific assays of enzymatic activity in which NOS activity was measured by 14C-citrulline generation from L-14C-arginine and CBS/3MST activity was assessed by sulfides formation from L-cysteine (both corrected by total protein amount). We observed in hypertonicity that tissues exposure to SNP effectively decreased AVP ($F_{3,34} = 3.3$; $p < .05$) and OT ($F_{3,37} = 4,3$; $p < .05$) release at the concentration of 600 μM, besides reducing NOS activity ($F_{3,27} = 9.6$; $p < .001$) at 150, 300 and 600 μM. Similarly, Na2S reduced CBS/3MST activity ($F_{3,15} = 5.9$; $p < .01$) at every concentration tested (0.1, 1, 10 m); however, it increased AVP ($F_{3,26} = 15.4$; $p < .0001$) release at 0.1, 1 and 10 mM, and OT ($F_{3,28} = 4.3$; $p < .05$) at 10 mM only. These data indicate that, although NO inhibits AVP and OT release and H2S stimulates the release of both hormones, both NO and H2S perform similar control by negative feedback on their own enzymatic systems in the condition of extracellular hyperosmolality.

Disclosures: **R. Coletti:** None. **J.B.M. Lima:** None. **F.M.V. Vechiato:** None. **G. Almeida-Pereira:** None. **F. Lucio-Oliveira:** None. **L.L.K. Elias:** None. **J. Antunes-Rodrigues:** None.

Poster

164. Thirst and Water Balance

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.05/S20

Topic: E.06. Thirst and Water Balance

Support: FAPESP

CNPq

CAPES

Title: Type-1 cannabinoid receptor agonist decreases hypothalamic and neurohypophyseal hormonal release in response to hyperosmolality *in vitro*

Authors: ***F. M. VECHIATO**[1], R. COLETTI[1], S. G. RUGINSK[2], J. B. M. LIMA[1], L. L. K. ELIAS[1], J. ANTUNES-RODRIGUES[1];

[1]Physiol., Univ. of São Paulo/ Sch. of Med. of Rib, Ribeirao Preto, Brazil; [2]Physiol., Federal Univ. of Alfenas, Alfenas, Brazil

Abstract: Vasopressin (AVP), oxytocin (OT) and atrial natriuretic peptide (ANP) released by the hypothalamus and AVP and OT by neurohypophysis (NH) play important roles in the maintenance of body fluid homoeostasis. The endocannabinoid (ECB) system, mainly the type-1 cannabinoid receptor (CB1R), has also been considered a potential modulator of hydromineral balance. However, the effect of CB1Rs on hyperosmolality-induced hormone release has not been yet well elucidated. Objectives: This study aimed to evaluate the effects of ACEA, a CB1R selective agonist, on AVP, OT and ANP release by the medial basal hypothalamus (MBH) and NH in response to hyperosmolality. Methods and Results: Adult male Wistar rats were euthanized by decapitation, the brain quickly removed and the MBH and NH collected and pre-incubated in 0.5 mL of cold KRBG (Krebs-Ringer bicarbonate buffer) in a Dubnoff shaker (50 cycles per min) at 37°C for 60 min in an atmosphere of 95% O2 and 5% CO2. The medium was replaced with fresh KRBG medium (280 or 340 mOsm/Kg H2O, the latter made by the addition of mannitol or NaCl) containing or not ACEA (0.1, 1.0 or 10 μM). Exposure of MBH and NH to the hyperosmotic medium, regardless of whether hyperosmolality was induced by mannitol or NaCl, resulted in a significant increase in AVP, OT and ANP release. The incubation with ACEA under basal conditions produced no effect on hormone release. However, ACEA promoted a dose-dependent decrease on AVP, OT and ANP release by the MBH and NH under mannitol-induced hyperosmolality. The lowest dose of ACEA that decreased the release of all hormones tested was 10 μM. Therefore, we investigated whether this effect was dependent on sodium extracellular concentrations. Interestingly, ACEA also decreased hormone release by the MBH and NH induced by hyperosmotic stimulus performed with NaCl. Conclusion: Our data showed for the first time that ACEA reduced osmolality-induced AVP, OT and ANP release by the MBH and AVP and OT by NH, regardless of sodium extracellular concentrations.

Disclosures: F.M. Vechiato: None. **R. Coletti:** None. **S.G. Ruginsk:** None. **J.B.M. Lima:** None. **L.L.K. Elias:** None. **J. Antunes-Rodrigues:** None.

Poster

164. Thirst and Water Balance

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.06/T1

Topic: E.06. Thirst and Water Balance

Title: TRPV channel regulation of supraoptic nucleus neuron activity in lactation

Authors: *C. H. BROWN, Z. JAQUIERY, G. T. BOUWER, R. A. AUGUSTINE;
Univ. Otago, Dunedin, New Zealand

Abstract: Vasopressin promotes water retention by the kidney. Vasopressin secretion is inhibited by reduced plasma osmolality, as well as by increased plasma volume. During lactation, plasma osmolality is reduced and plasma volume is increased but circulating vasopressin is not decreased. Vasopressin release from the posterior pituitary gland is triggered by action potential firing of magnocellular neurosecretory neurons that are mainly located in the hypothalamic supraoptic nucleus and paraventricular nucleus. Vasopressin neuron activity is increased by TRPV channels that are activated by acute increases extracellular osmolality. Here, we tested whether TRPV channels contribute to the spontaneous activity of supraoptic nucleus vasopressin neurons in virgin and lactating rats by microdialysis administration of the TRPV channel blocker, ruthenium red, into the supraoptic nucleus of anaesthetised rats during extracellular single unit recording of vasopressin neuron firing rate. In virgin rats, ruthenium red reduced the firing rate of vasopressin neurons ($P < 0.01$; $n = 9$), but not oxytocin neurons ($P = 0.65$; $n = 5$). Ruthenium red inhibited the firing rate of vasopressin neurons in lactating rats ($n = 7$) to similar degree to that evident in virgin rats ($P = 0.97$). Hence, it appears that the *in vivo* spontaneous activity of vasopressin neurons, but not oxytocin neurons, is driven, in part, by TRPV channel activity and that this intrinsic osmotic drive remains during lactation despite the decreased plasma osmolality evident during lactation.

Disclosures: C.H. Brown: None. **Z. Jaquiery:** None. **G.T. Bouwer:** None. **R.A. Augustine:** None.

Poster

164. Thirst and Water Balance

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.07/T2

Topic: E.06. Thirst and Water Balance

Support: CNPq

FAPESP

Title: Sodium intake induced by gabaergic activation in the lateral parabrachial nucleus depends on opioid mechanisms of the central nucleus of amygdala

Authors: G. M. F. ANDRADE-FRANZÉ, *C. A. ANDRADE, S. GASPARINI, L. A. DE LUCA JR., P. M. DE PAULA, D. S. A. COLOMBARI, E. COLOMBARI, J. V. MENANI; Sch. of Dent. - FOAr - UNESP, Araraquara, Brazil

Abstract: The lateral parabrachial nucleus (LPBN) and the central nucleus of the amygdala (CeA) are important central areas involved in the control of sodium appetite. GABAA receptor activation with bilateral injections of muscimol into the LPBN induces 0.3 M NaCl intake in normohydrated rats, an effect abolished by the deactivation of CeA neuronal activity, suggesting that CeA facilitatory mechanisms are important for sodium intake induced by muscimol into the LPBN. The activation of μ opioid receptors in the CeA facilitates 0.3 M NaCl induced by water deprivation or by fluid depletion. In the present study, we investigated the effects of bilateral injections of naloxone (opioid receptor antagonist) into the CeA on water and 0.3 M NaCl intake induced by muscimol injections into the LPBN in normohydrated rats or by sodium depletion (treatment with the diuretic furosemide subcutaneously followed by 24 h of sodium deficient diet). Male Holtzman rats (n = 07-08/group) with stainless steel cannulas implanted bilaterally in the CeA and in the LPBN were used. Bilateral injections of naloxone (40 μg/0.2 μl) into the CeA completely abolished 0.3 M NaCl intake (0.7 ± 0.3 ml/4 h, vs. saline into the CeA: 29.4 ± 2.7 ml/4 h) and water intake (0.3 ± 0.1 ml/4 h, vs. saline into the CeA: 15.0 ± 2.4 ml/4 h) induced by muscimol (0.5 nmol/0.2 μl) into the LPBN. However, naloxone into the CeA did not significantly change sodium depletion-induced 0.3 M NaCl intake (7.4 ± 1.8, vs. saline into the CeA: 13.2 ± 2.0) or water intake (0.2 ± 0.1, vs. saline into the CeA: 1.3 ± 0.6). The present results suggest that the activation of the opioid mechanisms in the CeA is essential for water and hypertonic NaCl intake induced by the blockade of the inhibitory mechanisms with injections of muscimol into the LPBN. Supported by CNPq and FAPESP.

Disclosures: G.M.F. Andrade-Franzé: None. **C.A. Andrade:** None. **S. Gasparini:** None. **L.A. De Luca Jr.:** None. **P.M. De Paula:** None. **D.S.A. Colombari:** None. **E. Colombari:** None. **J.V. Menani:** None.

Poster

164. Thirst and Water Balance

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.08/T3

Topic: E.06. Thirst and Water Balance

Support: FAPESP

CNPq

Title: Importance of cholinergic and angiotensinergic mechanisms of the subfornical organ for water and sodium intake by hyperosmotic rats treated with moxonidine into the lateral parabrachial nucleus

Authors: *C. F. RONCARI, R. B. DAVID, P. M. DE PAULA, D. S. A. COLOMBARI, L. A. DE LUCA JR., E. COLOMBARI, C. A. F. ANDRADE, J. V. MENANI;
Dept Physiol. and Pathol., UNESP, Araraquara, Brazil

Abstract: Central facilitatory and inhibitory mechanisms are involved in the control of water and sodium intake. Important inhibitory mechanisms for the control of water and sodium intake are modulated by the lateral parabrachial nucleus (LPBN). It has been previously demonstrated that plasma hyperosmolarity, a classic dipsogenic stimulus, can also facilitate sodium intake if the inhibitory mechanisms are deactivated by the injections of moxonidine (α2-adrenoceptor/imidazoline receptor agonist) into the LPBN. Central cholinergic and angiotensinergic mechanisms are involved in the responses induced by plasma hyperosmolarity. The subfornical organ (SFO) is an important forebrain site involved in the control of water and sodium intake. Therefore, in the present study, we investigated whether angiotensinergic and cholinergic mechanisms of the subfornical organ (SFO) are involved on water and 0.3 M NaCl intake by hyperosmotic rats treated with moxonidine into the LPBN. Male Holtzman rats (290-310 g, n = 8) with stainless steel guide-cannulas implanted into the SFO and bilaterally into the LPBN were used. Rats received an intragastric (ig) load of 2 M NaCl (2 ml) and injection of saline, atropine (muscarinic cholinergic antagonist, 2 nmol/0.1 μl) or losartan (AT1 receptor antagonist, 1 μg/0.1 μl) into the SFO, 45 minutes before bilateral injections of vehicle or moxonidine (0.5 nmol/0.2 μl) into the LPBN. Water and 0.3 M NaCl intake was measured for 2 h starting 15 min after LPBN injections. Hyperosmotic rats that received bilateral injections of moxonidine into the LPBN combined with injection of saline into the SFO ingested a significant amount of water (15.1 ± 3.6 ml/2 h, vs. vehicle: 6.3 ± 1.6 ml/2 h) and 0.3 M NaCl (21.4 ± 4.4 ml/2 h, vs. vehicle: 0.7 ± 0.4 ml/2 h). Atropine or losartan injected into the SFO reduced the ingestion of water (2.1 ± 0.9 ml/2 h and 2.5 ± 1.1 ml/2 h, respectively) and 0.3 M NaCl intake (5.0 ± 2.8 ml/2 h and 3.3 ± 2.0 ml/2 h, respectively) in hyperosmotic rats treated with moxonidine injected into the LPBN. The results suggest the involvement of cholinergic and angiotensinergic mechanisms of the SFO on water and sodium intake by hyperosmotic rats treated with moxonidine injected into the LPBN.

Disclosures: C.F. Roncari: None. **R.B. David:** None. **P.M. De Paula:** None. **D.S.A. Colombari:** None. **L.A. De Luca Jr.:** None. **E. Colombari:** None. **C.A.F. Andrade:** None. **J.V. Menani:** None.

Poster

164. Thirst and Water Balance

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.09/T4

Topic: E.06. Thirst and Water Balance

Support: CIHR Grant

Title: Mechanism of clock-dependent activation of thirst neurons in rat

Authors: *C. GIZOWSKI, C. W. BOURQUE;
Res. Inst. of the MUHC, Montreal, QC, Canada

Abstract: Under normal conditions, the sensation of thirst arises when osmosensitive “Thirst” neurons in the OVLT (Organum Vasculosum Lamina Terminalis) become electrically excited by hyperosmolality or hyperthermia, and relay this information to the rostral anterior cingulate cortex. We have shown that mice display a major peak of water intake just prior to sleep, which is independent of increases in core body temperature and plasma osmolality. Since the electrical activity of clock neurons in the Suprachiasmatic Nucleus (SCN) is rising during the corresponding period, we hypothesize that these neurons can mediate an increase in the electrical activity of Thirst neurons in the OVLT. We have previously shown whole-cell current clamp recordings of rat and mouse OVLT neurons that display a prolonged depolarization and excitation in response to repetitive electrical stimulation of the SCN (30s; 10Hz) in slice. Furthermore, we have established that this response is mediated by vasopressin (VP) via the metabotropic V1a receptor. The aim of the present study is to investigate the ionic basis of these events in OVLT neurons, using the patch clamp technique in whole-cell voltage clamp configuration. When tested under voltage clamp, repetitive stimulation of the SCN induced the appearance of a long-lasting inward current which could return to baseline after 20-25 minutes. The average amplitude of the current measured at a holding potential of -50 mV was -5.66±1.68 pA (p=0.008; n=10). Current voltage (I-V) analysis using voltage ramps revealed that the SCN-induced current displayed a reversal potential (Erev) of -24.57 ± 3.41 mV, which was associated with a mean increase in slope conductance, measured near -40 mV, of 0.22±0.04 nS in OVLT neurons (p=0.0006; n=10). This mean increase in slope conductance is significantly attenuated by bath application of SR-45059 (10 μM; selective VP V1a receptor antagonist; 0.05±0.05 nS; p=0.026; n=10). The Erev is unaffected in cells recorded using pipettes containing cesium instead of potassium (140 mM), or varying concentrations of chloride (155 mM or 4 mM). These results indicate the depolarization of OVLT neurons induced by stimulation of the SCN is mediated by the activation of a non-selective cation conductance.

Disclosures: C. Gizowski: None. **C.W. Bourque:** None.

Poster

164. Thirst and Water Balance

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.10/T5

Topic: E.06. Thirst and Water Balance

Support: Fapesp

Title: Muscimol injected into the lateral parabrachial nucleus increases potassium chloride intake in the rat

Authors: *J. C. CALLERA[1], L. A. DE LUCA JR[2], J. V. MENANI[2];
[1]Sao Paulo State Univ., Aracatuba, Brazil; [2]Physiol. and Pathology, Sao Paulo State Univ., Araraquara, SP, Brazil

Abstract: We previously demonstrated that the blockade of the inhibitory mechanisms with bilateral injections of muscimol, GABAA receptor agonist, into the lateral parabrachial nucleus (LPBN) results in strong ingestion of water and different mineral solutions containing sodium (0.3 M NaCl or 0.3 M NaHCO3) in a two-bottle test. In the present study, we investigated if muscimol injections into the LPBN would modify the ingestion of other mineral solution (potassium chloride, KCl) in normohydrated, in cell-dehydrated rats by an intragastric load of 2 M NaCl (IG 2 M NaCl) or in rats with sodium depletion (treatment with the diuretic furosemide subcutaneously + sodium deficient food for 24 h). Male adult Wistar rats with bilateral stainless steel guide-cannulas implanted in the LPBN were used (n=5-6/group). Deionized water and 0.3 M KCl intake was measured in two-bottle tests at every 30 min during 240 min, starting 15 min after bilateral injections of muscimol (0.5 nmol/0.2 µl) or saline into the LPBN. Bilateral injections of muscimol into the LPBN increased 0.3 M KCl intake in normohydrated rats (5.1 ± 1.4, vs. saline: 0.1 ± 0.1 ml/240 min), in rats treated with IG 2 M NaCl (20.6 ± 2.7, vs. saline: 1.0 ± 0.6 ml/240 min) and in sodium-depleted rats (8.3 ± 2.9, vs. saline: 0.5 ± 0.1 ml/240 min). Muscimol into the LPBN also increased water intake in normohydrated rats (8.7 ± 4.5, vs. saline: 0.4 ± 0.2 ml/240 min) and in sodium-depleted rats (7.5 ± 3.6, vs. saline: 1.9 ± 0.8 ml/240 min), however, muscimol reduced water intake in the first 150 min of test in rats treated with IG 2 M NaCl (4.9 ± 1.9, vs. saline: 11.2 ± 1.0 ml/150 min). Water and 0.3 M KCl intake was not modified by injections of methysergide (serotonergic receptor antagonist, 4 µg/0.2 µl) into the LPBN. These data show that besides sodium and water intake, muscimol injections into the LPBN also increase 0.3 M KCl intake in normohydrated, cell-dehydrated and sodium depleted rats, suggesting that the activation of GABAA receptors in the LPBN releases the ingestion of different mineral solutions.

Disclosures: **J.C. Callera:** None. **L.A. De Luca Jr:** None. **J.V. Menani:** None.

Poster

164. Thirst and Water Balance

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Program#/Poster#: 164.11/T6

Topic: E.06. Thirst and Water Balance

Support: FAPESP

CNPq

PROPE-UNESP

Title: Sodium intake induced by acute injection of aldosterone into the 4th ventricle in rats treated with moxonidine into the lateral parabrachial nucleus

Authors: ***S. GASPARINI**, P. A. NASCIMENTO, G. F. LEITE, M. R. MELO, J. V. MENANI, E. COLOMBARI;
Sao Paulo State University. Dep. of Physiol. and Pathology, Araraquara, Brazil

Abstract: Chronic infusion of low dose of aldosterone into the 4th ventricle (4th V) induces robust daily sodium intake, whereas acute injection of aldosterone into the 4th V usually produces no sodium intake. The blockade of the inhibitory mechanisms with bilateral injections of moxonidine (alpha2 adrenergic/imidazoline agonist) into the lateral parabrachial nucleus (LPBN) increases sodium intake induced by different stimuli like central injection of angiotensin II or chronic treatment with deoxycorticosterone. In the present study, we investigated the ingestion of 1.8% NaCl and water in rats treated with acute injections of aldosterone into the 4th V combined with moxonidine into the LPBN. Male Holtzman rats with stainless steel cannulas implanted in the 4th V and bilaterally in the LPBN were used. Rats received two injections of aldosterone (250 ng/2 µl each) or vehicle into the 4th V with one hour interval. Fifteen minutes before the second injection into the 4th V, moxonidine (0.5 nmol/0.2 µl) or vehicle was bilaterally injected into the LPBN. Burettes containing water and 1.8% NaCl were offered to the rats immediately after the second injection into the 4th V. Aldosterone into the 4th V combined with moxonidine into the LPBN induced strong ingestion of 0.3 M NaCl in the next two hours (17.6 ± 3.7, vs. vehicle 4th V + vehicle LPBN: 0.5 ± 0.5 ml/2 h) and also water intake (6.8 ± 2.1, vs. vehicle 4th V + vehicle LPBN: 0.4 ± 0.4 ml/2 h). These data suggest that the inhibitory mechanisms of the LPBN act against the facilitation of sodium intake produced by aldosterone

acutely injected into the 4th V restraining sodium intake in this condition, which is probably not the same in rats treated with chronic infusion of aldosterone into the 4th V.

Disclosures: S. Gasparini: None. **P.A. Nascimento:** None. **G.F. Leite:** None. **M.R. Melo:** None. **J.V. Menani:** None. **E. Colombari:** None.

Poster

164. Thirst and Water Balance

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Program#/Poster#: 164.12/T7

Topic: E.06. Thirst and Water Balance

Support: NIH AG-025465 to RLT

NIH HL-14388 to AKJ

Title: Effects of selective beta 1- and beta 2- adrenergic receptor activation on salt appetite of rats

Authors: *R. L. THUNHORST[1,2], B. XUE[1,2], T. BELTZ[1], A. K. JOHNSON[1,2,3,4];
[1]Dept Psychology, [2]Cardiovasc. Ctr., [3]Hlth. and Human Physiol., [4]Pharmacol., Univ. of Iowa, Iowa City, IA

Abstract: Isoproterenol is a mixed β1-, β2-adrenergic receptor agonist that causes hypotension, renin secretion and water drinking upon systemic administration. We previously have shown that isoproterenol inhibits the salt appetite of rats. We extended this work to examine the roles of receptor subtypes for this effect. Animals received either 24-hr of sodium depletion, acute diuresis/natriuresis with furosemide or daily injections of DOCA (5 mg/kg bw). Just prior to fluid access, we administered isoproterenol (30 μg/kg, sc) to simultaneously activate β1- and β2-adrenergic receptors, salbutamol (150 μg/kg) to selectively activate β2-adrenergic receptors, and the combination of isoproterenol and the β2-adrenergic receptor antagonist ICI 118,551 (1 mg/kg) to stimulate only β1-adrenergic receptors. Both isoproterenol and salbutamol significantly ($P < .05$) inhibited salt appetite after 24-hr of sodium depletion while the combination of isoproterenol with ICI compound had no effect. Both isoproterenol and salbutamol significantly ($P < .05$) increased thirst acutely after water and sodium depletion with furosemide (a time when animals normally ingest only water) while selective activation of β1 receptors did not. Lastly, selective activation of β2 receptors significantly ($P < .05$) inhibited salt appetite in response to daily mineralocorticoid treatment with DOCA while selective activation

of β1 receptors significantly (P <.05) increased DOCA-induced salt appetite. Therefore, selective activation of β2-adrenergic receptors was consistently associated with decreased salt appetite and increased thirst, while selective activation of β1-adrenergic receptors was associated with increased mineralocorticoid-induced salt appetite.

Disclosures: R.L. Thunhorst: None. **B. Xue:** None. **T. Beltz:** None. **A.K. Johnson:** None.

Poster

164. Thirst and Water Balance

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.13/T8

Topic: E.06. Thirst and Water Balance

Support: conacyt 295860-VVO

Title: Sugar consumption by male offspring of rats that consumed sugared water during pregnancy and lactation affects the renal expression of aquaporin 2 in the adulthood

Authors: *V. VELAZQUEZ[1], L. NICOLÁS[2], I. SOTO[3], F. CASTELÁN[2], J. RODRÍGUEZ[2]; [1]Univ. Autonoma De Tlaxcala, tlaxcala, Mexico; [2]Univ. Autonoma De Tlaxcala, Tlaxcala, Mexico; [3]Univ. Veracruzana, Veracruz, Mexico

Abstract: The physiology of the urinary tract achieves the elimination of toxic substances from the organism, particularly in the kidneys. The several types of aquaporins differentially expressed along the kidney are highly relevant for the water transport across the renal tubules. When this process is altered, the organism can present a wide range of renal injuries. The high consumption of sugared drinks have been related with metabolic deseases, including the diabetes mellitus type 2 that is linked to renal failure. The aim of this project was to determine whether the consumption of sugared water during the pregnancy and lactation, alters the expression of the aquaporin 2 in the kidney of adult male offspring. We use females rats that were mated and divided in a control group fed with standard diet and tap water and the experimental group fed with standard diet and 5 % sucrose diluted in tap water (sugared water). At weaning, two male rats were randomly selected per litter; one of them has free access to simple water while the other has free access to the sugared water). The male rats were sacrificed at four months old and the expression of aquaporin 2 of the left kidney was estimated by immunohistochemistry. Preliminary results show an overexpression of aquaporin 2 in the group that consumed sugared water during pregnancy, lactation, and postnatal life. It seems that the consumption of sugar water, even in low concentrations, modifies the renal expression of aquaporin 2. This could be

directly related to water transport in the renal tubules altering the function of the upper urinary tract. Acknoledgements: CONACyT fellowship (Reg. 295860) to VVO.

Disclosures: **V. Velazquez:** None. **L. Nicolás:** None. **I. Soto:** None. **F. Castelán:** None. **J. Rodríguez:** None.

Poster

164. Thirst and Water Balance

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Program#/Poster#: 164.14/T9

Topic: E.06. Thirst and Water Balance

Support: INCT - NanoBiofar

CNPq

FAPEMIG

CAPES

UFOP

Title: Sucrose intake is increased by central angiotensin II

Authors: M. H. PAES, L. M. CARDOSO, *L. B. OLIVEIRA;
Fed. Univ. Ouro Preto - UFOP, Ouro Preto, Brazil

Abstract: The effects of angiotensin (ANG) II on water and sodium, but not in sucrose, intakes are well known. So, this study investigated the effects of central injection of ANG II on sucrose intake. Therefore, male Wistar rats weighing 280-300g were anesthetized with ketamine (80 mg/kg) and xylazine (7 mg/kg) and a stainless steel cannula was implanted directed to the right lateral ventricle (LV) in the animal's brain. After five days of recovery (water and food ad libitum), 2% sucrose solution was offered to the rats (2h/day: from 2:00 p.m. to 4:00 p.m.). Water and sucrose intakes were measured. The experiments began when the volume ingested per rat during this 2 h seems to be more constant (approximately 15 days). After this period, food was removed and the rats received LV injection of 1 µL of PBS (control) or ANG II (400 ng/µL). After fifteen minutes, they had free access to two graduated burettes containing water and 2% sucrose solution. Water and 2% sucrose intakes were measured at 15, 30, 60, 90 and 120 minutes. The results are expressed as means±SEM. Two way RMANOVA and pos test

Dunnett's were used for statistical analyses. Differences were considered significant at $p<0.05$. ANG II injected into LV increased sucrose intake compared to control group (ANG II 18.2 ± 4.7 vs PBS 9.3 ± 4.2 mL/120min). No significant differences between groups were observed to water intake (ANG II 0.2 ± 0.0 vs PBS 0.2 ± 0.1 mL/120min). The results shows that, when water and sucrose were available, it was not observed the dipsogenic effect of ANG II, but a facilitation on sucrose intake.

Disclosures: **M.H. Paes:** None. **L.M. Cardoso:** None. **L.B. Oliveira:** None.

Poster

164. Thirst and Water Balance

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 164.15/T10

Topic: E.06. Thirst and Water Balance

Title: Characteristics of a consciousness disturbance in water intoxication in patients with psychoses

Authors: J. NAGAI[1], X. CAO[1], M. TAJITSU[1], A. FUKUI[1], T. YAMADA[1], Y. YAMBE[1], T. MURASE[1], *Y. SUGIMURA[2];
[1]Endocrinol. and Diabetes, Nagoya Med. Ctr., Nagoya, Japan; [2]Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan

Abstract: Objective: Water intoxication is a type of hyponatremia that frequently occurs in patients with psychoses, the characteristics of which were the focus of the current study. Materials and Methods: Data were retrospectively collected from the medical records of 13 patients with psychoses who developed hyponatremia due to self-induced polydipsia and visited the emergency department of a medical center in Japan between 2009 and 2012. The background of the patients, mental status, serum sodium (Na+) level, and urine output were analyzed. Results: Six of 13 patients were schizophrenic and 11 of 13 patients had taken anti-psychotic medications. The serum Na+ level on admission was 113±5 mEq/L (mean±SD). Patients were treated with hypertonic or normal saline, and the serum Na+ level rose to 129±6 mEq/L after 24 h. The mean increase in the serum Na+ level after 24 h was 15±6 mEq/L, and > 12 mEq/L in 9 patients, including 4 patients in whom the serum Na+ level increased by > 20 mEq/L. The urine output was 6010±2789 mL 24 h after admission. In 7 patients, the serum Na+ level rose to > 130 mEq/L after 24 h, but 4 of the patients had not yet recovered from the consciousness disturbance. Recovery of mental status required 5 days for 1 patient; however, all patients eventually had full recovery of mental status without neurologic complications. There was a positive correlation

between urine output and increased serum Na+ level 24 h after admission (r=0.66, P=0.027). There was no significant correlation between the serum Na+ level and the extent of consciousness disturbance on admission (r=-0.34, P=0.26). There was no apparent correlation between the serum Na+ level on admission and the time required for full recovery from the consciousness disturbance (r=-0.30, P=0.31). Conclusion: Patients with water intoxication had a prolonged consciousness disturbance, even after the serum Na+ level was corrected; however, all patients had full recovery of mental status without sequelae, thus indicating that hyponatremia itself does not cause irreversible neurologic damage. These results suggest that acute water intoxication does not lead to the osmotic demyelination syndrome, even when the Na+ level autocorrects rapidly via aquaresis.

Disclosures: J. Nagai: None. **X. Cao:** None. **M. Tajitsu:** None. **A. Fukui:** None. **T. Yamada:** None. **Y. Yambe:** None. **T. Murase:** None. **Y. Sugimura:** None.

Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Topic: E.08. Biological Rhythms and Sleep

Support: NSF Grant IOS-0920417

NIH Grant R01NS054794

Title: Developing and leveraging cell-based clock models in clock gene discovery

Authors: *C. RAMANATHAN, A. C. LIU;
Dept. Biol., Univ. of Memphis, Memphis, TN

Abstract: In mammals, circadian clocks regulate 24 hr rhythms in most aspects of physiological and behavioral processes. The circadian clocks are present in nearly all cells in the body. Cellular oscillators from different tissue types in mammals share a similar transcriptional and translational negative feedback mechanism. At the molecular level, a dozen core clock genes have been identified that underlie the generation of the circadian rhythms. However, experimental evidence supports the existence of additional clock components or modifiers and revealed cell and tissue type-specific clock functions. These observations prompted us to develop various cell type-specific clock models, including 3T3 fibroblasts, 3T3-L1 adipocytes, and MMH-D3 hepatocytes. Each model has an integrated luciferase reporter that allows for

longitudinal luminescence recording of rhythmic reporter gene expression in high-throughput formats on 96- or 384-well plates using an off-the-shelf luminometer. RNAi-mediated knockdown of canonical clock components in these clock models displayed expected cell-autonomous circadian phenotypes based on their known function, as well as cell type-specific characteristics. We have used these cell-autonomous clock models to screen for clock modifiers including new genes and small molecules. We will present and discuss these recent findings in a greater detail.

Disclosures: **C. Ramanathan:** None. **A.C. Liu:** None.

Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Support: Conacyt 183078

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PROMEP DSA/103.5/14/10476

Title: Relationship between circadian disruption and cancer: interaction between clock genes and cell cycle genes

Authors: *S. CÁRDENAS ROMERO[1,2], N. SADERI[1], O. RAMÍREZ-PLASCENCIA[1], C. ESCOBAR[2], R. SALGADO-DELGADO[1];
[1]Facultad de Ciencias, Univ. Autónoma De San Luis Potosí, San Luis Potosi, Mexico; [2]Univ. Nacional Autónoma de México, México, Mexico

Abstract: The suprachiasmatic nucleus of the hypothalamus (SCN) provides temporal order to the brain and the periphery, maintaining the oscillating structures synchronized to the light dark cycle. Several epidemiologic studies suggest that people exposed to Circadian Desynchronization (CD) due to shift-work, nocturnal work, light pollution or even jet lag, are more prone to develop certain diseases, some of which have an important inflammatory component and cancer. It has been proposed that these pathologic conditions are due to the uncoupling of the peripheral oscillators with the SCN. However, the mechanisms that link CD to

inflammation and cancer are not known, triggering the question: is there any alteration of clock genes (such as Per2 and Clock) and: a) the cell cycle genes involved in cancer development (such as c-Myc, Cyclin E, p53 and p-21?); b) the production of pro-inflammatory cytokines that are markers for cancer, such as IL-6?. To answer our question, we used male Wistar rats which were assigned to 4 groups: two of them were kept in a L/D photoperiod (12 hours light and 12 hours darkness), while the others two were kept in a L:L photoperiod (24 hours light). One of LD groups and one of the L:L groups were inoculated with 8 million of glioblastoma cancer cells (xenobiotic transplant) between the omoplates. The remaining groups were injected with vehicle. Some of the rats belonging to the last two groups were injected to LPS and IL-6 levels were measured in plasma 90 minutes after the inoculation. Results indicate that CD induced by constant light promotes a tumor growth faster in comparison with rats exposed to a normal light-dark cycle. Preliminary results about the LPS-induced inflammation, show that light is associated with an exacerbated inflammatory response. These results demonstrate that constant light exposure affects clock genes, as well as cell cycle genes and IL-6. On the base of these results, we suggest that many health problems associated with CD might be due to increased inflammatory responses, including cancer.

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Poster

165. Molecular Biology and Physiology of Circadian Clocks

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CNPq

CAPES

Title: Trpv1 desensitization disrupts circadian rhythm of clock gene expression in the suprachiasmatic nucleus and peripheral tissues

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Abstract: The search for new pain management/treatment drugs leads to the discovery that TRPV1 blockade increases the internal temperature as a side effect, giving raise to the hypothesis of TRPV1 role on thermoregulation. Circadian changes of internal temperature have been proposed as universal synchronizer of the peripheral clocks without altering the central paemaker through mechanisms that is still unclear. We investigated whether the loss of a functional TRPV1 induced by desensitization disrupts the molecular clock oscillation in peripheral tissues. Rats received an injection of 20 ug/kg body weight ip of resiniferatoxin (RTX) and after five days were euthanized at zeitgeber (ZT) 0 (time of light on), ZT6, ZT12 and ZT18. The total RNA was extracted from the liver, adrenal and microdissections of the suprachiasmatic nucleus (SCN). Relative analyses of clock genes, rPer1 and rBmal1 mRNA, were performed by qPCR. The desensitization abolished the adrenal circadian profiles of rPer1 and rBmal1 expression. This was also observed in liver only for rPer1 expression, although rBmal1 was increased at ZT0. Intriguingly, in the SCN (the central clock), TRPV1 desensitization altered the circadian profile of rBmal1 expression regardless of changes on rPer1 circadian expression. We previously observed that acute blockade of TRPV1 channels decreases corticosterone plasma levels, this may be a secondary signal from peripheral clocks to the central pacemaker, since RTX induced desensitization is restricted to the abdominal cavity. Thus, TRPV1 may mediate the temperature-induced rhythm of peripheral clocks.

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Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Topic: E.08. Biological Rhythms and Sleep

Support: NINDS Grant PO1 NS39546

Title: The purinergic receptor, P2X7, mediates cell-cell synchrony and clock gene expression rhythms among SCN astrocytes

Authors: *A. C. CAMACHO[1], D. J. EARNEST[2], M. J. ZORAN[1];
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Abstract: In mammals, physiological and behavioral rhythms are coordinated by the suprachiasmatic nuclei (SCN) of the hypothalamus. Astrocytes in rat SCN release ATP in a circadian manner. In SCN cell cultures, inhibitors of purinergic signaling disrupt ATP accumulation and clock gene expression rhythms. Further, clock-defective astrocytes are unable to accumulate ATP rhythmically, further suggesting clock-control. We have previously suggested that the ATP receptor, P2X7R, mediates cell signaling and synchronization among astrocytes. Here, we tested whether specific activation of the P2X7R alters ATP accumulation and gene expression rhythms. Application of ATP (10 nM) did not impact PER2::LUC bioluminescence rhythms in mSCN cell lines, which report expression of the clock gene, period2. In contrast, activation of P2X7R with the agonist BzATP (100 μM), a drug that induces robust increases of ATP release in mSCN astrocytes, affected rhythmic PER2::LUC expression in a time dependent way. An immediate, transient alteration in PER2::LUC reporter bioluminescence occurred at all circadian times. However, a sustained increase in rhythm amplitude was only present when BzATP was applied at the peak time of ATP accumulation. Interestingly, rat SCN astrocytes also show enhanced synchronization of ATP release following ATP treatment at this peak. The P2X7R functions in conjunction with the hemichannel protein, Pannexin1 (PANX1). We used immunocytochemistry of P2X7 and PANX1 proteins in mSCN astrocytes to determine expression across circadian time. Immunostaining of both proteins was rhythmic and peaked at times consistent with maximal ATP release. Thus, ATP signaling through the P2X7 purinergic receptor regulates both ATP release and clock gene expression rhythms in SCN astrocytes, suggesting a mechanism of cell synchrony that is both mediated by this purinergic signaling and under robust clock regulation.

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Poster

165. Molecular Biology and Physiology of Circadian Clocks

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MCubed

Title: A role for Krüppel-like factor 9 in the synchronization of peripheral circadian clocks by adrenal steroids

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Abstract: In mammals, cellular circadian rhythms are maintained in part by a core transcription-translation feedback loop driven by the CLOCK/BMAL1 transcription factors. While this loop runs independently of endocrine influences in the central neural pacemaker (the suprachiasmatic nucleus of the hypothalamus [SCN]), in other parts of the nervous system and in peripheral organs it can be reset by glucocorticoids (GC). The core clock genes Per1 and Per2 are directly and rapidly upregulated by GC via direct GC receptor (GR) action. This results in resetting of circadian rhythms, and has been hypothesized to be important for synchronizing circadian rhythms in extra-SCN tissues. The zinc-finger transcription factor Krüppel-like factor 9 (Klf9), an evolutionarily conserved immediate-early GR target gene, shows circadian expression in mouse liver and human keratinocytes. To identify its genomic targets in the hippocampus we conducted RNA-seq and chromatin immunoprecipitation (ChIP) followed by deep sequencing in a mouse hippocampal cell line (HT22). We found that Klf9 acted predominantly as a repressor, with 75% of differentially regulated genes showing reduced expression. In addition, we found that Klf9 associated with several circadian genes, including Per1-3, Dbp, Tef, and Nr1d1. We therefore tested the hypothesis that Klf9 acts as a feed-forward repressor of circadian clock gene expression. Forced expression of Klf9 in HT22 cells delayed GC induction of Per1, suggesting that Klf9 may act as a feed-forward brake on Per1 induction, which we hypothesize is necessary for resetting the clock under conditions of elevated GC. Forced Klf9 expression also repressed the clock output gene Dbp. ChIP assays on HT22 cells synchronized with GC showed association of Klf9 with a Dbp intronic enhancer at a time point that coincided with high Klf9 and low Dbp mRNA, consistent with rhythmic repression of this locus by Klf9. Furthermore, this region has an evolutionarily conserved Klf binding site in close proximity to a conserved CLOCK response element, suggesting that Klf9 and CLOCK/BMAL1 may interact at this locus. Klf9 also showed rhythmic association with the promoter of the circadian cell cycle regulator Wee1 in HT22 cells, suggesting a link between GC, circadian rhythms and cell proliferation. ChIP assays showed that Klf9 associated with the Klf9 binding sites discovered in HT22 cells in mouse hippocampus *in vivo*. We are now investigating whether Klf9 mutant mice display altered circadian gene expression, behavioral rhythms, and responses to stressors. Our findings support that Klf9 is a novel link between the hypothalamo-pituitary-adrenal axis and circadian rhythms.

Disclosures: J.R. Knoedler: None. **R.J. Denver:** None.

Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Title: The circadian modulation of physiology in the praying mantis: a novel insect model system

Authors: *A. E. SCHIRMER, F. R. PRETE, E. S. MANTES, W. BOGUE, A. F. URDIALES, S. A. PATEL, C. CARRION, G. M. PRETE, V. M. SKITAL;
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Abstract: Physiological processes function synergistically as a constellation of integrated, nested systems. Natural selection has shaped these systems such that they establish a dynamic equilibrium giving rise to emergent properties defining life. Understanding these relationships requires studying physiological systems across levels of analysis in sufficiently complex (but tractable) model systems. We have done so successfully using circadian rhythms and the praying mantis model system. Circadian rhythms and their effects on the behavior and physiology of insects have been well described in many common model systems. The influences of these clocks range from the modulation of whole organism behavior to the gating of ion channels in the interneurons of the brain; however, historically little has been done using the praying mantis model system. We used a multilevel experimental approach to determine to what extent circadian rhythms modulate several key physiological and behavioral parameters in the praying mantis, *Hierodula patellifera*. The experiments included chronic electroretinograms (ERG) to assess compound eye sensitivity, photographic colorimetric analyses of changes in compound eye color resulting from the migration of shielding pigments, assessment of the differences between responsiveness to prey-like, computer generated visual stimuli during periods of maximum vs. minimum compound eye sensitivity, and evaluation of temporal changes in gross locomotor

activity on a modified treadmill apparatus. Our results clearly indicate that circadian clocks modulate the target behaviors across all levels of our analyses. Further, when overlaid, graphs depicting all of these parameters peaked early in the dark or subjective dark phases of the light/dark cycle suggesting their functional synchrony. Recently, we have collected data on the patterning of respiratory related abdominal compressions in both restrained and unrestrained mantises. These data, too, suggest that respiratory patterns are synchronous with rest/activity cycles. Taken together, our data strongly suggest the existence of complex interactions between circadian clocks operating at the cellular, cellular systems, and organismal levels in this mantis. These results are an intriguing first step toward revealing the mechanisms that modulate praying mantis physiology and add a unique model system that can be utilized to better understand the organization of circadian systems in general.

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Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Topic: E.08. Biological Rhythms and Sleep

Support: MH75968

Title: Knockdown of prefrontal cortex period1/period2 gene expression impairs diurnal-dependent conditioned fear extinction learning

Authors: *L. R. WOODRUFF[1], L. E. CHUN[2], N. M. VARRA[2], L. R. HINDS[3], B. GREENWOOD[4], C. MCCLUNG[5], R. L. SPENCER[3];
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Abstract: Circadian rhythms are highly conserved 24h fluctuations in physiology and behavior. Optimal organismal health relies on the integrity of this system. Anxiety disorders such as post-traumatic stress disorder (PTSD) are often associated with impaired circadian functioning as well as poor conditioned fear extinction learning. We have previously shown that conditioned fear extinction but not conditioned fear acquisition is modulated by both time of day and the presence

of endogenous glucocorticoids (CORT) in adult male rats. Here we examined the relationship between auditory conditioned fear extinction learning, circadian phase, and prefrontal cortex (PFC) core clock gene (Per1/2) expression. Rats maintained on a 12h light:dark cycle were trained and tested across 4 sessions (conditioned fear acquisition, extinction, extinction recall, and fear renewal) that were administered either during the rats' active (zeitgeber time 16_ZT16) or inactive (ZT4) circadian phase. One week prior to testing rats received a microinjection in the infralimbic region of the PFC of either adenoassociated virus (AAV) containing a PER1/2-specific shRNA (PER1/2 KD) construct or a scrambled sequence shRNA (SCR). Neither PER1/2 knock down nor time of day had any effect on conditioned fear acquisition. However, rats trained and tested at ZT16 extinguished conditioned fear faster than those trained and tested at ZT4, regardless of PER1/2 status. Interestingly, SCR ZT16 rats showed superior extinction recall than SCR ZT4 rats, but this superior extinction recall was absent in PER1/2 KD ZT16 rats. In addition, PER1/2 KD rats trained and tested at ZT4 exhibited high freezing levels throughout most of the extinction recall session, suggesting impaired additional extinction learning compared to the other treatment groups. During session 4 (fear renewal) PER1/2 KD ZT4 rats showed much higher freezing and thus greater fear renewal compared to all other groups of rats suggesting an inability of these rats to generalize extinction to a novel context. In general, optimal conditioned fear extinction depends on the time of training/testing as well as normal clock gene expression within the infralimbic PFC. These results emphasize the importance of incorporating a consideration of circadian effects in fear conditioning research and psychotherapy for anxiety related disorders like PTSD.

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Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Topic: E.08. Biological Rhythms and Sleep

Support: BGSU Building Strength Grant

Title: Circadian clock genes are needed for normal adult neurogenesis

Authors: A. MALIK[1], R. V. KONDRATOV[2], R. J. JAMASBI[1], *M. E. GEUSZ[1];
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Abstract: Adult neurogenesis depends on regulatory signals originating from intracellular and extracellular events. Although daily cycles of neurogenesis have been described in the dentate gyrus (DG) of the hippocampus, the role of timing signals from endogenous circadian oscillator cells during neurogenesis and their importance in learning or memory remain unclear, partly because circadian clocks outside the DG modulate hippocampal functions. Nevertheless, circadian rhythms persist in isolated cultures of hippocampal tissue. To provide a simpler preparation in which circadian oscillators can be examined in greater isolation, neurosphere cultures were prepared from the DG of knockout mice that lack a functional circadian clock and from mPer1::luc mice to identify circadian oscillations. Neurospheres were also prepared from the subventricular zone, another adult stem cell-rich region. Circadian bioluminescence rhythms were recorded in neurospheres maintained in a culture medium that induces neurogenesis but not in one that maintains the stem cell state. Although the differentiating neural stem progenitor cells (NSPCs) of spheres were rhythmic, mature neurons were extremely sparse. Because they are clearly the most abundant cells it is likely that NSPCs are functional circadian clocks generating the bioluminescence rhythm. This conclusion was also supported by immunocytochemistry for mPER1 protein that was localized to the inner, more stem cell-like neurosphere core. Neurospheres from BMAL1 knockout mice displayed unusually high differentiation into glia rather than neurons according to GFAP and NeuN expression, respectively, and very few BetaIII tubulin-positive, immature neurons were observed. The knockout neurospheres also displayed more cell death than wild-type. Neurospheres from mice lacking Cry1 and Cry2 showed significantly reduced growth. This study provides insight into how the circadian clock could be used for gating stem cell proliferation to treat neurodegenerative disorders or impaired brain functions.

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Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH grant MH75968

Title: Characterization of diurnal core clock gene expression in forebrain glucocorticoid receptor knockout mice brains

Authors: *S. J. MORTON[1], L. E. CHUN[1], L. JACOBSON[2], L. R. HINDS[1], L. R. WOODRUFF[1], R. L. SPENCER[1];
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Abstract: Circadian rhythms are maintained through the self-regulatory, oscillatory molecular clock, which includes the core clock genes Per1, Per2, and Bmal1. Disruptions to clock gene expression have been associated with numerous mood and behavior disorders including major depression, anxiety disorders, and bipolar disorders. The molecular clock has been well-characterized in the suprachiasmatic nucleus (SCN), the master clock of the body. Many peripheral tissues and extra-SCN brain regions have also been shown to have circadian rhythms in these core clock genes, but considering that the SCN has few direct projections to these brain and body regions, the question remains how the SCN communicates to extra-SCN molecular clocks. Glucocorticoids (CORT) are a potential candidate by which the SCN signals to other brain and body regions as glucocorticoid receptors (GR) are found ubiquitously throughout the brain and body, with the notable exception of the SCN. Furthermore, CORT is released in a diurnal manner, with peak plasma levels occurring immediately upon the animal's active phase. Interestingly, there is a glucocorticoid response element (GRE) in the promoter regions of the Per1 and Per2 genes, which may be a mechanism by which CORT can induce Per1 and Per2 expression, and thereby entrain the molecular clock. We compared clock gene expression in mice that had a conditional forebrain glucocorticoid receptor knockout (FBGRKO) to GR floxed mice to determine the necessity of GRs in diurnal core clock gene expression. FBGRKO (C57BL/6 pure strain of the T29-1 founder line containing Cre+ recombinase transgene) mice have been previously well-characterized to have disruptions in GR expression in the forebrain including the hippocampus, cortex, and nucleus accumbens, while the central nucleus of the amygdala (CEA) had a 50% deletion and the paraventricular nucleus (PVN) was not affected. Mice were sacrificed under basal conditions in the light phase (AM) or dark phase (PM). *In situ* hybridization was used to measure mRNA. Our results show there is a time of day difference in the SCN and PVN for Bma1 and Per1 mRNA expression as well as for Bmal1 mRNA expression in the CA3 subregion of the hippocampus. There was no genotype difference for these brain regions. These results are expected in the SCN and PVN, as hypothalamic GRs would not be affected by the FBGRKO. The lack of evident FBGRKO effect in the CA3 may be due to the fact that GRs are not necessary for diurnal clock gene expression in this tissue. Examination of additional forebrain regions, including the prefrontal cortex, will be examined, as well as examination of Per2 mRNA expression in these mice.

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Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Topic: E.08. Biological Rhythms and Sleep

Support: R01 NS072337

F32 NS084582-01A1

Title: Circadian modulation of behavioral aggression

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Abstract: While the suprachiasmatic nucleus (SCN) is acknowledged to be the master biological clock, its influence on physiology and behavior is largely mediated via relays in the adjacent subparaventricular zone (SPZ). Most SPZ neurons are GABAergic, and the targets of the SPZ are known (Vujovic et al., JCN, in press). But the relays for specific biological functions remain poorly understood. We recently found a major input from the SPZ to the ventromedial nucleus of the hypothalamus (VMH), which has appositions onto estrogen receptor alpha (ERα) expressing neurons in the ventrolateral VMH. These VMHvl neurons have been implicated in promoting aggressive behavior by male mice. As inappropriate aggressive behavior plays an important role in psychiatric disorders, dementia, and a variety of social situations, it would be important to understand how aggression may be regulated by the circadian timing system. Here we tested the hypothesis that GABAergic transmission from the SPZ onto VMH neurons influences emotional state and gates the timing and scale of behavioral aggression across the 24-h day. Using the Cre-lox system and viral vector injections in transgenic mice, we genetically targeted and deleted VGAT, the vesicular GABA transporter, in the SPZ. This allows for the selective silencing of GABAergic transmission from these neurons *in vivo*. We then assessed levels of aggressive behavior in these mice using the resident intruder paradigm at four different time points: zeitgeber time (ZT)1, ZT7, ZT13, and ZT19 in mice on a 12:12 light-dark cycle (lights on at ZT0). We found that there is a peak of aggressiveness in male mice at ZT13, the early part of their (dark) active phase. VGAT-deletion in the SPZ substantially altered the daily pattern and overall level of aggressive behavior by increasing aggression throughout the day to levels near the peak in control mice. We are currently assessing corticosterone and sleep-wake rhythms in these mice as well, as alterations in these rhythms might also affect aggressiveness. Our data thus far suggest that the circadian timing system actively inhibits aggressiveness of male mice at

certain times of day. Manipulation of this pathway may hold promise for regulating aggressive behaviors.

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Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: MH75968

Title: Diurnal variations in rhythmic clock gene expression across brain regions important for emotional control of male and female rats

Authors: ***L. E. CHUN**, L. R. WOODRUFF, S. MORTON, L. R. HINDS, R. L. SPENCER; Psychology & Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: The molecular clock consists of a counter-regulatory transcription/translation cycle of positive (bmal1, clock/npas2) and negative (per1/per2, cry1/cry2) components, whose oscillatory nature consists of a 24-hour period. The molecular clock has been well-characterized in the body's master clock, the hypothalamic suprachiasmtic nucleus (SCN). Surprisingly, a limited number of studies have examined clock genes of both the positive and negative components in extra-SCN tissue. Furthermore, there has yet to be a direct comparison of basal clock gene expression in extra-SCN brain regions between female and male rodents. This comparison is warranted, as there are sex differences in circadian rhythms, as well as a greater prevalence of mood disorders associated with disruptions in clock gene expression (e.g., depression, anxiety, post-traumatic stress disorder). This study examined in male and female rats basal clock gene mRNA expression (*in situ* hybridization) of both the positive (bmal1) and negative (per1, per2) components of the molecular clock in brain regions important in emotional regulation (e.g., prefrontal cortex, hippocampus, amygdala), as well as the SCN and the hypothalamic paraventricular nucleus (PVN), the head of the HPA axis. Clock genes were examined at 4-h intervals across a 12:12h light:dark cycle. There was a significant rhythm of bmal1, per1, and per2 mRNA in the SCN, PVN, PFC, subregions of the hippocampus, and the amygdala with a 24-h period (two-way ANOVA, cosinor analysis, $p < 0.05$). Importantly, there were three distinct profiles of rhythmic clock gene mRNA acrophase relationship across the brain regions, suggesting diversity amongst molecular clocks. Furthermore, while the clock gene expression

profiles were generally similar between males and females, there were a few instances where the robustness of clock gene expression differed between the sexes (e.g., females had less robust per1 and per2 mRNA rhythms in the medial PFC, but more robust bmal1 mRNA rhythms in the CA1 and CA3 hippocampal subregions). There was also a general trend for females to be phase-delayed in nearly all brain regions for all clock genes compared to males. Additionally, females with a regular estrous cycle had altered bmal1 mRNA rhythms in the PFC compared to females that were not cycling. There results indicate that oscillatory clock gene expression is widespread throughout forebrain regions involved in emotional control, and that gonadal hormones may modulate these expression patterns.

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Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Topic: E.08. Biological Rhythms and Sleep

Support: Pritzker Neuropsychiatric Disorders Research Fund L.L.C.

Title: Clock gene rhythm disruption: comparison of major depressive disorder (mdd), bipolar (bp), and schizophrenia

Authors: *M. H. HAGENAUER[1], J. Z. LI[2], B. BUNNEY[4], D. WALSH[4], C. TURNER[3], D. ABSHER[5], F. MENG[3], M. VAWTER[4], S. EVANS[3], J. D. BARCHAS[6], A. F. SCHATZBERG[7], R. M. MYERS[5], W. BUNNEY[4], S. WATSON[3], H. AKIL[3];
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Abstract: One of the cardinal characteristics of many mood disorders, including Major Depressive Disorder (MDD) and Bipolar Disorder (BP), is the disruption of daily rhythms in sleep and activity. Recently we discovered that a similar disruption occurs in daily rhythms of clock gene expression in the brains of individuals with MDD. To perform this analysis, we organized microarray data from high-quality post-mortem brain tissue by the time of death of the subjects. As a group, the clock gene data from non-psychiatric control subjects showed a clear daily rhythm, and the molecular “time stamp” for any particular individual matched the time that

the individual had died. In contrast, as a group the clock gene data from MDD subjects had either a dampened or non-existent rhythm, and the molecular "time stamp" for MDD individuals differed widely from the time that they had died, suggesting that the MDD subjects were internally "jet lagged" from the rest of the population. We have now extended these analyses to include individuals with BP and Schizophrenia. As a group, we find that BP subjects exhibit disrupted clock gene rhythms in the brain similar to MDD subjects, whereas the disruptions present in Schizophrenia are not as severe. We also find that these patterns hold true even after controlling for other potential confounding factors, including age and gender. This work indicates that psychiatric interventions to improve patient sleep are likely to be enhanced if they simultaneously address the presence of severe circadian disturbance.

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Poster

165. Molecular Biology and Physiology of Circadian Clocks

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Topic: E.08. Biological Rhythms and Sleep

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FRQS award 22210

Title: Transcriptional regulation of Neuroligin-1 by core clock transcription factors

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Abstract: Introduction: Neuroligin-1 (NLGN1) is a postsynaptic adhesion molecule regulating glutamatergic transmission and sleep. NLGN1 can be expressed in different isoforms since it can contain inserts in two splice sites (A and B). We have shown that sleep deprivation decreases

Nlgn1 expression, in particular that of the Nlgn1 transcript variant containing an insert in splice site B, and the binding of core clock transcription factors to the Nlgn1 gene. Here, we verified the contribution of core clock transcription factors to Nlgn1 expression by measuring Nlgn1 expression in Clock mutant mice and the transcriptional regulation of Nlgn1 by CLOCK/BMAL1 *in vitro*. The contribution of GSK3beta, a regulator of the clock molecular machinery, to Nlgn1 expression was also assessed. Methods: Expression of four Nlgn1 transcript variants (e.g., with insert in splice site A, without insert in splice site A, with insert in splice site B, without insert in splice site B) was measured by quantitative PCR in the forebrain of ClockΔ19/Δ19 mutant and wild-type (WT) mice, sacrificed every 6h starting at ZT2 (2h after light onset). NLGN1 protein level was also measured by Western blot at the same 4 times in Clock mutant mice. To assess the control of Nlgn1 transcription by CLOCK/BMAL1 and GSK3beta, COS-7 cells were transfected with a luciferase reporter plasmid containing Nlgn1 E-boxes. Luciferase activity was measured and normalized to β-galactosidase activity and protein level. Results: The rhythmic expression of Nlgn1 transcripts with and without insert in splice site B was inverted in Clock mutant mice, and the overall expression of the two other variants (with and without insert in splice site A) was decreased compared to WT. Daily rhythm in NLGN1 forebrain protein level was also altered in Clock mutant mice. Luciferase assays revealed that CLOCK/BMAL1 activate transcription driven by the Nlgn1 promoter (4-fold), and this activation was reduced by GSK3beta. Conclusion: Our results suggest that the sleep/wake-dependent regulation of Nlgn1 expression is clock-controlled. The specific E-boxes involved in Nlgn1 transcriptional regulation by CLOCK/BMAL1 are being characterized using site-directed mutagenesis.

Disclosures: V. Mongrain: None. **E. Bélanger-Nelson:** None. **J. Beaulieu:** None. **N. Cermakian:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 166.01/U4

Topic: E.08. Biological Rhythms and Sleep

Support: CRSNG

FRQS

Title: The characterization of sleep in mutant mice for EphA4 reveals its implication in the circadian regulation of sleep

Authors: *M. FREYBURGER[1,2], A. PIERRE[1], E. BÉLANGER-NELSON[1], V. MONGRAIN[1,2];
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Abstract: Introduction: Sleep is essential for the maintenance of organism homeostasis in mammals and many non-mammalian species. The recovery aspect of sleep seems to be linked to mechanisms controlling synaptic strength. Synapses are finely regulated by the interaction of complex pre- and postsynaptic machineries where adhesion molecules have an important regulatory role. The EphA4 receptor is an adhesion molecule that has been implicated in the regulation of synaptic function and plasticity. The aim of the study is to understand the role of EphA4 in sleep regulation and the impact of sleep deprivation [SD] on its expression. Methodology: 1) Vigilance states (wakefulness, non-rapid eye movement [REM] and REM sleep) duration was measured using electroencephalography [EEG] in mice lacking EphA4 (provided by K. Murai and bred on site). Vigilance states and spectral analysis were computed during 24h baseline and after a 6 hour SD. Ten-week-old mice from the three genotypes (n=16 wild-type [WT], 15 heterozygous [HET] and 13 homozygous mutants [KO]) were implanted with EEG electrodes. EEG was recorded during baseline [BL] and 18h of recovery sleep after 6h SD. 2) Mice from 3 genotypes (wild-type, heterozygous and homozygous EphA4 KO mice) were submitted to SD followed by qPCR and microarray measurements of gene expression in the forebrain. 3) The expression of EphA4 and its partners (EphB2, EfnB2, EfnA3, EfnA3) was measured by quantitative PCR [qPCR] after a 6h SD in 3 different mouse brain regions (cortex [CTX], hippocampus [HP], and a thalamic/hypothalamic [TH/H] region). Results: 1) During the 24h baseline, the duration of wakefulness and non-REM sleep was similar in KO, HET and WT mice. However, KO mice showed a significant reduction in REM sleep duration compared to WT especially during the light period. Spectral analysis during BL reveal a genotype-by-time interaction effect on the non-REM sleep sigma band, and specially in 10-11Hz and 11-12 Hz bins which are related to circadian regulation of sleep, but no difference in the delta band. Preliminary analyses indicate that the EEG response to SD is similar in KO and WT mice. 2) The absence of EphA4 did not significantly impact on SD-dependent increase in Bdnf, Per2, Homer1A, Fos and Arc, but alters the expression of an enzyme involves in epigenetic regulation. 3) The mRNA expression of EphA4 and its partners was not changed by SD in the CTX and in the HP. However, SD significantly increased EphA4 mRNA in the TH/H region. Discussion: These results suggest that EphA4 acts to favor REM sleep and that it could be involved in the circadian regulation of sleep.

Disclosures: M. Freyburger: None. **A. Pierre:** None. **E. Bélanger-Nelson:** None. **V. Mongrain:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Title: Withdrawal from chronic intermittent exposure to ethanol is associated with insomnia in a mouse model of alcohol dependence

Authors: *C. A. BLANCO-CENTURION[1], L. RALSTON[2], M. F. LOPEZ[2], H. BECKER[2], J. WOODWARD[2], P. SHIROMANI[1,3];
[2]Charleston Alcohol Res. Ctr., [1]Med. Univ. of South Carolina, Mount Pleasant, SC; [3]Ralph Johnson VAMC, Charleston, SC

Abstract: The mechanisms behind alcohol-induced insomnia are poorly understood and there is little empirical evidence whether commonly used animal models of alcohol dependence faithfully reproduce the sleep disturbances reported by human alcoholics. The rodent model of Chronic Intermittent Ethanol Exposure (CIEE) produces an escalation in alcohol consumption and withdrawal symptoms that are hallmarks of alcohol dependency in humans. Data from previous animal studies also show that a brief exposure to CIEE can lead to insomnia yet it is unknown whether longer periods of CIEE would also cause protracted insomnia. Longer period of exposure to alcohol exposure is particularly important because in humans alcohol dependence takes years to fully manifest including its effect on sleep. The goal of the present study was to study the time course of sleep/wake behaviors before, during and after repeated cycles of chronic ethanol exposure in mice. In five adult male C57BL WT mice sleep was recorded for a week and then the mice underwent four weekly cycles of CIEE. In each CIEE cycle mice were exposed to ethanol vapors for 16 hr/day for four days. Blood ethanol concentrations (BEC) were measured once in every cycle (day 3). Sleep was continuously recorded across all four cycles of CIEE including the short withdrawals and for at least two months following the last CIEE cycle. BECs averaged around 177 mg/dL across the four cycles of CIEE. During CIEE, sleep was significantly increased during the night and day. However, as early as the first short withdrawal period, mice had insomnia (34% more wake; $P<0.017$) during the lights-on phase (rest period). The insomnia was still present two weeks after the end of the last CIEE cycle (+29%; $P<0.025$). Our results indicate that withdrawal from CIEE quickly produces insomnia that remains many

days after cessation of exposure to alcohol. It supports the use of the CIEE model to identify the mechanisms responsible for the sleep disturbance associated with alcohol dependence. Because insomnia during the withdrawal from alcohol dependence is a significant risk factor for relapse, pharmacological agents that can safely alleviate the insomnia are urgently needed.

Disclosures: C.A. Blanco-Centurion: None. **L. Ralston:** None. **M.F. Lopez:** None. **H. Becker:** None. **J. Woodward:** None. **P. Shiromani:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Program#/Poster#: 166.03/U6

Topic: E.08. Biological Rhythms and Sleep

Support: NIH R01 NS20246

NIH P20 GM110702

Title: Intracellular mechanisms modulating gamma band activity in the pedunculopontine nucleus

Authors: *B. R. LUSTER[1], F. J. URBANO[2], E. GARCIA-RILL[1];
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Abstract: The pedunculopontine nucleus (PPN) is a component of the reticular activating system, and is most active during both waking and REM (rapid eye movement) sleep. During waking, our cognitive function is driven by high frequency beta/gamma band activity. However, REM sleep manifests similar high frequency on the cortical EEG. We are interested in determining the differences between gamma activity in waking vs REM sleep. We showed that every cell in the PPN plateaus at beta/gamma band frequencies when depolarized. Moreover, this high frequency activity is mediated by high threshold, voltage-dependent N- and P/Q-type calcium channels. We discovered that the PPN contains cell populations which can manifest gamma band frequencies through only N-type, only P/Q-type, or both N- and P/Q-type calcium channels. Other studies suggest that N-type calcium channels are modulated by the cAMP/PK pathway, which also modulates REM sleep. This study was designed to determine the intracellular mechanisms subserving cells with N-type calcium channels in the PPN. Electrical responses were recorded using whole cell patch clamp electrodes on 11-17 day old sagittal rat

brain slices. Intrinsic membrane properties of cells were recorded at 37°C perfused with oxygenated aCSF in an immersion chamber containing the synaptic blockers (SB) gabazine (GABAA antagonist), strychnine (glycine antagonist), CNQX (AMPA/Kainate receptor antagonist), and APV (NMDA receptor antagonist), and also tetrodotoxin (TTX) to block sodium channels. We found that all rat PPN cells (n=13) showed beta/gamma oscillations in the presence of SB+TTX when the membrane potential was depolarized using current ramps. PPN neurons showed beta/gamma oscillations when depolarized above -30 mV, suggesting that their origin may be spatially located beyond voltage-clamp control. In a group of cells tested (n=7), the cAMP/PK inhibitor H-89 along with the P/Q-type calcium channel blocker ω-Agatoxin-IVA combined to inhibit the presence of gamma oscillations (assumed to be N+P/Q cells) ($df=13$, $F=7.04$, $p<0.05$). In another set of cells (n=4), the presence of H-89 had no effect on gamma oscillations while ω-Agatoxin-IVA completely blocked them (assumed to be P/Q-only cells) ($df=7$, $F=0.34$, $p>0.05$). In a final set of cells (n=2), H-89 completely blocked the presence of gamma oscillations while ω-Agatoxin-IVA had no effect on them (assumed to be N-only cells). These results suggest that cells in the PPN that manifest gamma band activity through N-type calcium channels are modulated by the cAMP/PK pathway. We hypothesize that N-only cells are equivalent to "REM-on" cells *in vivo*.

Disclosures: B.R. Luster: None. **F.J. Urbano:** None. **E. Garcia-Rill:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Program#/Poster#: 166.04/U7

Topic: E.08. Biological Rhythms and Sleep

Support: NIMH RO1 MH099231

NINDS P01N5083514

Title: Sleep contributes to double-strand DNA repair in *Drosophila* melanogaster mushroom body cells

Authors: *D. B. BUSHEY[1], G. TONONI[2], C. CIRELLI[2];
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Abstract: It was recently found that double-strand DNA breaks (DSB) are induced in young adult mice during natural exploratory behavior, an effect that appears to be mediated by an increase in neuronal activity and reverts the following day (1). In recent experiments we confirmed that exploration induced DSB in mouse cortex, and found that their repair occurred if extended wake with exploration was followed by sleep but not by wake (2). Since neuronal activity increases during wake relative to sleep also in flies, here we tested whether DSB frequency is also dependent on wake time in *Drosophila* melanogaster. We used an antibody targeting phosphorylated H2AV, which accrues at DSB, and measured with confocal microscopy the DSB frequency in the mushroom body cells of flies that were mostly asleep or awake (sleep deprived) during the 12h of the dark period, when flies are usually asleep. In flies that were sleep deprived H2AV foci tended to increase compared to flies that slept over the same time period, suggesting that being awake per se can increase the occurrence of DSB, consistent with the results in mice. In a second experiment, we gamma irradiated flies to induce DSB and then tested whether sleep contributed to DSB repair. As expected, 40 min after irradiation H2AV foci were elevated in the mushroom body cells. H2AV levels returned to baseline levels in flies that were allowed to sleep during the 18 h post-irradiation, but tended to remain elevated in flies that were sleep deprived over the same time period. These results suggest that sleep deprivation impairs the DSB repair pathway.

Disclosures: **D.B. Bushey:** None. **G. Tononi:** None. **C. Cirelli:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: 1R01MH099231

Title: Effects of sleep and wake on astrocytes

Authors: *M. BELLESI, L. DE VIVO, G. TONONI, C. CIRELLI;
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Abstract: Astrocytes can mediate neurovascular coupling, modulate neuronal excitability, and promote synaptic maturation and remodeling. All these functions are likely to be modulated by the sleep/wake cycle, because brain metabolism, neuronal activity and synaptic turnover change as a function of behavioral state. Yet, little is known about the effects of sleep and wake on

astrocytes. Here we show that sleep and wake strongly affect both astrocytic gene expression and ultrastructure in the mouse brain. Using translating ribosome affinity purification technology and microarrays (Affymetrix microarray chips; mouse 430 2.0) we find that 1.4% of all astrocytic transcripts in forebrain are state-dependent (3 groups, 6h of sleep during the day (S), 6h of spontaneous wake at night (W), and 4h of sleep deprivation (SD); 6 mice/group). Relative to W and SD, S upregulates few select genes, like Cirp and Uba1, whereas both W and SD upregulate many genes related to metabolism (Adcy10, Ppp1r3c), extracellular matrix (Sdc4, Hpse) and cytoskeleton (Trio, Gem, Synj2). Using serial block-face scanning electron microscopy (4 groups, sleep, wake, 4h sleep deprivation, 5 days of chronic sleep restriction; 3 mice/group) and tridimensional reconstruction of the ultrastructural neuropil of mouse frontal cortex (>100 spines/mouse) we then find that a few hours of wake are sufficient to bring astrocytic processes closer to the synaptic cleft, while chronic sleep restriction also extends the overall astrocytic coverage of the synapse, including at the axon/spine interface, and increases the available astrocytic surface in the neuropil. Wake-related changes likely reflect increased need for glutamate clearance, and are consistent with an overall increase in synaptic strength when sleep is prevented. The reduced astrocytic coverage during sleep, instead, may favor glutamate spillover, thus promoting neuronal synchronization during NREM sleep.

Disclosures: **M. Bellesi:** None. **L. de Vivo:** None. **G. Tononi:** None. **C. Cirelli:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Program#/Poster#: 166.06/U9

Topic: E.08. Biological Rhythms and Sleep

Title: Effects of ghrelin on hypothalamic tuberomammillary nucleus neurons in rats

Authors: D. SHIMA, Y. WAKABAYASHI, *J. KIM, K. NAKAJIMA;
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Abstract: Ghrelin is produced by stomach, other peripheral organs and brain, and acts on GH secretagogue receptors (GHS-Rs) which express in the some peripheral organs and also in some brain regions. Ghrelin has been known as a potent stimulator of growth hormone (GH) secretion and feeding. Recent study also indicates that ghrelin contributes to regulation of sleep-wakefulness, but the mechanisms are not known. Tuberomammillary nucleus (TMN), which involves histaminergic neurons that participates in induction and maintenance of arousal state, also expresses GHS-Rs. However, direct effects of ghrelin on TMN neurons are remained

unclear. Thus, we examined effects of ghrelin on TMN neurons using rat brain slice preparations and whole-cell patch clamp recording technique. Application of ghrelin depolarized TMN neurons in both absence and presence of tetrodotoxin, and the depolarization was blocked by [D-Lys3]-GHRP-6, a specific antagonist for GHS-Rs. The ghrelin-induced depolarization was accompanied by increase of membrane resistance, and decreased under high-K+ extracellular solution and/or presence of KB-R7943, an inhibitor of Na+/Ca2+ Exchanger (NCX). Furthermore, following histochemical study on the recorded neurons demonstrated that more than half of histaminergic neurons were depolarized by ghrelin. These results suggest that ghrelin depolarizes TMN histaminergic neurons postsynaptically via GHS-Rs with a dual ionic mechanism including a decrease in K+ conductance and an activation of NCX, and may contribute to the regulation of sleep-wakefulness via the excitatory effect on TMN neurons.

Disclosures: **D. Shima:** None. **Y. Wakabayashi:** None. **J. Kim:** None. **K. Nakajima:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

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Title: A delimited node of lateral hypothalamic GABAergic neurons promote wakefulness

Authors: *A. VENNER[1,2], C. B. SAPER[1,2], P. M. FULLER[1,2];
[1]Dept. of Neurol., Beth Israel Deaconess Med. Ctr., Boston, MA; [2]Neurol., Harvard Med. Sch., Boston, MA

Abstract: The lateral hypothalamus (LH) has long been suggested to play a role in promoting arousal. However, the neurochemical identity of the neurons responsible has remained somewhat elusive. Whilst orexin neurons have been shown to be critical for maintenance of arousal, loss of orexinergic transmission does not decrease overall daily wake quantities per se. Additionally,

melanin-concentrating hormone (MCH) neurons promote REM sleep, rather than wakefulness. We have recently reported that activation of an overlooked population of GABAergic neurons (containing the vesicular GABA transporter, VGAT) within the LH reliably induces continuous wakefulness for several hours in the mouse. It was therefore of interest to more precisely define this population anatomically and determine to what extent it is necessary for normal arousal. To define the wake-promoting LH VGAT population more precisely, we made small microinjections of AAV-hSyn-DIO-hM3Dq-mCherry in and around the LH in Vgat-IRES-cre mice. We implanted these mice for EEG/EMG recording and recorded sleep-wake following i.p. saline and clozapine-N-oxide (CNO; the ligand for hM3Dq) injections. The region of transfected neurons was detected by immunohistochemical labelling against the fusion protein, mCherry. For each mouse, this transfected area was manually projected onto a series of standard sections and correlated with the waking phenotype following CNO injection, enabling construction of a Z-score based heat map. From this heat map, we inferred a delimited node of LH VGAT neurons responsible for mediating the waking phenotype, which comprised a region lateral to the fornix, ventral to the zona incerta, medial to the optic nerve and centered at bregma -1.7. Moreover, these neurons did not co-express orexin or MCH. To determine the extent to which this delimited node of LH VGAT neurons was necessary for normal arousal, we made bilateral microinjections of AAV-hSyn-hM4Di-mCherry (hM4Di) into our defined field of LH VGAT neurons in Vgat-IRES-cre mice. Injections of CNO at 7pm resulted in an apparent reduction of c-Fos in hM4Di+ neurons, confirming that the ligand inhibited hM4Di-containing neurons. Analysis of the EEG in these mice revealed that CNO injection increased NREM sleep by ~60% over the 3-hour post injection period, concomitant with ~15% reduction in wake. Taken together with our previous finding that activation of LH VGAT neurons strongly promotes wakefulness, the reduction in wakefulness observed upon acute inhibition of these neurons suggest that this delimited node of LH VGAT neurons may constitute a previously unrecognised, yet key circuit element, of neural networks regulating arousal.

Disclosures: **A. Venner:** None. **C.B. Saper:** None. **P.M. Fuller:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: R01 NS073613

K99 MH103399

Title: The slow-wave-sleep promoting parafacial zone counteracts the wake-promoting action of both caffeine and modafinil

Authors: *C. ANACLET, P. M. FULLER;
BIDMC Harvard, Boston, MA

Abstract: We have previously reported that acute and selective activation of GABAergic parafacial zone (PZ) neurons is sufficient to produce slow wave sleep (SWS) and cortical slow wave activity (SWA), independent of the time of day. We further showed that PZ GABAergic neurons project to and inhibit the parabrachial nucleus -> basal forebrain -> cortex pathway, which is a major wake-promoting pathway, and hence may represent a circuit substrate through which GABAergic PZ neurons trigger SWS and modulate the cortical EEG. It however remains unclear if GABAergic PZ neurons are capable of modulating (inhibiting) other wake promoting pathways and circuits. To begin to explore this possibility, we employed two wildly used psychostimulants, caffeine, an adenosine A2A receptor antagonist, and modafinil, to promote wakefulness and asked whether these compounds, known to activate multiple brains areas, could produce wakefulness when PZ GABAergic neurons are active. To test this hypothesis, we placed small bilateral injections of an adeno-associated viral (AAV) vector containing a cre-enabled excitatory receptor system [hM3Dq-AAV10] into the PZ of Vgat-ires-cre mice. As previously reported, injection of the ligand (CNO, 0.3mg/kg, IP, ZT3) produced a rapid and strong increase in SWS and cortical SWA. Also as previously reported, modafinil and caffeine alone induce wakefulness in these same mice. Co-injection of CNO, however, with either caffeine or modafinil, reversed the waking response, resulting in sleep/wake quantity similar to control (saline injection) levels. These results 1) suggest that PZ GABAergic neurons promote SWS by inhibiting not only the parabrachial nucleus but also likely other wake promoting pathways, and 2) provide additional evidence that GABAergic PZ neurons represent a key inhibitory cell population for SWS induction and maintenance.

Disclosures: C. Anaclet: None. **P.M. fuller:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

Location: Hall A

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Program#/Poster#: 166.09/U12

Topic: E.08. Biological Rhythms and Sleep

Support: Department of Anesthesiology

Title: Cholinergic mechanisms in rat prefrontal cortex promote waking and inhibit sleep states

Authors: *D. PAL[1], D. C. FEDRIGON, III[2], F. ALAM[2], G. A. MASHOUR[3];
[1]Dept. of Anesthesiol., [3]Anesthesiol., [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Electroencephalographic (EEG) and behavioral arousal are associated with increased acetylcholine (ACh) levels in cortex [1]. In contrast, cortical ACh levels decrease during slow wave sleep (SWS) and general anesthesia, two states characterized by slowing EEG frequency and behavioral quiescence [1]. However, it is not known if cortical ACh plays a causal role in the EEG and behavioral state transitions. We hypothesized that cholinergic stimulation of prefrontal cortex (PFC) through nicotine (NIC) or carbachol (CARB) microinjection during SWS will cause EEG activation and increase in the time spent in waking state. Under surgical anesthesia, male Sprague Dawley rats (n=26; 300-350g, Charles River) were instrumented for sleep-wake recordings and unilateral microinjections into PFC (Bregma, anterior 3mm, lateral 0.5mm, ventral 4mm) [2]. Sleep-wake recordings were conducted after injection of either saline (VEH, vehicle control) or one of the following concentrations of cholinergic agonists: 10mM NIC (n=6), 100mM NIC (n=7), 1mM CARB (n=9) or 10mM CARB (n=4). Each rat received only one concentration of either NIC or CARB. All microinjections (200nL) were done remotely during SWS, as determined by electrophysiological and behavioral criteria. The sleep-wake recordings were continued for 4 post-injection hours. The site of injections was confirmed histologically. A t-test or Wilcoxon test was used for the comparison of the effect of the VEH with the cholinergic agonists on sleep-wake states. Compared to the VEH, CARB at 1mM caused a significant increase in wake state and a decrease in SWS during the first post-injection hour. The increase in wake state was due to an increase in the mean duration of wake epochs. There was no effect on rapid eye movement sleep (REMS). Higher concentration of CARB (10mM) produced immediate behavioral seizures. Nicotine, 10mM or 100mM, did not significantly affect the time spent in wake or SWS. However, 100mM NIC significantly decreased the time spent in REMS by reducing the number of REMS epochs. The decrease was significant only during the first post-injection hour. The latency to the onset of REMS was also reduced. Neither NIC nor CARB produced EEG activation. These data suggest that cortical ACh is not a simple correlate of wake state but contributes to the top-down modulation of sleep-wakes states by promoting waking and inhibiting sleep states. This is in contrast to the role of ACh in brainstem where it promotes REMS. References: 1.Lydic R, Baghdoyan HA (2005) Anesthesiology 103:1268-95. 2.Paxinos G, Watson C (1997) Australia: Academic Press.

Disclosures: D. Pal: None. **D.C. Fedrigon:** None. **F. Alam:** None. **G.A. Mashour:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 166.10/U13

Topic: E.08. Biological Rhythms and Sleep

Support: University of Missouri, Bridge funding

Title: Nicotine promotes wakefulness via activation of wake-promoting cholinergic neurons of the basal forebrain

Authors: *A. NAIR[1,2], A. SHARMA[1], I. RICE[1,3], P. SAHOTA[1,4], R. SHARMA[1,4], S. MURUGESAN[1], M. M. THAKKAR[1,4];
[1]Harry S Truman Mem. VA Hosp., Columbia, MO; [2]Ross Univ. Sch. of Med., Portsmouth, Dominica; [4]Neurol., [3]Univ. of Missouri, Columbia, MO

Abstract: INTRODUCTION: Nicotine disrupts sleep through a yet unclear mechanism. The corticopetal basal forebrain (BF) is a major wake-promoting center in the brain. Multiple neuronal subtypes are localized in the BF, including cortically projecting cholinergic and GABAergic neurons. BF wake-promoting neurons express high levels of $\alpha 4\beta 2$ and $\alpha 7$ nicotinic acetylcholine receptors (nACHRs). We hypothesized that one mechanism by which nicotine disrupts sleep is through activation of the BF. METHODS: We used Sprague-Dawley (SD) rats as our animal model and performed three experiments. Nicotine was administered to these nocturnal animals at light onset to simulate smoking in the evening. Experiment 1 examined BF activity following systemic nicotine infusion. c-Fos was used to measure neuronal activation. SD rats were divided in two groups: Control and Nicotine. Just before light onset, nicotine (nicotine group; 0.3 mg/Kg) or saline (controls) was administered subcutaneously. The animals were left undisturbed for two hours and then euthanized to examine c-Fos expression in the BF. Experiment 2 examined the effects of nicotine on sleep. SD rats, implanted with sleep recording electrodes, were given subcutaneous nicotine at light onset. 4 doses of nicotine (0, 0.15, 0.3. 0.6 mg/Kg; randomized order of dose administration) were used to examine the dose response effect of nicotine on sleep. Experiment 3 examined the effects of nicotine on sleep in rats pretreated with a nicotine receptor blocker in the BF. SD rats, implanted with sleep recording electrodes and bilateral guide cannulas in the BF, were given mecamylamine (15 µg/500nl/side) in the BF followed by nicotine administration (0.6 mg/Kg; subcutaneously) at light onset. Rats were left undisturbed for next six hours and sleep was examined. Results: Our results suggest that: 1) systemic administration of nicotine significantly ($p<0.05$) increased activation of the BF; 2) systemic administration of nicotine significantly ($p<0.05$) and dose-dependently disrupted sleep; 3) blockade of nicotinic receptors in the BF attenuated nicotine-induced sleep disruptions. Conclusion: We conclude that nicotine induced activation of the BF may play a role in its disruption of sleep.

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Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Support: NIAAA Grant AA020334 and AA0174720

Title: Nicotine administration in the wake-promoting basal forebrain attenuates sleep promoting effects of alcohol

Authors: *R. SHARMA, P. SAHOTA, M. THAKKAR;
Neurol., Harry S. Truman Mem. Veterans Hosp. and Uni, Columbia, MO

Abstract: BACKGROUND: Nicotine and alcohol co-abuse is highly prevalent. However, underlying causes are unclear. It has been suggested that nicotine enhances pleasurable effects of alcohol while reducing aversive effects. Recently, we reported that nicotine acts via the basal forebrain (BF) to activate nucleus accumbens and increase alcohol consumption. Does nicotine via the BF suppress alcohol induced aversive effects? We hypothesized that nicotine may act via the BF to suppress sleep- promoting effects of alcohol. **METHODS:** To test this hypothesis, adult male Sprague-Dawley rats were implanted with sleep-recording electrodes and bilateral guides targeted toward the BF. Nicotine [75 pmol/500 nL/side] or artificial cerebrospinal fluid (ACSF; 500 nL/side) was microinjected into the BF followed by intragastric alcohol (ACSF+EtOH and NiC+EtOH groups; 3 g/Kg) or water (NiC+W and ACSF+W groups; 10 mL/Kg) administration. On completion, rats were euthanized and processed to localize injection sites in the BF. **RESULTS**: Statistical analysis revealed a significant effect of treatment on sleep-wakefulness. While rats exposed to alcohol (ACSF+EtOH) displayed strong sleep promotion, nicotine pre-treatment in the BF (NiC+EtOH) attenuated alcohol induced sleep and normalized sleep-wakefulness. **CONCLUSIONS:** Our results suggest that nicotine acts via the BF to suppress the aversive, sleep-promoting effects of alcohol further supporting the role of BF in alcohol-nicotine co-use.

Disclosures: **R. Sharma:** None. **P. Sahota:** None. **M. Thakkar:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 166.12/U15

Topic: E.08. Biological Rhythms and Sleep

Support: HSTMV Hospital

School of Medicine, University of Missouri-Columbia

Title: Melatonin promotes sleep by inhibiting orexinergic neurons in the perifornical lateral hypothalamus

Authors: A. SHARMA[1], A. NAIR[1,2], I. RICE[3], S. MURUGESAN[1], R. SHARMA[3], P. SAHOTA[3], *M. M. THAKKAR[3];
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Abstract: BACKGROUND: Melatonin promotes sleep, however the mechanism is unclear. Since the perifornical hypothalamic (PF-LH) orexin system is critical for wake promotion, we asked: Does melatonin promote sleep by inhibiting orexin neurons? We used C57BL/6J mice as our animal model and designed four experiments to address this question. METHODS: Experiment 1 examined the presence of melatonin receptors on orexin neurons by double-label immunofluorescence. Experiment 2 examined the effects of melatonin on the activation of orexin neurons. Standard sterile surgical protocol was used to implant bilateral guide cannulas targeted toward the orexinergic PF-LH. Melatonin (500pmole/50 nl/side) was bilaterally infused into PF-LH at dark onset. The animals were euthanized two hour later to examine c-Fos expression (marker of neuronal activation) in orexin neurons. Experiment 3 examined the effects of bilateral PF-LH infusion of melatonin on sleep-wakefulness. Animals were implanted with bilateral guide cannulas and sleep recording electrodes. At the onset of active (dark) period, melatonin (500pmole/50 nl/side) was infused into the PF-LH and its effects on spontaneous bouts of sleep-wakefulness were examined. Experiment 4 examined the effects melatonin receptor blockade on spontaneous bouts of sleep-wakefulness. Surgical and experimental procedures used were as described in experiment 3 except at sleep (light) onset melatonin receptor antagonist, Luzindol, (10pmol/50 nL/side) was administered and its effects on sleep-wakefulness were examined. RESULTS: Orexin neurons express MT1, but not MT2 receptors. Melatonin infusion into the orexinergic PF-LH significantly ($p<0.05$) reduced the number of orexin neurons with c-Fos immunoreactivity, increased NREM sleep and reduced wakefulness during normal active period. Blockade of melatonin receptors by local infusion of Luzindol into the orexinergic PF-LH, during normal sleep period significantly ($p<0.05$) increased wakefulness. CONCLUSIONS: We

suggest that melatonin may act via the MT1 receptors to inhibit orexin neurons and promote sleep.

Disclosures: **A. Sharma:** None. **A. Nair:** None. **I. Rice:** None. **S. Murugesan:** None. **R. Sharma:** None. **P. Sahota:** None. **M.M. Thakkar:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Support: COETCYJAL-UDG:2009-PS-2009-827

CONACYT SCHOLARSHIP: 15826

Title: Prophylactic oral administration of Melatonin in adult Balb/C mice, before and during 96 hours of REM sleep deprivation improves hippocampal neurogenesis

Authors: ***G. L. ARMAS**[1,2], M. FLORES-SOTO[3], D. ACUÑA-CASTROVIEJO[4], S. LUQUIN DE ANDA[2], O. GONZALEZ-PEREZ[5], V. LÓPEZ-VIRGEN[5], S. SOTO-RODRIGUEZ[2], G. CHIPRES-TINAJERO[2], R. RAMOS-ZUÑIGA[2], R. GONZALEZ-CASTAÑEDA[2];
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Abstract: Sleep deprivation (SD) is consider part of new style of live in our modern society that affect children, adolescent, adults and older people. SD is a stress agent with a negative consequences like day sleepiness, alteration in cognitive process- attention, concentration, memorize and learning. SD affects brain plasticity, mood regulation and neurogenesis. This last one issue neurogenesis is well accepted that occurs in brain throughout life in selected areas of the adult mammalian brain, mainly in the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. Is well documented that consequences of SD at the molecular level are largely unexplored. Hippocampus is particularly region susceptible to ROS and oxidative stress

generation and one source mentioned is SD affecting cellular functions including proliferation, survival, apoptosis, motility, transcription, metabolism and differentiation. By other hand Melatonin (MEL) is an import indole released from the pineal gland, and it possesses important neurobiological actions because is a pleiotropic molecule, some roles include control of seasonal reproduction, regulation of circadian rhythms, besides is an excellent antioxidant. The aim of this work were investigate how MEL and Luzindole (antagonist MT1 and MT2 receptors) can modify MEL content at the hippocampus tissue and if has any influence over neuronal precursors. We administrated oral MEL at 10mg/kg and Luzindole I.P at 5mg/kg for 14 days before and during performance of 96 hours of REM SD by the flower pot technique. Groups were: 1. Control, 2. SD, 3. MEL+SD, 4. Luzindole +MEL+SD and Luzindole+SD. MEL was administrated daily in water consumption and Luzindole once daily at 17:30 and 30 minutes after MEL was given by oral cannula. After 96h of SD we sacrificed by decapitation and quickly we remove hippocampal tissue and stored -80°C until used for HPLC measures. For Immunofluorescence experiments animals received 2 hr. before sacrifice an single injection of BrdU(100mg/Kg) after that were deeply anesthetized (pentobarbital 100 mg/kg) and perfused transcardially with phosphate buffer followed by paraform¬aldehyde (4%). Our results suggest that mice treated with MEL shows major content of MEL in hippocampus tissue by HPLC measures, Luzindole groups shows a slight diminish content of MEL and SD group show minor concentration of MEL of all groups. To respect of neuronal precursors the group with MEL improves BrdU-U/Nestin positives cells in contrasted with SD group. Thus melatonin can be consider a neuroprotective agent against REM SD.

Disclosures: **G.L. Armas:** None. **M. Flores-soto:** None. **D. Acuña-castroviejo:** None. **S. Luquin de anda:** None. **O. Gonzalez-perez:** None. **V. López-Virgen:** None. **S. Soto-rodriguez:** None. **G. Chipres-Tinajero:** None. **R. Ramos-zuñiga:** None. **R. Gonzalez-castañeda:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Program#/Poster#: 166.14/U17

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Support: NIH grant NS27881

Title: Orexin receptor activation generates gamma band input to cholinergic and serotonergic arousal system neurons and drives an intrinsic Ca^{2+}-dependent resonance in LDT and PPT cholinergic neurons

Authors: *M. ISHIBASHI, I. GUMENCHUK, B. KANG, C. STEGER, E. LYNN, N. E. MOLINA, L. M. EISENBERG, C. S. LEONARD;
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Abstract: The ascending arousal system, including cholinergic laterodorsal (LDT) and pedunculopontine (PPT) tegmental neurons and serotonergic dorsal raphe (DR) neurons, plays important roles in regulating wakefulness and sleep. During the waking state, the EEG shifts toward higher frequencies with synchronized intracortical gamma activity (30-60 Hz) - a process associated with high-level cognitive functions. Recently, this ascending arousal system has been proposed as a gamma wave generator, in part, because some neurons produce high-threshold, Ca^{2+}-dependent oscillations at gamma frequencies. However, it is not known whether arousal-related inputs to these neurons generate such oscillations, or whether such oscillations are ever transmitted to neuronal targets. One key input that is critical for maintaining normal wakefulness arises from orexin neurons. Orexin is well known to induce a noisy, depolarizing current in these cholinergic and serotonergic neurons. However, little is known about the frequency composition or function of this orexin-induced noise. To investigate this problem, we used whole-cell patch clamp and dynamic clamp techniques in brain slices obtained from mice expressing Cre-induced fluorescence in cholinergic LDT and PPT, and serotonergic DR neurons. After first validating reporter expression accuracy, we found that the orexin current produced significant high-frequency, including gamma band, input to both cholinergic and serotonergic neurons. We next used a dynamic clamp to add a virtual noisy orexin conductance to cholinergic neurons. This revealed an intrinsic, Ca^{2+}-dependent resonance that peaked in the theta and alpha frequency range (4 - 14 Hz) and extended up to 100 Hz in LDT and PPT cholinergic neurons. This intrinsic resonance emerged at membrane potentials near spike-threshold but below the potential necessary for generating high-threshold, Ca^{2+}-dependent oscillations at gamma frequencies. We propose that orexin current noise and the Ca^{2+} dependent resonance that peaks in the theta and alpha frequency range work synergistically to boost the encoding of high-frequency synaptic inputs into action potentials and to help ensure cholinergic neurons fire during EEG activation. This activity could reinforce thalamocortical states supporting arousal, REM sleep and intracortical gamma.

Disclosures: **M. Ishibashi:** None. **I. Gumenchuk:** None. **B. Kang:** None. **C. Steger:** None. **E. Lynn:** None. **N.E. Molina:** None. **L.M. Eisenberg:** None. **C.S. Leonard:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: National Natural Science Foundation of China (Grant nos. 81172638)

Title: Influence of SKF38393 on changes of gene profile in rat prefrontal cortex during chronic paradoxical sleep deprivation

Authors: *W. MA;
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Abstract: Chronic paradoxical sleep deprivation (CSD) can induce dramatic physiological and neurofunctional changes in rats, including decreased body weight, reduced learning and memory, and declined locomotor function. SKF38393, a dopamine D1 receptor agonist, can reverse the above damages. However, the mechanism of CSD syndrome and reversal role of SKF38393 remains largely unexplained. To preliminarily elucidate the mechanism of the neural dysfunction caused by CSD, in the present study we used gene chips to examine the expression profile of more than 28 000 transcripts in the prefrontal cortex (PFC). Rats were sleep deprived by modified multi-platform method for 3 weeks. Totally 208 transcripts showed differential expressions in CSD group in contrast to controls; they included transcripts coding for cell killing, immune system and metabolism. Among the 208 transcripts, 120 ones increased their expression and 88 decreased. Fourteen of them could be specifically reversed with SKF38393, including those coding for apoptosis, calcium ion pathway and MAPK pathway. Our findings in the present study indicate that long-term sleep deprivation may trigger apoptosis, stress response and changes of rhythmic regulation in the PFC, and the activation of D1 receptor by SKF38393 might ameliorate these changes via calcium ion pathway and MAPK pathway.

Disclosures: W. Ma: None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Program#/Poster#: 166.16/U19

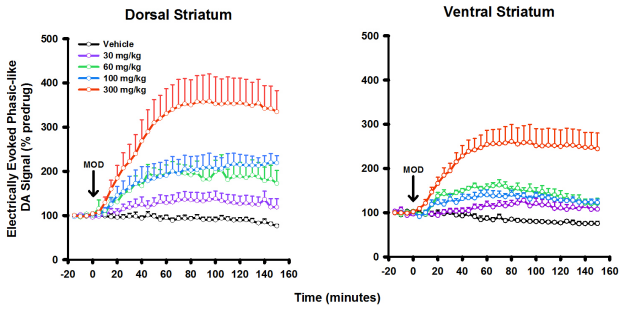
Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant DA036331

Title: Modafinil robustly activates phasic dopamine signaling in the striatum

Authors: M. J. BOBAK, M. W. WEBER, M. A. DOELLMAN, J. M. ATHENS, D. R. SCHUWEILER, *P. A. GARRIS;
Illinois State Univ., Normal, IL

Abstract: Modafinil (Nuvigil®) exhibits therapeutic efficacy for treating narcolepsy, work-related fatigue, and cognitive deficits. Like other psychomotor stimulants, such as amphetamine and cocaine, modafinil interacts with the dopamine transporter. However, its affinity is lower, and it is less potent in increasing tonic dopamine levels in the striatum as measured by microdialysis. Nevertheless, and also similar to other psychomotor stimulants, the ability of modafinil to increase wakefulness is abolished in dopamine transporter-knockout mice. Another potential target for modafinil is phasic dopamine signaling, which is implicated in reward learning and seeking. During phasic dopamine signaling, burst firing by dopamine neurons elicits transient dopamine signals in dopamine terminal fields. Moreover, amphetamine and cocaine robustly activate striatal dopamine transients. Here we investigated the effects of modafinil on phasic dopamine signaling in urethane-anesthetized rats with fast-scan cyclic voltammetry at a carbon-fiber microelectrode. Electrically and pharmacologically evoked phasic-like dopamine signals were assessed. As shown in Figure 1, modafinil robustly augmented the amplitude of electrically evoked phasic-like dopamine signals in dorsal and ventral striata. Increases were time- and dose-dependent and greater in the dorsal compared to ventral striatum. Electrically evoked phasic-like dopamine signals were further analyzed to assess effects of modafinil on the presynaptic mechanisms of dopamine release and uptake. This analysis demonstrated that modafinil increased dopamine release and decreased dopamine uptake. Furthermore, in the presence of the D2 dopamine antagonist, raclopride, which blocks somatodendritic dopamine autoreceptors and relieves anesthesia blunting of burst firing by dopamine neurons, modafinil elicited dopamine transients in both dorsal and ventral striata. Taken together, these results suggest that activation of phasic dopamine signaling is an important mechanism underlying the clinical efficacy of modafinil.



Disclosures: **M.J. Bobak:** None. **M.W. Weber:** None. **M.A. Doellman:** None. **J.M. Athens:** None. **D.R. Schuweiler:** None. **P.A. Garris:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH RO1 NS20246

NIH P20 GM110702

Title: Lithium decreases the effects of neuronal calcium sensor protein 1 on pedunculopontine neurons

Authors: *S. M. DONOFRIO[1], F. J. URBANO[2], E. E. GARCIA-RILL[1];
[1]Dept. of Neurobio. and Developmental Sci., Univ. of Arkansas For Med. Sci., Little Rock, AR;
[2]IFIBYNE-CONICET, Univ. of Buenos Aires, Buenos Aires, Argentina

Abstract: Whole-cell patch-clamp responses were recorded on 9-13 day old pups from adult timed-pregnant Sprague-Dawley rats. Slices were cut at 400 um and recorded at 370C perfused with oxygenated aCSF in an immersion chamber containing the synaptic blockers gabazine (GABAA antagonist), strychnine (glycine antagonist), 6-cyano-7-nitroquinoxaline-2,3-dione (AMPA/kainate receptor antagonist), APV (NMDA receptor antagonist), and mecamylamine (nicotinic receptor blocker), and also tetrodotoxin to block sodium channels. Our preliminary studies on a group of cells (n=5) show that NCS-1 at 1μM increased the amplitude of gamma band oscillations above control levels at 20, 25 and 30 min ($df=6$, $F=4.13$, $p<0.001$). In another group of cells (n=5) recorded with NCS-1 at 1μM, superfusion with lithium at low concentration (1 μM) reduced the effect of NCS-1 on gamma band oscillations, decreasing oscillations at 25 and 30 min ($df=6$, $F=3.93$, $p<0.007$). Lithium by itself had a blocking effect, reducing amplitude by 30 min ($df=6$, $F=5.5$, $p<0.002$). Human postmortem studies reported over expression of neuronal calcium sensor protein 1 (NCS-1) in the brains of some bipolar disorder patients. Reduced or aberrant gamma band activity has been reported in the same disorder. Bipolar disorder is also characterized by sleep dysregulation, suggesting a role for the reticular activating system (RAS). In a previous study examining the pedunculopontine nucleus (PPN), we found that NCS-1 at 1μM significantly increased the amplitude of gamma oscillations, whereas very high concentrations of NCS-1 (10μM) reduced or blocked gamma band oscillations in these

cells. Lithium been shown to effectively treat the mood disturbances seen in some bipolar disorder patients, although limited by side effects. Lithium was proposed to act by inhibiting the interaction between NCS-1 and inositol 1,4,5-triphosphate receptor protein (InsP). We hypothesized that one mechanism of action of lithium will be able to reduce the effects of over expression of NCS-1 and prevent the down regulation of gamma band activity and restore normal levels of gamma oscillations. These findings taken together resolve the 60 year mystery of one the mechanisms of action of lithium in bipolar disorder and suggest that lithium may reduce the effects of over expressed NCS-1 in bipolar disorder, thereby normalizing gamma band oscillations mediated by P/Q-type calcium channels modulated by NCS-1. In summary, these recent discoveries provide novel therapeutic targets for alleviating some of the arousal and sleep/wake disturbances in this devastating disease.

Disclosures: **S.M. Donofrio:** None. **F.J. Urbano:** None. **E.E. Garcia-Rill:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: NS084477

NS079940

NS052287

Veterans Administration Medical Research

Title: Optogenetic stimulation of astrocytes in the posterior hypothalamus increases sleep at night

Authors: *D. PELLURU[1], R. KONADHODE[1], N. R. BHAT[1], P. SHIROMANI[1,2];
[1]Psychiatry and Behavioral Sci., Med. Univ. of South Carolina, Charleston, SC; [2]Ralph Johnson VAMC, Charleston, SC

Abstract: A distributed network of neurons regulates wake, non-rapid eye movement sleep (NREM), and rapid eye movement sleep (REM sleep) (Pelluru et al., 2013). Optogenetics has been used to selectively manipulate some of these neurons to produce specific changes in wake, NREM and REM sleep. This has cemented the status of specific neurons in neural circuit models

of sleep-wake regulation. However, there are also glia in the brain, and there is growing evidence that neurons and astroglia communicate intimately to regulate behavior (Frank, 2013). We hypothesized that optogenetic stimulation of glia in posterior hypothalamus increases sleep. To selectively stimulate glial cells, rAAV-GFAP-ChR2-EYFP was microinjected into the posterior hypothalamus of WT mice (n=7) and orexin-KO mice (n=8). As controls a separate group of WT mice (n=5) received rAAV-GFAP-EYFP (no ChR2). Three weeks after gene insertion sleep was recorded and optogenetic stimulation (10 msec pulses; 1mW; one minute on-4 minutes off; for 6h) applied during the first 6h of the lights-off period. Optogenetic stimulation at 10 Hz compared to 0 Hz significantly increased NREM sleep in WT (+68%; $p<0.003$) and orexin-KO (+71.5%; $p<0.01$) mice, and decreased the length of wake bouts along with the time spent in wake in WT and orexin-KO mice. 10Hz stimulation significantly increased REM sleep in WT ($t=2.44$; $p<0.05$) and the orexin KO mice ($t=2.62$; $p<0.022$). There were no changes in delta power. In orexin-KO mice optogenetic stimulation of the astroglia did not change incidence of cataplexy. In control mice (no ChR2), 10Hz stimulation had no effect. This study demonstrates that direct stimulation of glia powerfully induces sleep during the active phase of the sleep-wake cycle and underscores the inclusion of glia in network models of sleep-wake regulation.

Disclosures: **D. Pelluru:** None. **R. Konadhode:** None. **N.R. Bhat:** None. **P. Shiromani:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: NS084477

NS079940

NS052287

Veterans Administration Medical Research

Title: Optogenetic activation of galanin neurons in the preoptic area (MnPO, VLPO) in galanin-cre mice does not increase sleep at night

Authors: *R. KONADHODE[1], D. PELLURU[1], C. BLANCO-CENTURION[1], M. LIU[1], C. ROBERTSON[1], P. SHIROMANI[1,2];
[1]Med. Univ. of South Carolina, Charleston, SC; [2]Ralph Johnson VAMC, Charleston, SC

Abstract: Neurons in the ventral lateral preoptic area (VLPO) are selectively active during sleep based on electrophysiology and c-FOS studies (Sherin et al., 1996; Szymusiak et al., 1998). Moreover, lesion of the VLPO neurons produces insomnia (Lu et al., 2000) suggesting that these neurons are necessary for sleep. However, their status in the sleep process is unknown since these neurons have never been selectively manipulated. Most (>80%) of the VLPO sleep active neurons express galanin (Gaus et al., 2002) and can be selectively activated with optogenetics. Therefore, we hypothesized that optogenetic stimulation of these neurons should increase sleep. Galanin-cre mice (n=4; 30 gm) were administered excitatory (rAAV-EF1a-DIO-hChR2 (H134R)-EYFP) opsin into the VLPO (bilateral injection; 750 nl each side) and implanted with sleep recording electrodes. Three weeks later a 48 h baseline sleep recording was obtained (0 Hz). At the start of the lights-off (night) period the mice were stimulated with 5, 10 or 30 Hz (random order) of blue light pulses (10 msec duration). The light pulses were delivered for 1 minute every 5 minutes for 6h, and 36h elapsed between the three stimulation rates. Preliminary data analysis indicates that activation of galanin neurons in POA (10Hz) for the 6h night period has no effect on sleep or wake when compared to baseline (0Hz). 5, and 30Hz stimulation also had no effect on sleep and wake. Histology is pending and will identify the number of galanin neurons containing the EYFP reporter gene, a proxy marker of the light-sensitive channelrhodopsin-2 gene. Our study demonstrates that optogenetic stimulation of galanin VLPO neurons does not increase sleep at night. This contrasts sharply with the optogenetic activation of MCH neurons which induces sleep at night in both mice and rats. It is possible that because VLPO neurons receive direct retinal input they might be prone to induce sleep during the day, the animal's normal sleep cycle.

Disclosures: R. Konadhode: None. **D. Pelluru:** None. **C. Blanco-Centurion:** None. **M. Liu:** None. **C. Robertson:** None. **P. Shiromani:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH RO1 NS20246

NIH P30 GM110702

Title: Correlation between the developmental decrease in REM sleep and N-type calcium channel expression

Authors: *E. E. GARCIA-RILL, S. MAHAFFEY, M. MACNICOL;
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Abstract: In the human, rapid eye movement (REM) sleep decreases from 8 hr or 50% of total sleep time at birth, to 1 hr or 15% of sleep time. In the rat, this decrease occurs between 10 and 30 days, with REM sleep increasing at the expense of slow wave sleep, without changes in waking time. We discovered that every cell in the pedunculopontine nucleus (PPN), which modulates waking and REM sleep, manifests beta/gamma band activity, and that this activity is mediated by voltage-dependent high threshold N- and P/Q-type calcium channels. We also found that PPN cells exhibit N only (30%), P/Q-only (20%), and N+P/Q (50%) channels, which suggests that these correspond respectively to "REM-on", "Wake-on", and Wake/REM-on" cells *in vivo*. Our findings suggest that N-type channels modulate REM sleep while P/Q-type channels modulate waking. This study was carried out to determine if N-type and/or P/Q-type calcium channel expression in the PPN changes during the developmental decrease in REM sleep in the rat. We analyzed N- and P/Q-type calcium channel expression at two ages, 10+/-2 days and 30+/-4 days in two regions, the PPN and the hippocampus (HIPP). Brainstem slices were cut at 400 um from anesthetized rat pups (n=30) and 1 mm punches of the PPN and the HIPP were sampled. Groups of 10 animals at each age were sampled and the analysis repeated 3 times. Tissue was harvested in RNAlater and 50 ng RNA was analyzed in triplicate reactions with primers specific to N-type or P/Q-type channels (sequences available upon request), and with control primers to GAPDH and Cyc1 using Power SYBR Green RNA-to-Ct assay mix and a QuantStudio 12k Flex instrument. Data was analyzed using the QuantStudio Software v1.1 and comparisons between groups were carried out using one-way ANOVA with Bonferroni post hoc testing, and differences were considered significant at values of $p \leq 0.05$. We found that the N-type channel was expressed at significantly higher levels in the PPN from 10 day old compared to 30 day old animals (Experiment 1: 410+/-11% SE, n=3, p=0.011; Experiment 2: 368+/-15% SE, n=3, p=0.001, in 10 day vs 30 day PPN). By comparison, expression of P/Q-type channels decreased only slightly over the same developmental period (Experiment 1: 156+/-0.1% SE, n=3, p=0.001; Experiment 2: 222+/-26% SE, n=3, p=0.001 in 10 day vs 30 day PPN). No significant decrease in expression of N-type channels was observed in HIPP (Experiment 1: 117+/-6% SE, n=3, p=0.06; Experiment 2: 122+/-15% SE, n=3, p=0.11, in 10 day vs 30 day HIPP). These results suggest that the developmental decrease in REM sleep may be caused by a decrease in the expression of N-type calcium channels in the PPN.

Disclosures: E.E. Garcia-Rill: None. **S. Mahaffey:** None. **M. MacNicol:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Program#/Poster#: 166.21/U24

Topic: E.08. Biological Rhythms and Sleep

Support: CONACYT 133178

Title: Sleep deprivation induces morphological changes in the prefrontal cortex in young and old rats

Authors: *F. A. GARCÍA-GARCÍA[1], E. ACOSTA-PEÑA[2], M. MELGAREJO-GUTIERREZ[2,3], I. CAMACHO-ABREGO[3], G. FLORES[3];
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Abstract: Sleep is a fundamental state necessary for maintenance of physical and neurological homeostasis throughout the lifespan. Many hypotheses about the functions of sleep have been focused on effects of sleep deprivation on synaptic plasticity at a molecular and electrophysiological level and only a few have studied sleep function from a structural perspective. Moreover, during normal aging sleep architecture displays some changes that could affect normal development in the elderly. In the present study, using a Golgi-Cox staining and posterior Sholl analysis we evaluate the effects of 24 h total sleep deprivation on neuronal morphology of pyramidal neurons from layer III of prefrontal cortex (PFC) from male Wistar rats at two different ages (3 and 22 months old). We found no differences in total dendritic length and branching length in the region analyzed after sleep deprivation. Interestingly sleep deprivation increased spine density in PFC from aged animals. Taken together, our results show that sleep deprivation have different effects on synaptic plasticity and could play a beneficial role in cognition during aging.

Disclosures: F.A. García-García: None. **E. Acosta-Peña:** None. **M. Melgarejo-Gutierrez:** None. **I. Camacho-Abrego:** None. **G. Flores:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Program#/Poster#: 166.22/U25

Topic: E.08. Biological Rhythms and Sleep

Support: NIH/NIMH R21MH103775

Title: Long lasting global suppressed states during slow wave sleep

Authors: *H. MIYAWAKI, K. DIBA;
Dept. of Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Slow wave sleep (SWS) is characterized by alternations between network UP and DOWN states each lasting on the order of 100 milliseconds. Using large-scale recording from large populations of neurons in multiple brain regions, we observed and characterized an additional state during SWS, which we call the "LOW" state. LOW states were marked by sporadic epochs of suppressed activity with strongly diminished power < 50 Hz in the local field potential and lasting several seconds. During LOW states we observed decreased firing rates, lower incidences and amplitudes of oscillatory events, such as sharp wave ripples, sleep spindles, and slow waves. We initially identified the LOW state in hippocampal region CA1. However, by analyzing additional data from multiple brain regions recorded by Mizuseki et. al. (Neuron 2009) and Peyrache et. al. (Nature Neuroscience 2015) obtained from http://crcns.org, we also identified similar LOW states in the entorhinal cortex in rats, and the post-subiculum and the medial prefrontal cortex, but not the thalamus, in mice. Cross correlation analysis revealed that LOW state were global; they began and ended at the same time across multiple brain regions. LOW states occurred most frequently at the beginning of SWS episodes and were modulated by sleep and waking history. Across extended sleep, the fraction of time spent in LOW states gradually increased. On the other hand, the fraction of LOW states decreased following waking. These results suggest that LOW states may play a function in sleep, by effectively decreasing spiking activity throughout the brain.

Disclosures: H. Miyawaki: None. **K. Diba:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 166.23/U26

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant R01NS072431

Title: A novel assay to screen for genes involved in sleep homeostasis

Authors: N. HOFFNER, L. K. SATTERFIELD, G. A. SEIDNER, *W. JOINER;
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Abstract: Sleep is an evolutionarily conserved behavior that is essential for survival. However, the need(s) fulfilled by sleep are unknown. The mechanism by which sleep need is sensed and converted to suppression of behavioral arousal is also unknown. This process, which is referred to as sleep homeostasis, can be measured by depriving animals of sleep at night and measuring the duration and intensity of compensatory (or rebound) sleep the next day. To identify genes that contribute to sleep homeostasis we established a novel, high throughput thermogenetic assay in *Drosophila* melanogaster. In this assay, heat-activated TrpA1 channels are expressed transgenically in arousal-promoting neurons, where they are activated at night by a moderate increase in ambient temperature, leading to increased waking. The next morning, temperature is then returned to normal, shutting off TrpA1 channels and presumably returning excitability of targeted neurons to baseline, thus allowing robust homeostatic recovery sleep to occur. Using this assay we then conducted an RNAi-based forward genetic screen to identify mutants that can be sleep-deprived but are unable to mount a compensatory sleep response the next day. The results of our screen confirm that sleep homeostasis is indeed under genetic control. Further elucidating the genetic basis for sleep homeostasis may lead to novel approaches to treat diseases in which sleep structure is affected as well as promoting overall health and well-being.

Disclosures: N. Hoffner: None. **L.K. Satterfield:** None. **G.A. Seidner:** None. **W. Joiner:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 166.24/U27

Topic: E.08. Biological Rhythms and Sleep

Title: Altered EEG response to psychomimetic agents in a novel Han Wistar rat strain lacking metabotropic glutamate receptor 2 expression

Authors: *C. M. WOOD[1], D. LODGE[2], A. P. MCCARTHY[3], E. SHANKS[4], E. S. J. ROBINSON[2], K. A. WAFFORD[5];
[2]Sch. of Physiol. and Pharmacol., [1]Univ. of Bristol, Bristol, United Kingdom; [3]Discovery Data Sci., [5]*In vivo* Pharmacol., [4]Eli Lilly and Co., Windlesham, United Kingdom

Abstract: Patients suffering from psychiatric disorders such as schizophrenia, often have sleep disturbances and simultaneous changes in their EEG profiles. Group II metabotropic glutamate receptors (mGluRs) have been implicated in these disorders, with preclinical work showing mGluR2/3 compounds have efficacy in reducing psychosis-like behaviours following NMDA receptor antagonist and hallucinogen administration. Pharmacological and genetic manipulation of mGluR2/3 has also been shown to alter sleep-wake architecture in mice. Recent work has highlighted rat strains lacking mGluR2 protein expression[1]. This study aimed to investigate any changes in sleep/wake or EEG spectral profile in a novel rat strain lacking mGluR2 receptor expression, and examine their response following treatment with the NMDA receptor antagonist ketamine, and $5HT_{2A/C}$ agonist DOI. To assess sleep and EEG waveform activity the SCORE-2000^{TM} bioassay was used. Briefly, individually housed and tethered Charles River Wistar (CR) and HSD Han Wistar rats were monitored for EEG, EMG, locomotor activity, food and drink-related activity and body temperature. These data were recorded concurrently 24 hours before treatment as a baseline and for 30 hours post treatment for subsequent drug effects. Ketamine (10mg/kg, s.c.) and DOI (3mg/kg, i.p.) were administered 5 hours after lights on. No significant baseline differences were seen in sleep/wake parameters between the two strains. Ketamine and DOI both promoted wakefulness following treatment, decreasing NREM and REM sleep in both rat strains, with a reduced effect on REM sleep in the Han Wistar rats following ketamine treatment. Ketamine induced an increase in spectral power of the gamma frequency (30-80Hz) and high frequency oscillation bands (HFO; 130-160Hz). This increase in HFO was potentiated in the Han Wistar rats. Food and drink-related activity were increased in the Han Wistar rats alone following ketamine treatment. DOI induced an increase in HFO in both rat strains, with an attenuated effect in the Han Wistar rats. Psychomimetic drugs increase cortical spectral activity in the high frequency range. Our data demonstrates a divergence in this measure and associated behaviours elicited by the $5HT_{2A/C}$ agonist DOI and NMDA receptor antagonist ketamine, highlighting the importance of mGluR2 receptors in eliciting this response. This correlates with previous reports showing the importance of mGluR2 in the behavioural response to DOI using mGluR2$^{-/-}$ mice. Further work is required to elucidate the precise role played by mGluR2 in these pharmacological effects. 1. Ceolin L et al. (2011) J Neurosci. 31:6721-31.

Disclosures: **C.M. Wood:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BBSRC CASE studentship with Eli Lilly. **D. Lodge:** None. **A.P. McCarthy:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **E. Shanks:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **E.S.J. Robinson:** None. **K.A. Wafford:** A. Employment/Salary (full or part-time):; Eli Lilly and Company.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 166.25/U28

Topic: E.08. Biological Rhythms and Sleep

Support: ANR OPTOREM ANR-13-BSV4-0003

Title: Modulation of hypothalamic MCH-expressing neurons by pharmacogenetic tools strongly alters the sleep-waking cycle in mice

Authors: *C. VARIN[1], S. JEGO[2], S. ARTHAUD[1], M. LAZARUS[3], T. GALLOPIN[4], P.-H. LUPPI[1], A. ADAMANTIDIS[2,5], P. FORT[1];
[1]SLEEP team, Lyon Neurosci. Res. Center, UMR 5292 CNRS/U, Lyon, France; [2]Dept. of Psychiatry, McGill Univ., Douglas Inst., Montreal, QC, Canada; [3]Intl. Inst. for Integrative Sleep Med., Univ. of Tsukuba, Tsukuba, Japan; [4]Brain plasticity unit, ESPCI ParisTech, Paris, France; [5]Dept. of Neurol., Inselspital Univ. Hospital, Univerity of Bern, Bern, Switzerland

Abstract: Evidence supports a role of hypothalamic neurons expressing melanin-concentrating hormone (MCH) in sleep. These neurons are indeed maximally active during paradoxical sleep (PS) based on Fos immunostaining and juxtacellular unit recordings across the natural sleep-waking cycle in rats. Further, intracerebroventricular MCH infusion induces an increase in both PS and Slow-Wave Sleep (SWS) amounts in rats, whereas acute optogenetic activation of MCH neurons at PS onset extends the duration of PS, but not SWS episodes. To decipher the precise contribution of MCH neurons to SWS and/or PS regulation, we used a pharmacogenetic approach with DREADD tools in male transgenic MCH-cre mice (12 weeks old). These mice were bilaterally injected within the tuberal hypothalamus with either inhibitory (AAV-hSyn-hM4di-mCherry) or excitatory (AAV-hSyn-hM3dq-mCherry) DREADDs and prepared for chronic polysomnographic recordings. Four weeks later, effects on vigilance states were analyzed in response to i.p. CNO treatments. Inhibition of MCH neurons at light onset dose-dependently increased SWS quantities compared to saline, due to a dramatic increase in bout duration whereas excitation of MCH neurons conversely reduced SWS bout duration. Regarding PS, inhibition of MCH neurons did not produce any strong effect. In contrast, their excitation facilitated PS maintenance at light onset and both PS promotion and maintenance when performed at light offset. These data interrogating the circadian control of MCH neuron excitability, we further analyzed effects on sleep of excitation applied at different time across the light-dark cycle. The present results suggest that MCH-expressing neurons may play a key role in the fine-tuning of the sleep-waking cycle by modulating both SWS and PS, in particular by facilitating SWS consolidation and PS promotion and maintenance. Moreover our results indicate that the excitability of MCH neurons is under a strong circadian control.

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Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Program#/Poster#: 166.26/U29

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant MH59839

Title: Development of REM sleep homeostatic drive requires activation of BDNF TrkB receptor in the pedunculopontine tegmentum (PPT)

Authors: *A. BARNES[1,2], R. KOUL TIWARI[1,2], S. DATTA[1,2,3];
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Abstract: Selective REM sleep deprivation (RSD) is a novel method previously developed in our laboratory that successfully eliminates 85-95% of REM sleep without reducing slow-wave sleep. Recently, we have shown that RSD increases BDNF expression in the PPT, which potentially contributes to REM sleep homeostasis. In the present study, we investigated the functional role of BDNF TrkB receptor signaling in the homeostatic regulation of REM sleep. Adult male Sprague-Dawley rats were chronically implanted with sleep-wake (S-W) recording electrodes and bilateral guide cannulae for microinjections into the PPT. After surgical recovery, habituation, and baseline S-W recordings, the rats were randomly separated into control and experimental groups (Group 1 and Group 2, respectively). All animals were recorded from 9:00 am to 3:00 pm. Immediately before the recordings, Group 1 rats were microinjected with vehicle control (0.1 µl of 0.9% saline), and Group 2 rats were microinjected with a TrkB receptor inhibitor (30 µmol of K252a). For the first three hours of the recording period, half of each group subjected to RSD, and the other half was allowed to have undisturbed S-W activity. All animals were allowed to have undisturbed S-W activity for the final three hours. To determine the level of BDNF expression in the PPT, half of the animals from each subgroup were sacrificed immediately after the first 3 hours of recording. In the remaining animals, brains were marked and perfused for histological verification of the injection site. Results demonstrated that: a) In the PPT, BDNF expression had a positive correlation with homeostatic drive for REM sleep; b) inhibition of the TrkB receptors in the PPT minimized BDNF expression in the PPT; and c) inhibition of the TrkB receptors in the PPT suppressed selective RSD-induced homeostatic drive for, and rebound of REM sleep. These results, for the first time, suggest that the activation of PPT BDNF TrkB receptors plays a causal role in development of REM sleep homeostatic drive.

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Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: University Research Priority Program "Integrative Human Physiology" of the University of Zurich (Switzerland)

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Title: Association between cognitive performance, sleep EEG and the BDNF Val66Met polymorphism in children and adolescents

Authors: *F. PUGIN[1], A. METZ[2,3], A. BAUMER[4], M. WOLF[2], A. RAUCH[4], P. ACKERMANN[5], O. JENNI[1], R. HUBER[1];
[1]Child Develop. Ctr. - Children's Res. Ctr., Univ. Children's Hosp. Zurich, Zuerich, Switzerland; [2]Biomed. Optics Res. Lab. - Dept. of Neonatology, Univ. Hosp. Zurich, Zuerich, Switzerland; [3]Univ. of Berne, Institute of Complementary Medicine, Switzerland; [4]Inst. of Med. Genet., [5]Inst. of Pharmacol. and Toxicology - Chronobiology and Sleep Res., Univ. of Zurich, Zuerich, Switzerland

Abstract: Objective: In adults, the single nucleotide polymorphism (Val to Met substitution) on codon 66 of brain-derived neurotrophic factor (BDNF Val66Met) has been linked to differences in brain plasticity (e.g., differences in brain structure and higher cognitive performance in Val/Val allele carriers (VV) compared to heterozygotes (VM)). Evidence is accumulating that EEG sleep slow-wave activity (SWA, EEG power < 4.5 Hz) reflects cortical plasticity. Thus, we tested in this study whether the association between cognitive performance and sleep SWA differs between young VV and VM allele carriers. Methods: 27 subjects were recruited; none of them was a Met/Met carrier. In 16 Val allele carriers (VV; age mean ± SD, 13.1y ± 16m) and 11 Val/Met allele carriers (VM; 12.9y ± 19m), fluid intelligence (FI) was assessed with the TONI-IV, and sleep high-density electroencephalography (HD EEG; 128 electrodes) was measured during a night in the sleep laboratory. Results: The VV-group showed a trend towards higher fluid intelligence scores (110.5 ± 13) compared to the VM-group (101.9 ± 7; $p = 0.07$). No

significant group differences in sleep SWA were found. Correlating SWA with FI revealed three regions of interest that were further analyzed. In a frontal and parietal region, SWA correlated positively with FI ($r = 0.4$, $p < 0.05$; both groups pooled). In an occipital region, a negative correlation was found ($r = -0.7$, $p < 0.001$) for both groups pooled, as well as in the VV-group ($r = -0.8$, $p < 0.001$). Conclusion: The trend level group difference in cognitive performance is in accordance with current literature. The associations between FI and sleep SWA may highlight regions of intense plastic changes involved in the maturation of higher cognitive functions. The negative correlation between SWA and FI in the occipital region seems to be driven by the VV group, possibly reflecting differential mechanisms of cortical plasticity in relation to cognition during development in VV carriers with high FI. These preliminary results in children and adolescents indicate that sleep SWA topography shows a distinct relation to cognitive performance in VV and VM allele carriers.

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Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: NHLBI Grant HL095491

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Title: Glutamatergic projection from the medial Prefrontal Cortex to Basal Forebrain - a ChR2-assisted circuit mapping study

Authors: *L. FERRARI, D. PARK, E. ARRIGONI;
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Abstract: The basal forebrain (BF) is an important component of the ascending arousal system and contains a heterogeneous population of neurons, including neurons producing acetylcholine, GABA and glutamate. BF cholinergic neurons are the main cholinergic input to the cerebral cortex, they fire in association with wakefulness and REM sleep and, when activated, they promote cortical activation. These neurons also receive a robust glutamatergic input, which

might contribute to their behavioral state-dependent activity. Here we used a ChR2-assisted-circuit-mapping approach to identify BF neurons targeted by the medial prefrontal cortex (mPFC). We injected an adeno-associated viral (AAV) vector expressing GFP (rAAV8/hSvn-DIO-eGFP) into the BF (MCPO/SI region) of Vglut-ires-cre, Vgat-ires-cre or ChAT-ires-cre mice to express GFP in cholinergic, GABAergic and glutamatergic neurons respectively. We also injected AAV-CaMKIIa-hChR2(H134R)-mCherry into the medial prefrontal cortex (mPFC) to express ChR2 in glutamatergic cortical neurons. We performed whole-cell recordings in BF slices and we targeted MCPO/SI neurons that expressed GFP. We photostimulated cortical axons/terminals that expressed ChR2 using blue-light (473 nm) pulses. Photostimulation of the mPFC input failed to evoke synaptic responses in BF cholinergic neurons but evoked EPSCs in GABAergic and glutamatergic MCPO/SI neurons. These photo-evoked EPSCs were abolished by DNQX, indicating that they were mediated by the release of glutamate and by the activation of AMPA postsynaptic receptors. Previous studies have shown that BF neurons diffusely innervate the cortex, and our results demonstrate functional projections back from the mPFC to the BF. These projections mainly contact GABAergic and glutamatergic neurons but there appears to be no direct innervation of BF cholinergic neurons. Much sleep research emphasizes ascending arousal signals. Our results now demonstrate functionally-important descending projections from the mPFC which may influence behavioral state.

Disclosures: **L. Ferrari:** None. **D. Park:** None. **E. Arrigoni:** None.

Poster

166. Sleep: Molecular, Cellular, and Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 166.29/U32

Topic: E.08. Biological Rhythms and Sleep

Title: The novel zfhx3 circadian clock gene influences sleep and cognitive features

Authors: E. BALZANI[1], *V. TUCCI[1], G. LASSI[1], S. MAGGI[2], S. SETHI[3], M. J. PARSONS[3], M. SIMON[3], P. M. NOLAN[3];
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Abstract: The recently-identified circadian mouse mutant, *Zfhx3*$^{Sci/+}$, caused a shortening of the circadian period in mice due to a novel AT motif-dependent circadian axis. Here we provide evidence that this new circadian axis influences processes that are outside the canonical circadian

system. Analysis of the *Zfhx3*$^{Sci/+}$ transcriptome revealed links between the circadian clock, sleep and cognition. *In vivo* assessment of sleep and cognition in *Zfhx3*$^{Sci/+}$ mice demonstrated a defect of sleep homeostasis and short-interval time perception in mutants. We studied how circadian clock differences modify genetic noise in simulated neural networks. Thus, we assessed the dynamics among genetic noise, sleep and circadian rhythms in conditioning daily behaviors. We show here, for the first time, statistical dependency between behavioral/cognitive measures and various biological processes that varies with time of the day. Remarkably, the *Zfhx3*$^{Sci/+}$ mutation changes the dynamics of how the circadian clock and sleep can influence cognitive functioning in time.

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Poster

166. Sleep: Molecular, Cellular, and Pharmacology

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Topic: E.08. Biological Rhythms and Sleep

Support: Department of Veterans Affairs Medical Research Service Awards (RB and RWM)

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R01 MH099180 (AVK)

R01 MH039683 (RWM)

R01 AA020183 (PH)

R01 NS037585 (PH)

Title: Sleep homeostasis, adenosine and glia cells

Authors: *A. V. KALINCHUK[1], J. ZANT[1], P. HAYDON[2], R. W. MCCARLEY[1], R. BASHEER[1];
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Abstract: Extracellular adenosine (AD) is an important homeostatic sleep factor which acts in the basal forebrain (BF) via the A1 receptor. During sleep deprivation (SD) the level of AD increases 1.5-3-fold as compared to spontaneous wake, and this increase correlates with the

homeostatic sleep pressure (HSP). The cellular source of AD increase during SD is not well understood, although recent data indicate the importance of AD-triphosphate (ATP) released from glia in SD-induced AD increase. On the other hand, our data show that SD-induced AD depends on nitric oxide (NO) produced in wake-active BF neurons. In this study, to investigate the role of glia in SD-induced AD increase and homeostatic sleep response, we measured AD, NO and ATP in mice with conditional astrocyte-specific expression of the dnSNARE dominant negative protein. Using doxycycline (DOX)-dependent regulation of gliotransmission, we performed measurements in the same animals while they were on the DOX diet (DOX-ON, normal gliotransmission) and 2 weeks after the DOX diet removal (DOX-OFF, reduced gliotransmission). We also compared the homeostatic mechanisms triggered during the light (high HSP) and dark (low HSP) periods. Mice were implanted with EEG/EMG electrodes and microdialysis guide cannula targeting the BF nuclei. Microdialysis samples were collected during the baseline (non-disturbed sleep) days or SD days. In the microdialysates we measured (i) ATP by luciferin-luciferase based assay, (ii) AD using HPLC coupled to fluorescent detector, (iii) NO using a Nitrate/Nitrite (NOx) fluorometric assay kit. During the light period, the SD-induced increase in ATP level observed in DOX-ON condition was eliminated after the removal of DOX diet. SD-induced increases in AD (2X) and recovery NREM sleep/delta power observed in DOX-ON condition were attenuated in DOX-OFF condition, but they were still significantly higher than baseline (AD, 1.6X). SD-induced increase in NOx was not affected by expression of dnSNARE. During the dark period, in DOX ON condition, the SD-induced increases in AD (1.4X) and recovery NREM sleep/delta were significantly lower as compared to changes observed during the light period. In DOX OFF condition, these increases were completely eliminated. Most notably, NOx level was unchanged during the dark period. We conclude that different mechanisms contribute to the SD-induced increase in AD and homeostatic sleep response under high and low sleep pressure. During the light period, when HSP is high, AD increase is preferentially mediated by NO with a moderate contribution of gliotransmission. During the dark period (low HSP) the increase in AD is mostly dependent on ATP released from glia.

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Poster

167. Sleep: Behavior

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Program#/Poster#: 167.01/U34

Topic: E.08. Biological Rhythms and Sleep

Support: I-CORE Grant 51/11

Title: Comparing neuronal tracking of speech across wakefulness and sleep

Authors: *S. BEKER[1,2], S. MAKOV[1], O. SHARON[1], N. DING[3], Y. NIR[1], E. ZION GOLUMBIC[2];
[1]Fac. of Med., Tel Aviv Univ., Tel Aviv-Yafo, Israel; [2]Gonda Multidisciplinary Brain Res. Ctr., Bar Ilan Univ., Ramat Gan, Israel; [3]Col. of Biomed. Engin. and Instrument Sci., Zhejiang Univ., Hangzhou, China

Abstract: During sleep, external stimuli fail to elicit meaningful behavioral responses and rarely affect perceptual awareness. However, the extent to which the sleeping brain continues to process external stimuli remains unclear. Studies examining ERP responses to brief sounds during sleep yielded mixed results and their interpretation is complicated by the occurrence of k-complexes. Instead, focusing on neuronal tracking of speech may allow (a) to probe sleep processing with continuous stimulation, and (b) to determine how sleep affects different levels of processing (e.g. acoustic vs. phoneme/word segmentation vs. semantic). To test this, we used a variety of speech and vocoded speech stimuli, to manipulate intelligibility to different degrees. In addition, we presented speech in an unfamiliar language. Building on previously established findings that neural tracking of intelligible speech is more robust than non-intelligible speech, here we compared neuronal tracking of intelligible and non-intelligible speech across wakefulness and sleep using high-density (256-channel) EEG. Replicating previous results, in wakefulness we observe neural phase locking to the stimuli around 3-5Hz, which monotonically increased as a function of speech intelligibility at medial-frontal electrodes. In contrast, preliminary results during full-night sleep reveal that the speech tracking response is significantly reduced and does not vary strongly with intelligibility, suggesting that only limited linguistic processing of speech occurs during sleep. More generally, we hope that these paradigms can be adapted to assess residual cognitive processing in other non-responsive states such as anesthesia, disorders of consciousness and neurodegeneration.

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Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant R43NS83218

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Title: A comparative study of circadian rhythms and sleep between African spiny mice (Acomys cahirinus) and house mice (Mus musculus) in single and group housing conditions

Authors: *C. WANG, T. R. GAWRILUK, M. C. KEINATH, S. K. BISWAS, J. J. SMITH, A. W. SEIFERT, B. F. O'HARA;
Biol. Dept., Univ. of Kentucky, Lexington, KY

Abstract: The study of circadian and sleep behavior in different organisms can provide valuable insight for understanding behavioral, physiological and environmental influences on these processes. Interestingly, two species of African spiny mice, Acomys russatus (Golden spiny mouse) and Acomys cahirinus (Cairo spiny mouse) have been reported to exhibit different circadian rhythm patterns in locations where the two species overlap. Both species are primarily nocturnal when not in direct competition, but in areas of overlap A. cahirinus exhibit nocturnal behavior, while A. russatus become more diurnal. However, very few studies on the circadian activity of these species are available and nothing is known of their sleep behavior, which can be the dominant force in driving other diurnal variables. Therefore, we have begun to study one of these species (A. cahirinus) in greater detail alongside the well-studied house mouse (Mus musculus) using a well validated, non-invasive, piezoelectric system, that picks up all movements during wake, and the breathing rhythms during sleep. In these studies, we found A. cahirinus and M. musculus to be primarily nocturnal, but with clearly distinct behavioral patterns. Specifically, the activity of A. cahirinus sharply increases right at dark onset, which is common in nocturnal species, but surprisingly, decreases sharply just one hour later in single cage studies. In group cage studies, the activity of A. cahirinus remained high for a few hours after dark onset. Similarly, the activity of A. cahirinus is more active before middle of the night period than after middle of the night period. These differences may be related to foraging differences between these species, or may be related to the socialized behavior of A. cahirinus and its poorer adaptation to isolation as compared to Mus musculus. Also, we have sequenced and assembled a low coverage genome for A. cahirinus and explored genes known to influence sleep and circadian rhythms in A. cahirinus and M. musculus. We are currently investigating these and other variables that might explain A. cahirinus sleep behavior including a comparison of genomic sequences between these species.

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Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: R01 MH092273-01A

Title: Proboscis extensions during sleep: a new sleep stage in *Drosophila* that clears the brain?

Authors: *B. VAN ALPHEN, A. AUGUSTIN, R. ALLADA;
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Abstract: Despite its relaxed appearance, sleep is an active process where the brain cycles through different stages of activity. Although the exact function of sleep remains the topic of a lively debate, its proposed functions include memory consolidation and metabolite clearance. Sleep in *Drosophila* has all the hallmarks of mammalian sleep, including homeostasis, altered brain activity, stages of lighter and deeper sleep, increased arousal thresholds and a characteristic posture. We discovered a novel sleep stage in *Drosophila*, during which the fly repeatedly and spontaneously extends its proboscis (mouth parts) in a stereotypical manner during inactivity and without any apparent stimulus, e.g, food exposure. Experiments in tethered flies showed that, during these proboscis extensions, arousal thresholds are even higher than during regular sleep, suggesting an even deeper sleep stage. Proboscis extensions are normally an appetitive response, where a hungry fly extends its mouthparts when its gustatory receptors come in contact with sugars. Also, proboscis extensions have also been shown to correlate with CO2 release during flight, suggesting that proboscis extensions facilitate respiration. So why do flies extend their proboscis during sleep? We found that proboscis extensions increase with starvation duration, suggesting a link to appetitive mechanisms, where either gustatory receptors become more sensitized or (more tantalizing) hungry flies replay wake experience. However, we also found substantial evidence that proboscis extensions facilitate a clearing mechanism. Increasing temperature, which would increase a fly's metabolic rate and CO2 production among other effects, increased the rate at which proboscis extensions occur. Also traumatic brain injury, which increases waste products among other changes, resulted in an immediate six-fold increase in proboscis extension rate. These data suggest that, besides for feeding, a fly uses its proboscis as a pump to increase the rate at which hemolymph flows through its body, increasing both gas exchange and metabolite clearance rate. We are currently investigating whether proboscis extension during sleep accomplishes waste clearance to optimize waking brain function and recovery from injury.

Disclosures: B. Van Alphen: None. **A. Augustin:** None. **R. Allada:** None.

Poster

167. Sleep: Behavior

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Program#/Poster#: 167.04/U37

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant NS082242 (YYL)

Restless Legs Syndrome Foundation (YYL)

NIH Grant MH064109 (JMS)

Title: Effect of striatal lesions on sleep and motor activity

Authors: *Y.-Y. LAI[1], K.-C. HSIEH[3], Y.-H. CHENG[2], J. M. SIEGEL[2];
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Abstract: Periodic leg movements (PLMs) in sleep have been reported in 10% of the general population. PLMs are also commonly seen in patients with striatal pathology, such as Parkinson's disease, Huntington's disease, rapid eye movement (REM) sleep behavior disorder, and attention deficit hyperactivity disease. We hypothesize that abnormal basal ganglia activity may cause PLMs. Our previous study showed that cats with lesions of the substantia nigra pars reticulata (SNR) generate PLMs in sleep. The SNR receives GABAergic projections from the striatum via direct and/or indirect pathways. In this study, we show that neurotoxic lesions of the striatum increase phasic motor activity in SWS in the rat. Thirteen young adult male Sprague-Dawley rats, weighing 280-400 g, were used. Among them, 8 rats were NMDA lesioned, 2 rats were sham lesioned, and 3 rats were controls. Under isoflurane anesthesia, electrodes were implanted in the skull to record EEG. Flexible wires were implanted in the hindlimb and neck musculatures to record muscle activity. Striatal or sham lesioned rats were given 50 nl NMDA (0.5 M) or 50 nl artificial cerebrospinal fluid injections into the striatum. Three days of sleep polysomnography were performed after the animal recovered from the surgery. No signs of tremor, rigidity, or bradykinesia in wake were observed in the lesioned animals. However, motor hyperactivity in slow wave sleep (SWS) was observed in all NMDA lesioned rats. The PLM index (PLMI; total number of PLM/total time of SWS/24 hour) in SWS in NMDA lesioned rats was significant different between control and sham lesioned rats ($F(2,10)=53.85$, post hoc $p<0.001$; PLMI, control rats: 9.6 ± 1.5, sham lesioned rats: 6.2±3.6, NMDA lesioned rats: 40.8 ± 12.9). The NMDA lesioned rat showed a circadian pattern of PLM, with higher PLMI in the dark phase than in the light phase (light phase: 36.7 ± 10.2, dark phase: 52.5 ± 17.8; paired T-test,

p<0.01). However, the number of isolated leg movements (ILMs) index in SWS (total number of ILM/total time of SWS/24 hour) was not different between NMDA lesioned and control/sham lesioned rats (F(2,10)=0.103). Striatal NMDA lesioned rats also showed a significant decrease in wake time (F(2,10)=5.27, post hoc p<0.05, minute/24 hour; control rats: 726.9 ± 61.4, sham lesioned rats: 810.2 ± 125.1, NMDA lesioned rats: 644.2 ± 73). We hypothesize that the striatum plays an important role in the control of sleep and motor activity in sleep.

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Poster

167. Sleep: Behavior

Location: Hall A

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Topic: E.08. Biological Rhythms and Sleep

Support: PO1 NS 083514

Title: A nap but not quiet rest prevents performance deterioration induced by intensive training

Authors: ***A. B. NELSON**[1], C. MOISELLO[1], D. BLANCO[1], J. LIN[1], P. PANDAY[1], J. P. BORKOWSKI[1], H. H. CHEN[1], M. GADALLA[1], C. FONTANESI[1], G. TONONI[2], C. CIRELLI[2], M. F. GHILARDI[1];
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Abstract: There is substantial evidence that sleep need occurs locally as a result of the cellular consequences of synaptic plasticity and learning during wake. Recent work in humans and animals showed that intense training in a task leads to a progressive increase in performance errors that are related to the occurrence of local sleep during wake. It is not known whether local sleep is triggered by plasticity like sleep itself or by a temporary neuronal exhaustion triggered by intense activity. In the first scenario, the cumulative cellular costs of increased synaptic strength leads to “tiredness”, i.e., higher energy consumption, greater need of cellular supplies, increased number of synapses. Tiredness can only be overcome by a process of synaptic renormalization that requires sleep. The other scenario implies a temporary neuronal exhaustion or “fatigue” due to momentary rundown of energy resources, depletion of synaptic vesicles/calcium or accumulation of adenosine. Such effects should be rapidly reversible by rest without sleep. Here we test the hypotheses that: 1. extended training on a task that requires intense learning leads to an increase in errors on tests that demand similar neural/cognitive

resources; 2. “tiredness” is the cause of such errors and thus performance can be restored by a nap but not by quiet rest. 16 subjects performed a task involving significant attentional and working memory load (VSEQ) in blocks of 45 minutes in two 4-hour sessions, one in the morning and the other in the afternoon. After each block, we assessed performance in a brief visual working memory test (MEM), which shares the same neural substrates and characteristics as VSEQ and in a motor reaching test (MOT), which taps into motor function. After the morning session, 8 subjects slept for 90 minutes (NAP) and 8 subjects rested quietly with eyes closed (REST). Performance in MEM was similar in the two groups in the morning. In the afternoon session, MEM performance in the REST group significantly degraded. Meanwhile, the NAP group maintained the same performance level across the two sessions, resulting in a significant difference between the NAP and REST groups in the afternoon session. Performance in MOT did not change across group or session. These results demonstrate that intense training leads to task-specific impairments, as performance decayed in MEM but not in MOT. Most importantly, we found that sleep but not quiet rest protects against performance deterioration induced by intensive training. Therefore, performance deterioration is likely due to neuronal “tiredness”, that is, to the increased cellular costs of accumulated synaptic strength, which can only be reversed by sleep.

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Poster

167. Sleep: Behavior

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Pilot award 8G12MD00760 to J.C.E.

Title: Effects of social stress on behavior are sleep-dependent

Authors: *C. L. GRAY[1], L. PINCKNEY[1], E. OLIVER[2], K. N. PAUL[1], J. C. EHLEN[1]; [1]Neurosci. Institute, Morehouse Sch. of Medici, Atlanta, GA; [2]Ross Univ. Sch. of Med., Picard, Dominica

Abstract: Sleep disorders are often comorbid with psychological disorders like post-traumatic stress disorder (PTSD). However, the relationship between sleep impairments and the development and maintenance of the behavioral hallmarks that accompany these disorders are mostly unknown. In the present study, we used social stress to induce social avoidance in a mouse model to investigate the role of sleep in the regulation of behavior. Our hypothesis is that sleep changes are necessary for the development and maintenance of social avoidance behavior. We implanted C57Bl6/J mice with electroencephalography and electromyography electrodes, recorded baseline sleep and then subjected the implanted mice to 10 days of social defeat stress using a resident-intruder paradigm. Social avoidance testing and sleep recordings were then performed immediately and 3 weeks after social defeat. We found pronounced changes in sleep-wake architecture that persisted for the 3 weeks following the stress. These changes were characterized by a redistribution of sleep during the 24-hour day and decreased REM-sleep amount. To our knowledge, this is the first mouse model demonstrating sustained changes in sleep and behavior. In addition, both NREM and REM sleep amount during baseline sleep recordings during the first four hours of the dark period significantly increased in mice that developed social avoidance after social defeat. Thus, our findings also provide evidence that changes in sleep-wake architecture predict susceptibility to social avoidance. We are currently investigating the effects of sleep manipulations in this model on behavioral responses to social defeat in order test our hypothesis that sleep changes are necessary for the development and maintenance of social avoidance. Understanding how sleep regulates the neural pathways responsible for social avoidance in our current model is likely to reveal new targets for the treatment of psychological disorders such as PTSD.

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Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: ANR-1 1-IDEX-0007

Title: Behavioral and wireless electrophysiological characterization of sleep in a lizard, the Argentine Tegu (Tupinambis merianae)

Authors: *P.-A. LIBOUREL[1], S. ARTHAUD[1], B. MASSOT[2], E. VAN REETH[3], A.-L. MOREL[1], M. SDIKA[3], O. BEUF[3], A. HERREL[4], P.-H. LUPPI[1];
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Abstract: Based on behavioral and electrophysiological characteristics, two distinct sleep states: NREM and REM sleep (or active and quiet sleep) have been identified in terrestrial mammals and birds, both homeotherms. However, whether reptiles, key taxa in understanding the evolution of sleep given their position at the base of the amniote tree, display those states remains unresolved. In order to precisely implant electrodes in specific brains regions, we first constructed a stereotaxic atlas for the argentine Tegu (Tupinambis merianae), based on MRI, micro CT Scanning, and immunohistochemistry. Next, wireless electrophysiological recordings were performed simultaneously with behavioral recordings obtained through four infrared cameras. We recorded EMG, EEG, ECG, EOG, brain and body temperature, and LFP with bundles of 35 µm diameter tungsten electrodes in the medial cortex (hippocampus homologue), the dorsal cortex, the dorso ventricular ridge, and the nucleus sphericus. The first lizard recorded displayed the behavioral characteristics of sleep during the entire night (12h/12h), including a stereotypical posture and a rebound of behavioral sleep after 9 h of gentle handling deprivation compared to the baseline. During the day, the animal displayed a different resting posture compared to sleep. The arousal threshold, or the delay to induce an arousal, was evaluated by activating a vibrator fixed on the head of the animal during 5 s every hour during 3 days. The arousal threshold was significantly higher during the sleep period compared to the diurnal rest. This behavioral state was associated with frequent eye movements (EM), whereas rare phasic EM and slight twitches of the toes where observed during the sleep period. Although the EEG of the lizard did not allow a differentiation between waking and sleeping behavior, the LFP recordings revealed a polymorphic signal in amplitude and frequency. A preliminary spectral analysis show differences depending on the area recorded during the two states. In particular, phasic oscillations in the 9-16 Hz spectral band appeared specifically during sleep. The rebound of sleep that follows the total deprivation was associated with an increase of the low frequency (0-4 Hz) and of the 9-16 Hz bands. Our preliminary results suggest that this lizard displays the behavioral characteristics of sleep. Some behavioral and electrophysiological phasic phenomena were observed during sleep, suggesting that this lizard has a non-homogenous sleep, raising the possibility that active sleep and a quiet sleep could be both present in reptiles.

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Poster

167. Sleep: Behavior

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Title: Sex differences in sleep following footshock stress in mice: an animal model for sleep disturbances after trauma exposure

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Abstract: Posttraumatic Stress Disorder (PTSD) is a serious psychiatric condition that develops in a significant minority of people exposed to trauma. Women are twice as likely as men to develop PTSD following trauma, but the mechanisms underlying women's greater vulnerability are poorly understood. Specific sleep impairments, including fragmented rapid-eye-movement (REM) sleep, have been implicated in the development of PTSD. In women, poor sleep maintenance was associated with subsequent PTSD in a single study with a small sample size. Increased interest in the role of sleep in the pathogenesis of PTSD has stimulated the development of rodent models that investigate sleep patterns following a trauma-like experience. However, these models have thus far examined only male animals. Therefore, we have expanded this approach to examine sex differences and hormonal influences in post-trauma sleep in mice. Male and female C57BL/6 mice were implanted with bipotential telemetric EEG recorders, and baseline sleep was continuously recorded for 5-6 days for males or one complete estrous cycle for females. Mice then received 15 footshocks over 15 minutes (0.5 mA, 0.5 sec duration) during the first half of their 12 hour light period. Following the footshock session, sleep was again recorded for 5-6 days for males or one complete estrous cycle for females. Sleep data were scored using the EEG recordings and locomotor activity to identify wake, REM sleep, and non-REM sleep (NREM) stages for each 10-sec epoch of recording. Preliminary data based on one male and one female across two days of baseline recordings and two days of post-shock recordings indicated that the percentage of REM sleep during the post-shock light period increased nearly twofold for both animals. During the post-shock dark periods, REM sleep increased in the male and decreased in the female. Analysis of wake and NREM sleep revealed that during both the baseline and post-shock periods, the female had a greater percentage of wake and a lower percentage of NREM sleep during dark periods than the male. During the 1st post-shock light and dark periods, both the male and the female mice had increased wake and decreased NREM sleep compared with their baselines, and this change appeared to be greater in the female than in the male. During the 2nd post-shock light and dark periods, wake and NREM sleep reverted, attaining levels beyond those seen during baseline recording. Our continuing

work will be determining if this possible sex difference in REM sleep during the post-shock dark periods persists, and if the stage of the estrous cycle at the time of footshock influences the sleep responses of females.

Disclosures: I. Kobayashi: None. **T.A. Mellman:** None. **E.K. Polston:** None.

Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: 2014 Swarthmore College NSE Surdna Summer Research Fellowship

2014-15 Swarthmore College Faculty Research Support Grant

Title: Sleep plasticity due to social interactions and mating in *Drosophila* melanogaster

Authors: A. E. DOVE, *C. G. VECSEY;
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Abstract: Sleep behavior is universal across taxa but the regulatory mechanisms underlying it remain mysterious. *Drosophila* sleep is plastic, and increasing literature suggests that social conditions are a source of such behavioral plasticity. We specifically examined the way sexual interactions affect *Drosophila* sleep behavior, at the level of individual mating events and general cohabitation, or socio-sexual interactions (SSI) between males and females. It was recently demonstrated that female flies experience a post-copulatory reduction in daytime sleep mediated by Sex Peptide (SP) transferred to the female during mating via seminal fluid. After successfully replicating these findings using electronic activity monitors to measure the sleep of individual flies, we discovered that the daytime sleep reduction lasted 6 days post-mating and had no apparent critical period with regard to female age or previous mating experience. We also established that *Drosophila* mating status has no bearing on circadian rhythmicity. This line of research provides evidence that sexual experience can temporarily modify an organism's sleep behavior and raises interesting questions regarding the mechanism by which SP reduces female daytime sleep. Social enrichment via increased population density causes flies to consolidate their sleep into a small number of long-duration episodes - an architectural pattern thought to be beneficial for memory formation. Analogously, we found that increased SSI caused a trend toward more consolidated sleep in both male and female flies - a result consistent with the

presumably increased memory demands in a cohabitating environment. In another non-significant trend, parental SSI appears to promote less consolidated sleep in offspring. These preliminary findings offer the intriguing possibility that social effects on sleep plasticity may involve epigenetic mechanisms. These results expand our understanding of the connection between an organism's sexual experience and its subsequent sleep behavior. Due to the neurochemical similarities between *Drosophila* and mammalian sleep, a biochemical understanding of the molecular basis of these forms of plasticity in *Drosophila* may provide mechanistic insight about social control of our own sleep patterns.

Disclosures: **A.E. Dove:** None. **C.G. Vecsey:** None.

Poster

167. Sleep: Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 167.10/V1

Topic: E.08. Biological Rhythms and Sleep

Title: Sleep and recreational psychostimulant use

Authors: ***M. M. SCHADE**[1], M. KRAYNOK[2], H. E. MONTGOMERY-DOWNS[1];
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Abstract: Objective: Psychostimulants (PS) used in the treatment of attentional deficit disorders have iatrogenic effects on sleep quality and quantity. Whether these effects extend to recreational (nonmedical) PS use has not been objectively assessed. We aimed to assess the sleep of college student nonmedical PS users and nonusers. **Methods/Analyses**: 14 self-identified PS users and 14 nonusers (N=28) contributed daily urine samples, self-reported PS use and wore a wrist actigraph for one week to quantify sleep. Participants were included as "users" in analyses if they had ≥1 positive urinalysis (n=8). On some days users self-reported (SR) PS but had a negative urine screen; these were considered independently from urine-positive days. Some self-reported users did not use per urine screen or SR (n=6) and were considered separately. Among users who used PS, one night from each of the four night categories (preceding/following use based on urine/SR) was randomly selected for each participant for analyses. Effect sizes were analyzed. **Results**: Effect sizes were large for differences in sleep efficiency (SE) on nights preceding nonuse days among users, users-who-did-not-use and nonusers per both urine (η^2=.10) and SR (η^2=.12). The same was true among users, users-who-did-not-use and nonusers for total sleep time (TST) on nights preceding nonuse per urine (η^2=.12) but not per SR. Among users only, SE preceding use vs. nonuse did not differ meaningfully per urine (d=0.02) or SR (d=0.30);

however, SE on nights following use was worse than nonuse, with considerable effect sizes per urine (*d*=0.55) and SR (*d*=1.08). Users' TST on nights preceding use was shorter vs. nonuse with large effects per urine (*d*=1.03) and SR (*d*=1.07). TST after SR was shorter vs. nonuse with a large effect (*d*=0.82) but this was not found based on urine-positive days (*d*=0.05). **Summary**: Differences in the sleep of PS users, users-who-didn't-use and nonusers may go beyond day-to-day use behavior based on the large effects observed in a comparison of their SE and TST preceding nonuse. For users, SE was lower *after* PS than after nonuse but TST after use did not consistently change. Instead, TST may be a valuable *predictor* of use behavior because of the large effect between nights preceding use vs. nonuse. Users may be attempting to compensate for less sleep by using PS for functional recovery. Alternatively, risk behavior is affected by sleep disruption and could open an avenue for sleep to influence day-to-day use choices.

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Poster

167. Sleep: Behavior

Location: Hall A

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Program#/Poster#: 167.11/V2

Topic: E.08. Biological Rhythms and Sleep

Title: Effects of acute sleep debt on decision-making in mice

Authors: *E. PITTARAS[1,2,3], M. CHENNAOUI[1,3], S. GRANON[2], A. RABAT[1,3];
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Abstract: Total sleep deprivation impairs many cognitive functions in healthy humans (Chee and coll., 2008) and especially risky decision-making (Killgore and coll., 2006). No studies have questioned if a sleep debt affects such cognitive processes in mice, if individual differences emerge and what are the neurobiological basis behind them. Therefore, in this work, we studied the effect of acute sleep debt on risky decision-making processes in mice. The Mouse Gambling Task (MGT) is a decision-making task under uncertainty and risky situations that lasts 5 days (Pittaras and coll., 2013). In this task, options are more or less advantageous in the long term. MGT is organized in two phases: a phase of exploration (discovery of options) and a phase of exploitation (better knowledge of options). Usually, during the exploration phase, mice do not show any preference between options. However, during the exploitation phase preferences for long-term advantageous options emerge with inter-individuals differences i.e. some animals

show marked preference for long-term advantageous options (safe) or for long-term disadvantageous options (risky) while others make choices in between (average). In this study, we have combined the MGT with an acute sleep debt (23 hours) by using a mobile platform that bounces at random frequency and intensity. We performed this protocol in two different ways: in the first case, one group of mice was sleep deprived before the beginning of the exploitation phase and in the second case, another group of mice was deprived at the end of the exploitation phase. MGT response latency was reduced in both cases. An acute sleep debt had a significant effect on the global animal's preference only when it's applied before the beginning of the exploitation phase. In such situation, inter-individuals differences persisted and behavioral profiles were amplified by the lack of sleep e.g. risky mice insisted even more for choosing long-term disadvantageous option. Finally, based on these observations we assumed that 1) an acute sleep debt has a deleterious effect on risky decision-making processes only when it occurs between the two MGT's phases by reducing global animal's preferences for long-term advantageous options and by amplifying inter-individual choice patterns and 2) such acute sleep debt seems to either increase mice locomotors reactivity (impulsivity?) or appetitive reinforcement.

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Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: KAKENHI 26870547

Title: Time-related changes of jaw-opening reflex excitability in quiet sleep in rats during post-surgical recovery

Authors: *R. ODAI[1], K. ADACHI[2], S. HINO[3], T. SIMOYAMA[3], H. SAKAGAMI[2], S. WATANABE[1], G. J. LAVIGNE[4], B. J. SESSLE[5];
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Abstract: Motor excitability and sleep stability are both influenced by the impact of surgery in laboratory animals and this can induce variability in outcomes of interest. For example, the excitability of the jaw sensorimotor system is depressed during quiet sleep (QS) compared with quiet awake before sleep (QWBS) in both non-human primates and rats, and the density of slow-wave sleep, a marker of sleep restorative function, is compromised by inflammation, neuropathic pain and post-operative pain. The aim of this study was to determine, in the post-surgical recovery period, the time course of the excitability of the jaw sensorimotor system and the stability of slow-wave/delta electroencephalographic (EEG) activity between awake and sleep states. Six-week-old male Sprague-Dawley rats (n = 9) received wire implantations, under isoflurane general anaesthesia, for electrocardiographic (EKG) recording, electromyographic (EMG) recordings from anterior digastric and masseter muscles, and EEG and electrooculographic (EOG) recordings. The jaw-opening reflex (JOR) was triggered by electrical stimulation of the tongue. Rats were allowed to recover from the surgical procedure for a week to become habituated to the observation environment. Three recording sessions were carried out at post-operative days 7 (D7), 12 (D12) and 16 (D16). Sleep-related electrophysiological features (e.g., EMG, EOG, EEG and EKG) were scored with epochs of 4 sec. During QWBS, the tongue was stimulated (200 μs) to define the threshold of the JOR in three stimulation trials separated by more than 5 min intervals. Then the animal was allowed to sleep freely and the JOR threshold was determined three times during QS as well as during QW after sleep. At D7, the JOR threshold was significantly lower during QS in comparison to QWBS (mean ± SEM: 86.7 ±63.9%, $P < 0.01$). At D12, the JOR threshold was similar between both states. At D16, the JOR was significantly increased during QS in comparison to QWBS (113.4 ±110.0%, $P < 0.05$), suggesting a recovery. Furthermore, during QS, the delta band (a marker of the sleep recovery process) occupied at D7 75.9 ±72.1% of the total EEG distribution of all 4 (delta, theta, alpha and beta) bands, and was reduced at D12 and D16 to 71.1 ±12.6% and 65.0 ±57.9% (NS), respectively, suggesting a return towards normality in association with the recovery process in the post-operative period. These findings suggest that both the excitability of the jaw sensorimotor system and sleep quality are recovered around post-operative days 12 to 16, and that it is essential to control for the effect of surgery on the outcomes of interest in sleep studies.

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Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: KTIA_13_NAP-A-I/1 (LA)

KTIA_NAP_13-2-2015-0010 (FM)

Title: Quantitative control of arousal via the midline thalamic nuclei

Authors: G. KOMLOSI[1], C. PORRERO[2], P. BARTHO[1,3], A. BABICZKY[1], C. DAVID[1], B. BARSY[1], F. CLASCA[2], L. ACSADY[1], *F. MATYAS[1,4];
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Abstract: Setting the right level of alertness is a key step for shaping behavioral actions. Midline thalamus (MT) was believed to play a crucial role in regulation of arousal. However, no direct experimental evidence was provided to support this assumption and, in addition, the role of thalamus to control arousal has recently been challenged. In this study we identify a midline thalamus selective marker (MSM) and use *in vivo* electrophysiology and optogenetics in MSM-Cre transgenic mice to quantitatively evaluate its role in arousal. MSM was expressed in over 98% of MT cells expressing cFos as a result of elevated arousal states. MSM also specifically labeled the thalamic cells (95-98%) which project to the main forebrain targets of midline thalamus (prefrontal cortex, amygdala and nucleus accumbens). Sindbis-Pal-GFP electroporation of MSM-positive thalamic cells visualized branching axons in multiple forebrain targets. Optogenetic activation of MSM-expressing cells in the paraventricular nucleus of MSM-Cre transgenic mice under urethane anaesthesia induced short latency, reliable multi-unit activation in the target regions, together with cortical desynchronization. In drug-free sleeping condition, brief (10 sec) optogenetic stimulation of MSM-expressing cells at 10 Hz resulted in persistent arousal accompanied by intense locomotion, lasting up to tens of minutes. Stimulation at 1 Hz was not effective. Shorter (1 sec) stimulations with 10 or 20 Hz induced brief micro-arousals, (transient EEG and muscle activation without overt sign of behavioral arousal). These effects were significantly stronger during NREM-sleep than in REM-sleep. Varying the strength of laser pulse demonstrated tight correlation between the parameters of stimulation and the magnitude of evoked effect (duration and onset of EMG/EEG changes) with little variability among the animals. Our results provide direct evidence that selective activation of MT could effectively initiate arousal in naturally sleeping mice. Furthermore, the present findings show MSM-Cre mice can be a powerful tool to investigate questions which require precise regulation of sleep states and arousal, as well as to study the role of MT in various behavioral contexts at the cellular and network levels.

Disclosures: **G. Komlosi:** None. **C. Porrero:** None. **P. Bartho:** None. **A. Babiczky:** None. **C. David:** None. **B. Barsy:** None. **F. Clasca:** None. **L. Acsady:** None. **F. Matyas:** None.

Poster

167. Sleep: Behavior

Location: Hall A

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Program#/Poster#: 167.14/V5

Topic: E.08. Biological Rhythms and Sleep

Support: Dept. of Veterans Affairs merit (RWM, RES)

Dept. of Veterans Affairs CDA (JMM)

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Title: Sleep spindles and memory: optogenetic manipulation of parvalbumin containing GABAergic neurons in mouse thalamic reticular nucleus

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Abstract: Sleep spindles are an 8-15 Hz waxing and waning brain oscillation observed during non-rapid eye movement sleep. Electroencephalographic (EEG) recordings during sleep show that schizophrenia patients have sleep spindle abnormalities, including reduction in spindle number, along with memory consolidation impairment. An increase in spindle activity following learning has also been reported in healthy humans and animals. However, the specific cellular mechanisms controlling spindles and a causal link between spindles and cognitive function are still to be determined. Rhythmic inhibition of thalamocortical neurons by the thalamic reticular nucleus (TRN) is likely the central regulator of spindles. In particular, parvalbumin (PV) containing GABAergic TRN neurons, known to be reduced in schizophrenia patients, may play a key role in the control of spindle generation. Here we tested whether optogenetic manipulation of TRN PV neurons during sleep following learning alters spindle generation and memory performance in mice. AAV-ArchT-GFP was injected into the TRN of PV-Cre mice. In order to assess memory, mice were tested with the novel object recognition (NOR) task which consists of two phases; the familiarization phase and the recall phase. The task measures recognition memory based on the mice's natural tendency to investigate a novel object. To decrease spindles, inhibition of TRN PV neurons was performed via laser illumination (532 nm, 1-min on, 4-min

off) during the 4-hr retention interval between the familiarization and recall phases, which is commonly accepted as a memory consolidation period. EEG/EMG recording was performed during the retention interval to monitor sleep-wake states and spindle activity. Memory performance was compared between inhibition (laser on) and control (no laser) conditions (N=4). Our data suggest that animals spent more time to investigate a novel object than a familiar object in control condition (no laser), indicating that animals successfully recalled an object presented during the familiarization phase. In contrast, recognition memory was significantly impaired following inhibition of TRN PV neurons during the 4-hr retention interval (laser on). Spindle density (a number of spindles divided by the length of retention interval) decreased with inhibition of TRN PV neurons and was positively correlated with NOR performance, whereas average delta power did not decrease with inhibition of TRN PV neurons. These results show that manipulation of spindles by optogenetic inhibition of TRN PV neurons following learning alters memory performance, indicating the role of sleep spindles controlled by TRN PV neurons in memory.

Disclosures: F. Katsuki: None. **J.M. McNally:** None. **S. Thankachan:** None. **R.E. Brown:** None. **J.T. McKenna:** None. **R.E. Strecker:** None. **R.W. McCarley:** None.

Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: Posgrado Ciencias Biologicas

CONACYT Fellowship to AMPB

Title: Cortical hyperarousal state in patients with insomnia comorbid with sleep apnea syndrome

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Abstract: Different studies have reported the coexistence of insomnia and obstructive sleep apnea syndrome (OSAS), in asignificant number of patients. Around 40% to 50% of patients with OSAS suffer insomnia symptoms, nevertheless no much is known about the

physiopathology of this comorbidity. On the other hand, primary insomnia is considered as a sleep disorder caused by combined physiological, cortical and cognitive hyperarousal states, which can interfere with the sleep onset or its maintenance and could produce alterations in diurnal functioning. The cortical hyperarousal, is understood as an extension of physiological alterations, it is defined as elevated alpha, beta and gamma power frequencies (which are typical during wakefulness), as well as in the wake period previous to sleep onset, and in the NREM and REM sleep periods compared to normal sleepers. With regards to insomnia comorbid to OSAS, some authors propose that patients are under a hyperarousal state, in the same way to primary insomnia patients, however only one study has looked forward to characterize its psychological and behavioral pattern with psychometric tests, authors concluded that these patients have a similar arousal pattern that primary insomnia patients. Considering the limited knowledge about the relationship between insomnia and OSAS, we considered important to study this coexistence by means of the characterization of the electroencephalographic (EEG), frequency pattern during the sleep onset period (which consists of the wake previous to sleep onset and the first N1 sleep period), compared to patients with OSAS, with the objective to determine if these patients are under a cortical hyperarousal state proved by means of the quantitative EEG analysis with the Fast Fourier Transform (FFT). For this purpose, we selected 60 seconds EEG artifact free segments, from the wake previous sleep onset and the first N1 sleep stage, in two groups, comorbid insomnia (n=4) and OSAS patients (n=8). Segments were analyzed by one second for each subject, derivation and vigilance states were determined by means of quantitative analysis program POTENCOR. Preliminary results are presented, we observed an elevated beta absolute power and a decreased theta absolute power in the wake previous sleep onset. In the N1 period, we observed an elevated alpha and beta relative power, as well as a relative theta and delta power reduction. We hope to obtain more conclusive results when we complete the proposed subjects sample (n=10), for each groups.

Disclosures: **A.M. Paramo-Brando:** None. **P. Duran:** None. **I.Y. Del Rio-Portilla:** None. **M. Miranda-Anaya:** None. **R. Haro:** None.

Poster

167. Sleep: Behavior

Location: Hall A

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Program#/Poster#: 167.16/V7

Topic: E.08. Biological Rhythms and Sleep

Title: Estradiol modulates sleep, thermoregulation, and cognition in ovariectomized female marmosets

Authors: *N. GERVAIS[1], S. S. VIECHWEG[2], J. A. MONG[3], A. LACREUSE[1];
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Abstract: Menopause in women is often associated with hot flashes, sleep disturbance and memory deficits. Reduced circulating levels of 17β-estradiol (E2) during this period may contribute to these symptoms. Studies using animal models support this idea. In the female rat, E2 replacement modulates normal sleep-wake patterns, nighttime core body temperature (Tc), and working-memory performance. However, it is not known whether E2 has similar effects in a more translational primate model. The common marmoset (Callithrix jacchus) is a small diurnal primate well suited for such studies, due to a short lifespan (~ 10 years), high cognitive abilities, and monophasic sleep patterns comparable to those of humans. E2 replacement influences working memory in the marmoset but no study to date has examined whether E2 influences sleep patterns and thermoregulation in this species. The main goal of the present study was to conduct a preliminary examination of the effects of E2 manipulations on sleep patterns, working memory, and Tc in middle-aged ovariectomized female marmosets. Two 6-yr old ovariectomized females were implanted with a telemeter (DSI TL11M2-F20-EET) that records EEG, EMG, and Tc. Sleep patterns (including total duration of REM, NREM, and wake, duration and number bouts), Tc, and performance on the Delayed Response (DR) task were recorded under baseline and E2 replacement (12 μg/kg/day, p.o.) conditions. Compared to baseline, E2 replacement modulated performance on the DR task in a delay-dependent manner. E2 replacement was also associated with lower Tc during the night. The number of nighttime arousals and delta power were both higher in the E2 replacement condition relative to baseline, which is consistent with the idea that E2 improves sleep quality. These preliminary results support the marmoset as a model for studying the relationships between E2, sleep disturbances, working memory deficits and Tc changes that may serve as indicators of hot flashes.

Disclosures: N. Gervais: None. **S.S. Viechweg:** None. **J.A. Mong:** None. **A. Lacreuse:** None.

Poster

167. Sleep: Behavior

Location: Hall A

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Program#/Poster#: 167.17/V8

Topic: E.08. Biological Rhythms and Sleep

Support: Integrative Neuroscience Initiative on Alcoholism West AA-20893

Title: Effects of ethanol consumption and withdrawal on the sleep/wake cycle of high alcohol preferring (cHAP) mice

Authors: *S. HUITRON-RESENDIZ[1], N. J. GRAHAME[3], A. J. ROBERTS[2];
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[3]Grad. Studies IUPUI, Indianapolis, IN

Abstract: Sleep disruptions in alcoholics are more common than in non-alcoholic individuals. One of the major problems with using rodents as a model to study the effects of alcohol on sleep is their relatively low voluntary alcohol intake. Recently, crossed High Alcohol Preferring (cHAP) mice were generated, and under a free-choice drinking paradigm these mice show escalating drinking behavior accompanied by intoxication and tolerance. To further characterize the behavioral and physiological profile of these mice, we examined the sleep/wake cycle along an ethanol consumption protocol in male and female cHAP mice. Under isoflurane anesthesia, a standard set of stainless steel screw electrodes was implanted for chronic EEG and EMG recordings. The EEG was recorded from electrodes placed in the frontal and parietal bone over the hippocampus. One wk after surgery, mice were recorded for 24 hr for baseline data, and then recorded at 2, 6, and 10 wk after being exposed to a continuous ethanol/water two bottle choice protocol. The mice consumed roughly 20 g/kg ethanol per day (with females drinking more than males) and blood alcohol levels taken 4 hr into the dark cycle were about 100 mg/dl. Following 1, 2, and 30 days of abstinence, mice were recorded again for 24 hr. The EEG and EMG were stored with a resolution of 128 Hz in the hard drive of a computer for the off-line analysis of the vigilance states and spectral analysis. There were no overall changes in wakefulness or slow wave sleep associated with ethanol drinking or withdrawal/protracted abstinence. However, cHAP mice had a significant decrease in REM sleep 6 wk into drinking ($p = 0.004$), which reversed by 10 wk, suggesting a possible tolerance to the effects of ethanol on REM sleep. Interestingly, the amount of REM sleep was significantly reduced again relative to baseline levels at 1 ($p < 0.0001$), 2 ($p = 0.003$) and 30 ($p = 0.02$) days into withdrawal. These results support other studies showing that the amount of REM sleep is reduced by chronic alcohol exposure, and also suggest that the mechanisms involved in the regulation of REM sleep are uniquely compromised by ethanol.

Disclosures: S. Huitron-Resendiz: None. **N.J. Grahame:** None. **A.J. Roberts:** None.

Poster

167. Sleep: Behavior

Location: Hall A

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Topic: E.08. Biological Rhythms and Sleep

Support: SNF

ERC

Title: Analysis of electrophysiological cortical activity during sleep in turtles and lizards

Authors: *J. M. ONDRACEK, A. KOTOWICZ, M. SHEIN-IDELSON, G. LAURENT;
Max Planck Inst. For Brain Res., Frankfurt, Germany

Abstract: Sleep in birds and mammals has been characterized electrophysiologically as the waxing and waning of two alternating phases of brain activity. Slow wave sleep (SWS) consists of high-voltage, low frequency, synchronized activity. In contrast, paradoxical sleep (PS) resembles activity in the awake brain: erratic, desynchronized oscillations of low voltage and high frequency. In some species PS is accompanied by concurrent rapid eye movements (REMs). Reptiles, like birds, belong to the class of sauropsids and probably share a (now extinct) stem amniote ancestor with mammals. Because a homogeneous 3-layer cortex is found in all living reptiles, it is believed to have been present in the common ancestor to mammals and sauropsids. These evolutionary relationships make reptilian brains great models to explore the structural and functional evolution of vertebrate neural circuits [1]. Furthermore, comparative studies of brain structure and development suggest that the reptilian cortex may be homologous to mammalian hippocampus, which has been shown to be involved in memory consolidation during sleep [2]. Although reptiles display clear evidence of behavioral sleep and often assume stereotypical sleep postures, most early investigators failed to detect evidence of SWS in the reptilian cortex. The most consistent EEG correlate of behaviorally defined sleep was reported to be the appearance of intermittent high-voltage spikes (large sharp waves, LSW) arising from background activity, and early studies reported pharmacological similarities between LSWs and spikes arising from the mammalian hippocampus during sleep [3]. To investigate sleep-related electrical activity in the reptilian cortex and its possible similarity to mammalian hippocampal activity, we chronically implanted subdural surface arrays and penetrating depth electrodes to record from the dorsal cortex (DC) of two species of reptiles (Trachemys scripta and Pogona vitticeps). Our data suggest that LFP dynamics during sleep contain, among others, clear bouts of LSWs. In contrast, LFP during active states was characterized by frequent narrow spikes of smaller amplitude. Autocorrelations calculated over long (20 min.) durations of LFP revealed a wide central peak (5s) and multiple sidebands during sleep, and a narrow central peak (<1s) with no sidebands during active states. These results suggest the existence of functional similarities between reptilian cortex and mammalian hippocampus, with potential significance for our understanding of the evolution of sleep brain dynamics in amniotes. [1] Naumann et al., 2015 [2] Siapas & Wilson, 1998 [3] Hartse & Rechtschaffen, 1982

Disclosures: **J.M. Ondracek:** None. **A. Kotowicz:** None. **M. Shein-Idelson:** None. **G. Laurent:** None.

Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: CONACYT Grant MAMG- I0007-2014-01-232334

Title: Relation between sleep time duration and increase body mass index in a Mexican population

Authors: ***Á. PAVÓN ROSADO**[1], J. E. MOLINA-ALFONSO[2], E. AGUILAR-SÁNCHEZ[2], M. SAAVEDRA-VÉLEZ[3], C. ESCOBAR-BRIONES[4], M. A. MELGAREJO[2];
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Abstract: Sleep is considered a restorative, essential and periodic phenomenon in humans that is able to modulate various physiological processes. It is known that sleep plays important roles in the endocrine regulation of hormones involved in the metabolism and as a modulator of all the organism. In this regard, some studies have confirmed that expression of metabolic hormones is affected with decreased total sleep time. Metabolism of glucose and lipid degradation as well as modulation in expression of hormones as ghrelin, leptin, insulin, growth hormone, cortisol, among others, depend on ultradian sleep cycles. The goals of this study were 1) to determine whether a relationship exists between total sleep time and increased body mass index. This latter was considered as a parameter of overweight and obesity, and 2) to correlate the sleep time and anthropometric measurements and clinical tests, as glucose, triglycerides and cholesterol, to associate the parameters of overweight and obesity in a university student population. For these purposes, three surveys were applied to 3128 students of both sexes to measure quality and quantity parameters of sleep, the Epworth scale for measuring daytime sleepiness, the Athens scale for measuring insomnia, and Pittsburgh scale for quality and quantity of parameters of total sleep. Data from these surveys were collected, analyzed (by sex) and were related to anthropometric and clinical parameters. All procedures were conducted following the declaration of Helsinki, and the participants signed an information form. The results showed that subjects who slept less than 4h presented overweight it is related with the increasing of Body mass index

(BMI) in comparison with those males sleeping 8 h. In contrast, there were not statistical differences between sleep hours and BMI in females. In addition, males short sleep increased glucose in comparison with men who slept from 8-9 h presented lower glucose levels. Conversely, there were not statistical differences in females, more clinical data are analyzed. Overall, these results suggest that the reduction of sleep contributes to weight gain and increases the risk to develop metabolic syndrome and diabetes type 2 in a young Mexican population.

Disclosures: Á. Pavón Rosado: None. **J.E. Molina-Alfonso:** None. **E. Aguilar-Sánchez:** None. **M. Saavedra-Vélez:** None. **C. Escobar-Briones:** None. **M.A. Melgarejo:** None.

Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: CIHR

Polaris, AIHS

NeuroTek Fund

Title: An unsupervised brain machine interface to study the maintenance of wakefulness in rodents

Authors: *H. W. STEENLAND[1,2];
[1]Univ. of Lethbridge, Lethbridge, AB, Canada; [2]NeuroTek, Toronto, ON, Canada

Abstract: Sleep can be set aside if a superseding interests, yet there are no rodent models or methods to study how this is possible. Previous studies in rodents show that they can forgo sleep to explore an environment (Gompf et al., JNS 2010) and can even be trained to wake-up from sleep to receive rewards (Wilcox, Physiol & Behav. 1975). A software-hardware platform was developed to detect when an animal is getting drowsy in real-time (based on electroencephalogram (EEG) and electromyographic (EMG) data. Upon detection of drowsiness, one of two tones is played to arouse the animal before they fall asleep. After the tone is played the rodent must maintain arousal in order to receive a reward in the form of medial forebrain stimulation. Should the rodent fail to maintain arousal as detected by desynchronized electroencephalogram, they will not be rewarded. Mice were found to learn this task with 24 hours of unsupervised training (~200trials) in which rodents sleep through the neutral tone and

are aroused and maintain wakefulness for the rewarded tone. Future studies will aim at uncovering the neurobiological mechanism for the maintenance of wakefulness.

Disclosures: H.W. Steenland: None.

Poster

167. Sleep: Behavior

Location: Hall A

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Topic: E.08. Biological Rhythms and Sleep

Title: Does circadian rhythm contribute towards sleep inertia: a meta - analysis

Authors: *S. NAIR[1], P. SAHOTA[2], M. THAKKAR[2];
[1]Univ. of Missouri-Kansas City Med. Sch., Columbia, MO; [2]HSTMV Hospital/University of Missouri-Columbia, Columbia, MO

Abstract: INTRODUCTION: Sleep inertia is a transient decrease in performance and alertness that occurs immediately after awakening. The effects of sleep inertia are variable and can last from 1 minute to approximately 4 hours upon awakening. These cognitive and performance decrements are severe and can have profound impact on decision making and/or performing critical tasks. Sleep is regulated by the interaction of two processes: Process S or the homeostatic drive that maintains the “constancy” in the amount of sleep and Process C that maintains the timing of sleep. Since the nadir of the Process C occurs in the early morning hours, some previous studies have implicated that circadian rhythm as a major contributing factor in sleep inertia. However, others have been unable to demonstrate that sleep inertia has any circadian rhythm. Thus, to further examine the role of circadian rhythm in sleep inertia, we have decided to perform a meta-analysis. METHODS: Meta-analysis is a statistical method of combining results of individual studies to obtain the most reliable conclusions. In clinical medicine, the power of meta-analysis is indispensable for building feasible and practical guidelines. We’ve started reviewing the literature and are in the process of performing a meta-analysis to further understand the role of circadian rhythm in the development and maintenance of sleep inertia. We have conducted a “PubMed” search using free-text search term “sleep inertia” to identify studies. In addition, we will also perform searches using the first, last, and corresponding authors of any of the reports identified in the initial search. RESULTS: To date, our search has retrieved 149 publication. Our initial preliminary results indicate a role of circadian rhythm in sleep inertia. However, at this time our results are preliminary and the study is ongoing. We expect to

complete the review and the meta-analysis in couple of months and present our findings at the SFN meeting in October.

Disclosures: **S. Nair:** None. **P. Sahota:** None. **M. Thakkar:** None.

Poster

167. Sleep: Behavior

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European Union ERC-2010 AdG 268911

Forschungskredit Univrsität Zürich

Title: Ultradian dynamics of electrical brain activity in a zebra finch during sleep

Authors: *A. L. VYSSOTSKI, A. STEPIEN, R. H. R. HAHNLOSER;
Inst. of Neuroinformatics, Univ. of Zurich and ETH Zurich, Zurich, Switzerland

Abstract: Both mammals and birds have two distinguished phases of sleep: slow-wave sleep (SWS), characterized by slow-waves of the large amplitudes in electroencephalogram (EEG), and rapid-eye movement (REM) sleep, characterized by small fast wakefulness-like waves. In humans typically four or five SWS/REM cycles with a period of 70-90 minutes each occur within a night. In zebra finches similar cycles with a shorter period have been detected. However, the mechanisms of this self-organized ultradian periodicity are poorly understood. To get better description of the process we analyzed wide-band electrical brain activity, brain temperature and body movements in sleeping zebra finches. In the first experiment we recorded wide-band (1 Hz - 10 kHz) electrical brain activity from a pair of differential epidural AgCl electrodes (5-25 kOhm) placed over left and right hemisphere respectively in four male zebra finches during several nights. The brain temperature was recorded by the thermistor placed over the left hemisphere, and locomotor was quantified with the help of infrared camera. Recordings were done in sound-proof metallic chamber shielding electromagnetic disturbances. We have found that changes of high-frequency activity (HFA, 0.3-3 kHz) happened synchronously in both hemispheres with the main period of approximately 30 min in all investigated animals. Synchronous changes of activity pointed at centralized control of HFA, suggestively intermediated by the thalamus. Brain temperature changed co-directionally with the HFA,

indicating, as expected, that HFA is associated with increased metabolic activity. Slow-wave activity (SWA, < 4 Hz) dynamics was opposite to those of HFA, consistent with the concept that SWA occurs in the gaps of prolonged neuronal hyperpolarizations and firing. Shorter periods HFA dynamics undergo power law distribution that can be explained by a simple cellular automaton model. In the second experiment, to check how forebrain HFA can be controlled by mesencephalon and diencephalon, we recorded local filed potentials (LFPs) and neuronal activity from nucleus interfacialis (NIf), a part of avian vocal system receiving direct input from the thalamic nucleus uvareformis in a zebra finch during natural sleep. We exploited 16-channel single chunk silicon probe (Neuronexus Inc.) placed in a way that its tip was below NIf in the Field L2a, the middle part was in NIf, and the top-most part was above NIf in the Field L1. Neuronal firing and HFA in NIf had similar to recorded in the previous experiment ultradian rhythm with the main period about 30 min, suggesting common mechanisms of activity modulation in the superficial and deep brain structures.

Disclosures: **A.L. Vyssotski:** None. **A. Stepien:** None. **R.H.R. Hahnloser:** None.

Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: CONACYT Grant MAMG- I0007-2014-01-232334

Title: Misalignment time of sleep affect the glucose levels and Body Mass Index in a young Mexican population

Authors: ***E. AGUILAR**[1], J. E. MOLINA-ALFONSO[1], Á. PAVÓN-ROSADO[2], M. SAAVEDRA-VÉLEZ[3], C. ESCOBAR-BRIONES[4], M. A. MELGAREJO[1];
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Abstract: Sleep is an essential process for the brain and the maintaining of metabolism. Inadequate sleep and obesity are important public health problems. Studies provide consistent evidence that recurrent sleep curtailment and shifting time of sleep may increase the risk of obesity and diabetes. In this sense the circadian clock, controls sleep, onset of sleep, also regulates energy homeostasis and the disruption is associated with obesity. The fact that many

people in our society shift their sleep and activity times several hours between the work week and the weekend is comparable to disruption like jet lag. The aim of the present study was to investigate associations between time shift of sleep on working days and free days in the Mexican population, for this purpose a total of 1346 students of Universidad Veracruzana (18-25 years) participated. The study was conducted in according to standards established by the declaration of Helsinki as well as their applied the informed consent. All subjects completed a questionnaire Epworth scale for measuring daytime sleepiness, Athens scale for measuring insomnia and scale parameters Pittsburght for quality and quantity of total sleep. Also to investigate, onsets sleep on working days and free days. Data were classified and related to anthropometric and clinical parameters. The results suggest that time shift of sleep between work days and free days increases the glucose levels(mg/dl), in the group with 4 hours of shift time sleep (90.50+1.5) compared with group 1hour shift time sleep (88.09+1.24) $p<0.05$ and delayed time of sleep increases the Body Mass Index (BMI) . Our data suggest that the difference in a midpoint of sleep on working days and free days, may contribute to weight gain and modify glucose levels, this condition might promote development of obesity and diabetes mellitus.

Disclosures: **E. Aguilar:** None. **J.E. Molina-Alfonso:** None. **Á. Pavón-Rosado:** None. **M. Saavedra-Vélez:** None. **C. Escobar-Briones:** None. **M.A. Melgarejo:** None.

Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Title: Intermediate decrease of CSF orexin (hypocretin) and significant obesity in Prader-Willi syndrome patients compared with narcolepsy and idiopathic hypersomnia

Authors: *T. KANBAYASHI[1,2], M. OMOKAWA[1], T. AYABE[3], T. NAGAI[3], A. IMANISHI[1], Y. OHMORI[1], K. TSUTSUI[1], J. TAKAHASHI[1], Y. KIKUCHI[1], Y. TAKAHASHI[1], E. NARITA[1], S. SATO[1], K. TSUKAMOTO[4], S.-I. UEMURA[5], Y. SAGAWA[1], T. SHIMIZU[1]; [1]Akita Univ. Sch. Med., Akita City, Japan; [2]Intl. Inst. for Integrative Sleep Med. (WPI-IIIS), Univ. of Tsukuba, Tsukuba, Japan; [3]Dept of Ped, Dokkyo Med. Univ. Koshigaya Hosp., Koshigaya, Japan; [4]Akita Kaiseikai Hosp., Akita City, Japan; [5]Akita Univ. Grad. Sch. of Hlth. Sci., Akita City, Japan

Abstract: Introduction: Prader-Willi syndrome (PWS) is an acquired neurodevelopmental disorder caused by deletion in chromosome. Patients with PWS often exhibit excessive daytime

sleepiness (EDS), increased appetite, and obesity. As well as in narcolepsy, orexin (hypocretin) may be responsible for the symptoms. However, report regarding the correlation between obesity and orexin level in PWS is scarce. Here we discuss the relationship between obesity and orexin level in PWS patients, compared with narcolepsy and idiopathic hypersomnia (IHS) patients. Methods: We examined orexin levels in the cerebrospinal fluid (CSF) of 10 patients with clinically and genetically confirmed PWS with EDS, compared with 37 cases of narcolepsy patients with cataplexy, and 13 cases of IHS patients. Patients' body mass index (BMI) at the time of CSF examination was determined. All patients are Japanese and aged 15 to 50 years old. Results: CSF orexin level (mean ± S.D.) in PWS, narcolepsy, and IHS was 194 ± 45 pg/ml, 79 ± 75 pg/ml, and 291 ± 74 pg/ml, respectively. BMI (mean ± S.D.) in PWS, narcolepsy, and IHS was 34.4 ± 8.5, 23.4 ± 4.0, and 22.6 ± 2.1, respectively. Orexin level in PWS was significantly higher than narcolepsy, and lower than IHS. BMI was significantly higher in PWS than narcolepsy and IHS. Conclusion: Orexin level and BMI was higher in PWS than narcolepsy patients. Intermediately decreased orexin level in PWS patients suggests that both impaired secretion and receptor function of orexin may play a role in exhibiting symptoms such as sleepiness, increased appetite and obesity.

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Poster

167. Sleep: Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 167.25/V16

Topic: E.08. Biological Rhythms and Sleep

Support: NSU President's Faculty Development and Research Grant: Index 335325

Title: The effects of short- and long-term sleep loss on biological and psychological measures of health

Authors: *J. L. TARTAR[1], A. I. FINS[2], L. D. HILL[1], M. R. LORENZETTI[2], T. J. A. CRADDOCK[1];
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Abstract: Despite strong associations between sleep loss and health, there is no clear understanding of the mechanisms through which sleep loss alters the physiological processes that can lead to poor health. In an effort to shed light on the complex sleep-immune-stress relationship we focused on biochemical and psychological factors that previous research suggests might be essential to uncovering the role of sleep in health. To that end, we analyzed the effect of self-reported volitional chronic sleep restriction as well as short term sleep deprivation (SD) on a series of sleep and psychological health measures as well as biomarkers of health including immune functioning/inflammation (IL-1β), stress (cortisol), and sleep regulation (melatonin) in young adult females. We found that across multiple measures, ongoing sleep loss was associated with decreased psychological health and a reduced perception of self-reported physical health. New to our study, we found that volitional CSR was related to increased cortisol and increased inflammation as measured through IL-1β levels. We separately looked at individuals who experienced CSR with and without a delayed sleep time and found that IL-1β levels and cortisol levels were significantly elevated in both groups, relative to those who did not experience CSR or a late sleep time. We did not observe any changes in melatonin across groups and melatonin levels were not related to any sleep measures. Unlike CSR, we did not observe any changes in cortisol with 24 hours of SD. Analyses of inflammation and associated biomarkers of health with SD are ongoing, but preliminary analyses suggest that the effects of SD on inflammation and psychological health mimic those of CSR. Overall, our results show how an increase in a pro-inflammatory process and HPA axis activity relates to volitional CSR, with and without a delayed sleep time and how these mechanisms relate back to psychological and self-reported health.

Disclosures: **J.L. Tartar:** None. **A.I. Fins:** None. **L.D. Hill:** None. **M.R. Lorenzetti:** None. **T.J.A. Craddock:** None.

Poster

167. Sleep: Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: The Grants-in-Aid for scientific research (C) (24592816)

The Grant-in-Aid for Research Activity start-up (26893270)

Title: The effects of citalopram on masseter and neck muscle activities in mice

Authors: Y. NOGAWA[1,3], *A. MOCHIZUKI[1], K. KATAYAMA[2], M. IKEDA[2], Y. ABE[2], S. NAKAMURA[1], K. NAKAYAMA[1], M. KIYOMOTO[1], T. KATO[4], K. BABA[2], N. WAKABAYASHI[3], T. INOUE[1];
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Abstract: Bruxism is characterized by involuntary masseter muscle activity during not only sleep state but also awake state. Recently it is reported that depressed patients who take citalopram, which is one of the selective serotonin reuptake inhibitors (SSRIs), for long periods have been frequently associated with bruxism. However, it is not clear whether SSRIs are involved in the induction bruxism. In this study, we first examined the effects of citalopram on activity of the masseter and neck muscles during wakefulness (W), non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep in mice. Mice were prepared for chronic recording of electroencephalography (EEG), electro-oculography (EOG), and electromyography (EMG) of the masseter and neck muscles. After recovery and training periods, mice were recorded for 24 h as the control condition. Subsequently osmotic minipumps, which were filled completely with saline (n = 4) or citalopram hydrobromide (16.5 mg/ml, n = 5), were implanted under the skin of the back and saline or citalopram hydrobromide (10 mg/kg/day) were continuously delivered from the minipump. After 5 days of saline/citalopram administration, the recording sessions of the administration conditions were acquired for 24 h. Activities of EEG, EOG and EMG were quantified for each 10-s epoch, and EMG measurements of masseter and neck muscles were normalized using mean activity during total W over 24-h period under control condition. Under the control condition, histograms of masseter and neck activities during W and NREM sleep showed bimodal and unimodal distributions, respectively, and it seemed that the masseter and neck muscle activities during W slightly increased by citalopram administration. Both in the dark and light periods, the mean activities of the masseter muscle during W, NREM sleep, or REM sleep after citalopram administrations were not different from the activities during W, NREM sleep, or REM sleep after saline administration, respectively. Citalopram did not alter the ratios of numbers of the two clusters that were composed of greater and lesser masseter activities in any vigilance state compared with the ratios of the saline administrated group. Similarly, citalopram did not alter the mean activities of the neck muscle in any vigilance state both in the dark and light periods. These results suggest that citalopram using osmotic minipump administration for 5 days may not affect activities of the masseter and neck muscles.

Disclosures: Y. Nogawa: None. **A. Mochizuki:** None. **K. Katayama:** None. **M. Ikeda:** None. **Y. Abe:** None. **S. Nakamura:** None. **K. Nakayama:** None. **M. Kiyomoto:** None. **T. Kato:** None. **K. Baba:** None. **N. Wakabayashi:** None. **T. Inoue:** None.

Poster

167. Sleep: Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 167.27/V18

Topic: E.08. Biological Rhythms and Sleep

Support: Max Planck Society

Human Frontiers Grant RGP0004/2013

Title: Can sleeping birds preen? Dissociation between sleep-related EEG activity and behavior in pigeons

Authors: *N. C. RATTENBORG[1], D. MARTINEZ-GONZALEZ[1], J. VAN DER MEIJ[1], G. J. L. BECKERS[2], A. L. VYSSOTSKI[3], M. POMPEIANO[4], E. BALABAN[4];
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Abstract: Dissociations between sleep-related brain and behavioral states have been observed in mammals, including humans. Like mammals, birds exhibit rapid eye movement and slow-wave sleep (SWS), the later associated with homeostatically-regulated EEG slow-wave activity (SWA). Although elevated SWA has also been observed during some episodes of feather preening in birds, movement artifacts have not been ruled out as an explanation for this activity. Consequently, preening has usually been taken as an indicator of waking in birds. We demonstrate that slow-waves during preening are not movement artifacts, and preening with slow-waves reflects a dissociated state between the forebrain and brain regions controlling preening. EEG and head movements were recorded in domestic pigeons (Columba livia) with a 4-EEG-channel head-mounted data logger (Neurologger 2A) including a 3D accelerometer. Two epidural electrodes were placed on each hemisphere (mesopallium and hyperpallium) and referenced to an ipsilateral electrode on the cerebellum. All signals were sampled at 200 Hz. Behavior was video recorded. During preening, pigeons typically closed their eyes and exhibited rapid, repetitive movements of the head. Preening could occur with waking EEG activity or with high-amplitude, slow-waves (1-3 Hz) similar to those occurring during normal SWS. Individual slow-waves were not correlated with individual preening head movements. Moreover, changes in interhemispheric and intrahemispheric coherence between wakefulness and normal SWS followed a similar pattern during preening with slow-waves. During wakefulness, interhemispheric coherence was higher than intrahemispheric coherence, whereas during normal SWS and preening with slow-waves, interhemispheric coherence decreased and intrahemispheric

coherence increased relative to wakefulness. In conclusion, preening can occur together with SWS-like EEG activity recorded from the forebrain. Preening is likely to involve central-pattern-generating circuitry in the midbrain, brainstem and/or spinal cord that does not require forebrain control, which is why it may be activated in a dissociated fashion during sleep. From an adaptive perspective, given that birds sacrifice visual vigilance during preening, presumably to protect the cornea, it might make sense for them to simultaneously discharge some sleep pressure in the forebrain.

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Poster

167. Sleep: Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 167.28/V19

Topic: E.08. Biological Rhythms and Sleep

Support: NIHAA017718

R43NS83218

Title: The relationship of sleep-wake and anxiety phenotypes analyzed using genetic mouse models and mice of different ages

Authors: *K. M. HAMRE[1], S. LATTIMER[2], S. S. JOSHI[3], K. D. DONOHUE[3], B. F. O'HARA[3];
[2]Dept. of Anat. & Neurobio., [1]Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN; [3]Univ. of Kentucky, Lexington, KY

Abstract: Sleep and anxiety are interrelated phenotypes with anxiety having the ability to cause sleep disruptions and sleep disruptions contributing to heightened anxiety. While the relationship between these two variables is evident when one is disrupted, the relationship in normal, baseline states has not been fully evaluated. The evaluation of this relationship may provide information about whether individuals with specific sleep-wake characteristics are more or less likely to show increased anxiety phenotypes and vice versa. To test this relationship, 2 experiments were run in mice of differing genetic backgrounds, ages and sexes. Sleep-wake phenotypes were assessed using the non-invasive Piezo system. In experiment 1, adolescent (beginning at approximately 35 days of age) and adult (over 90 days of age) male and female C57BL/6J,

DBA/2J and BALB/c mice (n = 10/ strain/ sex/ age) were tested. The same mice were tested in both to assay sleep-wake and anxiety phenotypes assessed using elevated plus mazes and activity chambers. Sleep-wake phenotypes were measured for 5 days and the Piezo system was easily able to assess sleep in adolescent mice. As expected, strain and sex differences were observed across many of the measures. In comparing sleep-wake phenotypes, there was little difference across the ages suggesting that sleep-wake phenotypes are stable by adolescence in mice. Correlational analyses between sleep-wake and anxiety phenotypes showed only a few significant correlations. The exception was the measure of primary wake onset (when wake percentage increases dramatically, usually near dark onset) which was significantly correlated with many anxiety measures across sexes and ages. Experiment 2 used BXD mice and bioinformatic analyses to assess the relationship between sleep-wake phenotypes and anxiety measures using archived measures found on GeneNetwork.org. Different mice were tested for each phenotype and both males and females were examined for many of the measures. Similar to the results observed in experiment 1, the majority of anxiety measures were not significantly correlated with any of the sleep measures. However, there were several interesting relationships, some of which occurred in a sex-specific manner, including the relationship between adrenal weight and sleep parameters. These results suggest that while globally, the relationship among sleep-wake and anxiety phenotypes are relatively weak, individual measures show strong relationships between the two. This further suggests that these phenotypes may be useful predictors of which individuals with sleep problems are at higher risk for developing anxiety disorders and vice versa.

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Poster

167. Sleep: Behavior

Location: Hall A

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Program#/Poster#: 167.29/V20

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant R01DE22912

Title: Caffeine prevents the development of pain hypersensitivity after acute and chronic sleep restriction in mice

Authors: ***C. ALEXANDRE**[1,2], A. LATREMOLIERE[3,2], G. MIRACCA[1], T. E. SCAMMELL[1,2], C. J. WOOLF[3,2];

[1]Neurol., Beth Israel Deaconess Med. Ctr., Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]Neurobio., Boston Children's Hosp., Boston, MA

Abstract: The average American adult obtains 15-25% less sleep than optimal, and up to 10% of the adult population have fragmented, non-restorative sleep from sleep apnea or other sleep disorders. Experimental sleep deprivation increases pain sensitivity in healthy, pain-free volunteers, and healthy but sleepy individuals experience hyperalgesia. We examined how sleep disturbance affects pain sensitivity in mice and how it could be reduced. All mice (c57Bl6/j) were instrumented for sleep recordings (EEG/EMG) and allowed 15 days of recovery. After collection of baseline pain measures and sleep-wake behavior, we deprived mice of sleep for 6, 9 or 12 hours (using gentle, minimally-stressful procedures) and then immediately tested their mechanical (von Frey filaments) and heat sensitivity (hotplate at 52C and acetone). Sleep deprivation (SD) sessions were separated by at least 10 days. Control mice (not sleep-deprived) were tested together with the sleep-deprived animals in a blinded fashion. Both mechanical and heat sensitivity dose-dependently increased with the duration of SD, while no changes occurred in control mice allowed to sleep ad libitum. In addition, 5 days of chronic sleep restriction (6 hours SD daily) increased pain sensitivity to a similar degree as 12 hours of acute SD, suggesting that the changes in pain sensitivity are cumulative. As these interventions increase sleepiness, we tested whether medications that improve alertness could reduce or prevent pain hypersensitivity in sleep-deprived animals. We treated mice with caffeine (20 mg/kg i.p.) two hours before the end of an acute SD session (9 hours) and on the 5th day of repeated sleep restriction. Caffeine fully prevented the development of mechanical and heat pain hypersensitivity in both paradigms, but it had no effect on pain sensitivity in mice allowed to sleep ad libitum. We also subjected a group of mice to chronic sleep fragmentation (CSF; 9 hours of automated CSF over 5 consecutive days) to test if disrupting sleep continuity increases pain sensitivity. CSF severely disrupted normal sleep architecture by shortening sleep bouts (mean duration of NREM sleep episode reduced by ~50%) without reducing the total amount of NREM sleep. Sleep fragmentation did not affect pain sensitivity for any modalities tested. Altogether these results suggest that sleep loss, rather than sleep fragmentation, is responsible for the development of pain hypersensitivity. Most likely, sleep loss increases pain hypersensitivity by affecting nociceptive processing within the CNS, and importantly this can be prevented by promoting alertness and reducing sleepiness.

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Poster

167. Sleep: Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 167.30/V21

Topic: E.08. Biological Rhythms and Sleep

Support: RFH Grant 14-06-00963

Title: Circadian and sleep-related gene polymorphisms are associated with chronotypes and road accident history in professional bus drivers

Authors: *V. DOROKHOV[1], A. N. PUCHKOVA[1], A. O. TARANOV[1], T. V. TUPITSYNA[2], P. A. SLOMINSKY[2], V. V. DEMENTIENKO[3];
[1]Sleep and Wakefulness Neurobio. Lab., IHNA RAS, Moskva, Russian Federation; [2]IMG RAS, Moskva, Russian Federation; [3]Neurocom, Moskva, Russian Federation

Abstract: Homeostatic mechanisms of the brain as well as circadian clock can significantly influence the person's alertness level. For the drivers reduced alertness may manifest as unperceived increase in road accident risk. Due to individual differences in sleep, biological clock functions and sleep deprivation resistance a person may be better suited to work at a particular schedule. Thus appears the need to assess objective data and search for genetic correlates of fatigability. This study has looked for possible connections between single nucleotide polymorphisms (SNP) and chronotypes and accident history in Russian bus drivers. 237 bus drivers (all male) participated in the study, 188 of them had traffic accident records. The drivers worked on rolling schedule with shifts starting at 3:30, 6:30, 9:30, 12:30, 15:30, 17:30. To assess chronotype we have used a Russian version of Munich Chronotype Questionnaire (MCTQ) and a Sleep-Wake Pattern Assessment Questionnaire (SWPAQ; Putilov, 1990, 2000). SWPAQ measured the scales of morning lateness (M, high score means low activation in the morning) and evening lateness (E, high score means high activation in the evening). 4 SNPs were tested: rs12649507 in CLOCK (associated with sleep length), rs1159814 in RORA and rs4851377 in NPAS2 (associated with chronotype), rs324981 in NPSR1 (associated with sleep onset time). MCTQ assessment revealed high variance in sleep duration (3,9 to 9,9 h), average parameters were close to normal (6,9±1,3 h), and prominent social jetlag (-6,8 to 7,2 h, average 1,7±1,7 h) caused by the shift work. In the SWPAQ correlation between M and E was found ($r=0,34$, $p<0,001$). Correlation analysis (gamma correlation) demonstrated significant results in midsleep time for SNPs in RORA ($r=-0,13$ for A allele) and NPSR1 ($r=0,22$ for C allele). Social jetlag showed connections with SNPs in NPSR1 and CLOCK ($r=0,20$ for A allele and $r=-0,17$ for A allele). Average sleep duration showed no significant connection with the tested SNPs. C allele in RORA gene was also negatively connected with M subscale ($r=-0,16$). We have compares the same SNPs against the drivers' road accident history (presence and number of road accidents, driver's fault). For minor alleles in CLOCK and RORA genes there was a significant negative correlation with the "driver's fault" parameter ($r=-0,35$ and $r=-0,20$ respectively). Carriers of a minor C allele in RORA gene tended to be earlier choronotypes and less prone to

cause road accidents Based on our results we can say that SNPs in CLOCK, NPSR1 and especially RORA genes may contribute to individual differences in chronotype and sleep parameters and through them - to road accident risk.

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Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 168.01/V22

Topic: E.08. Biological Rhythms and Sleep

Title: Analysis of splicing factor Rbfox2 isoforms in the rat cortex and hypothalamus

Authors: *D. A. CARTER, L. M. M. PARTRIDGE;
Cardiff Univ., Cardiff, United Kingdom

Abstract: Rbfox proteins are regulators of gene splicing that bind pre-mRNAs at UGCAUG elements, and effect either exon inclusion, or exclusion, depending upon the relative position of the target site. All three proteins in the Rbfox2 family, RBFOX1 (A2BP1), RBFOX2 (RBM9) and RBFOX3 (NeuN) are widely, although differentially, expressed in the brain, but the diversity of functional activity is not fully appreciated because antibodies cannot distinguish the numerous isoforms of individual Rbfox proteins. We are interested in expression of Rbfox in the adult rat hypothalamus where the post-mitotic neuron marker, NeuN/RBFOX3, is absent in a major population of neurons in the suprachiasmatic nucleus (SCN). Focusing on Rbfox2, we initially showed that cellular RBFOX2 immunoreactivity is abundant across NeuN-negative regions of the rat SCN; therefore RBFOX2 may, at least partially, substitute NeuN-mediated splicing regulation in this cellular population. We have also found evidence of SCN-specific populations of Rbfox2 mRNAs - using RNA sampled from either adult SCN or somatosensory cortex, we amplified and sequenced mRNAs with primers designed around annotated start/stop codons in Rbfox2 ORFs. mRNA population analysis revealed a diversity of sequences; these exhibit both inclusion and exclusion of different exons as well as differential use of cryptic splice sites. Sequence variation in both brain populations is primarily located in C-terminal domain (CTD) coding sequence, changing both the reading frame and use of alternative stop codons in this region. In contrast, the RNA Recognition Motif (RRM) domain was maintained in all Rbfox2 variants sequenced to date. The CTD sequence variations potentially affect a number of CTD functional activities including protein-protein interaction, sub-cellular localization and exon-

inclusion activity. This potential functional variety is interesting in light of our finding that SCN and cortex samples exhibit distinct populations of Rbfox2 mRNAs. As NeuN is known to regulate splicing of Rbfox2, the differential occurrence of Rbfox2 isoforms in the two brain regions may relate directly to NeuN availability. Analysis of intronic sequence around alternative Rbfox2 exons revealed both upstream and downstream UGCAUG elements that are positioned to mediate possible Rbfox family cross-regulation. These studies indicate that the known diversity of Rbfox expression in the adult brain extends to involve cell-type-specific, Rbfox2 CTD activity. A distinct Rbfox2 cohort in the SCN may contribute to a selective splicing program required for functional activity in populations of these hypothalamic neurons.

Disclosures: D.A. Carter: None. **L.M.M. Partridge:** None.

Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 168.02/V23

Topic: E.08. Biological Rhythms and Sleep

Support: DGAPA-PAPIIT grants IN216015; IN215513

CONACYT grant 236908.

Title: The suprachiasmatic nuclei, asymmetries and the involvement on non-proestrous events in the regulation of ovulation

Authors: C. C. SILVA[1], D. P. BENITEZ[1], J. C. MUÑOZ[1], G. D. CORTÉS[1], M.-E. CRUZ[1], A. FLORES[1], *R. DOMINGUEZ[2];
[1]BRRU, [2]FES Zaragoza UNAM, Mexico City DF, Mexico

Abstract: GnRH release, and hence ovulation, is timed by neural circadian signals arising from the suprachiasmatic nucleus (SCN) that can only trigger it when converge with estradiol levels characteristic of proestrous day. There is compelling evidence about asymmetries in the involvement of left- and right-hypothalamic structures and also regarding the participation of neural signals regulating ovulation in days other than proestrous, but nothing is known about the participation of the SCN. In order to test the possibility that the SCN sends asymmetric information essential for the regulation ovulation before the preovulatory surge of GnRH, we injured the left- or right-SCN of adult cyclic female rats at 09:00 h of estrous or metaestrous. Animals were sacrificed the next predicted estrous and the ova shed was counted. Sham surgery,

barbiturate anesthetized (25 mg/kg) and intact animals were used as controls. Sample size was 7 but only animals displaying lesions without damage to the contralateral SCN or ipsilateral kispeptin centers were considered for this study. Lesion and sham surgery of either side of the SCN blocked ovulation independently of the stage of the cycle (the proportion of ovulating animals between total animals was: estrous; intact 7/7, anesthesia 7/7 R-sham 3/7, L-sham 1/7, R-lesion 0/7, L-lesion 3/7; metaestrous; anesthesia 6/7 R-sham 0/7, L-sham 2/7, R-lesion 0/7, L-lesion 0/7). This result shows the involvement of the SCN and neural pathways above it in the progress of ovulation. To depict the specific signaling controlled by the SCN on these stages of the estrous cycle, a second batch of rats received the same treatments and then were hormonally primed with either, GnRH (3.7 μg/kg) or estradiol benzoate (10 μg total) at 14:00 h of the predicted proestrous or diestrous, respectively. GnRH restored ovulation, suggesting that both sides of the SCN are needed for proper triggering of the preovulatory GnRH surge and that pituitary and ovarian sensitivity to their ligands remains intact after unilateral ablation of the SCN. Estradiol treatment was effective in rats with surgery performed on estrous (R-sham 4/5, L-sham 4/5, R-lesion 3/3, L-lesion 5/7), but not on metaestrous (R-sham 4/5, L-sham 0/3, R-lesion 0/3, L-lesion 0/3). This result supports our idea that SCN tunes ovulation by regulating other events in addition to the preovulatory gonadotropin surge in an estrous cycle stage-dependent fashion. We suggest that the lack of responsiveness to estradiol priming is the consequence of an impairment in estrogen receptor-α expression in the periventricular area, known to be involved in the integration of circadian and estrogenic signals gating ovulation.

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Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 168.03/V24

Topic: E.08. Biological Rhythms and Sleep

Support: BBSRC

Title: Functional properties of intergeniculate leaflet inputs to the suprachiasmatic nucleus in a novel *in vitro* slice preparation

Authors: ***L. HANNA**, M. HOWARTH, T. M. BROWN;
Neurosci., Univ. of Manchester, Manchester, United Kingdom

Abstract: A subset of intrinsically photosensitive melanopsin expressing-retinal ganglion cells (mRGCs) target an interconnected network of nuclei that together define behavioural and physiological responses to changes in ambient illumination: the suprachiasmatic nucleus (SCN), intergeniculate leaflet (IGL) and the pretectal olivary nucleus (PON). At present, the functional significance of interactions between these various nuclei is unclear. To address this issue, we set out to establish an *in vitro* slice preparation that retains optic tract projections to, and network connectivity between these anatomically dispersed nuclei. We first modelled the axonal trajectories linking the SCN, IGL and PON via X-gal histochemistry in slices prepared from melanopsin reporter mice (Opn4tauLacZ). With this method, we determined that a 600μm thick slice, with a slicing angle of 30 degrees off the coronal plane, was required to preserve retinal projections and most of each nucleus intact. Perforated multi-electrode array recordings revealed that the resulting slices remained viable for >24h *in vitro*, with the SCN exhibiting robust circadian variation in spontaneous electrical activity. Moreover, electrical stimulation of the optic chiasm reliably evoked excitatory (glutamatergic) responses across all retinorecipient nuclei, indicating that retinal projections were maintained in this slice preparation. Importantly, electrical or optogenetic stimulation of the IGL revealed inhibitory (GABAergic) responses in the SCN region and modulated responses to optic tract stimulation, indicating the presence of a functional geniculohypothalamic tract (GHT). In summary, this novel 'non-image forming' (NIF) visual slice preparation represents a useful new tool for understanding the functional connectivity and network organisation of the extended circadian system.

Disclosures: L. Hanna: None. **M. Howarth:** None. **T.M. Brown:** None.

Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 168.04/V25

Topic: E.08. Biological Rhythms and Sleep

Support: UAB Department of Ophthalmology

Title: Photic responses of suprachiasmatic nucleus neurons in the Rhesus monkey

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Abstract: The suprachiasmatic nucleus (SCN) is the “master clock” that regulates circadian rhythms in mammals including primates. In rodents, neurons of the SCN are photically modulated by input from the retina that arises predominantly from intrinsically photosensitive ganglion cells (ipRGCs) (Do and Yau, 2010). In macaque monkey, ipRGCs also provide a major input to the SCN (Hannibal et al., 2014), but the photic response characteristics of primate SCN neurons have not been studied to date. Thus, in primates, it is not known how the ipRGC signals that influence SCN neurons are combined and transformed to photically entrain circadian rhythms. Therefore we have initiated a study of the photic response properties of SCN neurons in awake, trained Rhesus monkeys. Using a 6-primary, extended Maxwellian view system, an animal was trained to fixate a central target while we presented stimuli of varying size, irradiance, and spectral composition. Eye movements and pupil diameter were measured binocularly with a video eye tracking system (ISCAN). Once the animal was trained, a recording chamber was placed, and single-unit recording sessions occurred between ZT6-10. The SCN was targeted using MRI and familiar landmarks. Recording locations within the SCN were verified postmortem by micro-lesions combined with Nissl stain and immunohistochemical labeling of SCN neurons for vasoactive intestinal polypeptide. We found that SCN neurons (6 cells) possess a low spontaneous firing rate, display both transient and tonic ON responses to light onset, and possess extensive, generally bilateral, receptive fields. Sustained firing rate increased with increases in retinal irradiance but, for some SCN neurons, the threshold for evoking these responses was substantially greater than that required to evoke a robust sustained pupillary response. Our preliminary data reveal that many SCN neurons possess bilateral receptive fields, which is unusual for retinorecipient neurons in primates, but is seen for some neurons in the pretectal olivary nucleus (Clarke et al., 2003). Furthermore, our data suggests that the majority of SCN neurons in primates are light-activated in contrast to other mammals in which many are reported to be light-suppressed (Jiao et al., 1999). In summary, there appear to be significant differences in the photic responses of primate SCN neurons compared to other mammals. Our findings may provide insight into the human circadian rhythm photic entrainment as well as dysfunction such as jet lag and psychiatric disorders.

Disclosures: **K.Q. Chang:** None. **P.D. Gamlin:** None.

Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 168.05/V26

Topic: E.08. Biological Rhythms and Sleep

Support: VA BLR&D MERIT AWARD

VA RR&D MERIT AWARD

Title: CK1 Inhibitor, Longdaysin, has mild effect on behaviors under normal lighting conditions

Authors: *P. FENG[1], A. AKLADIOUS[2], Y. HU[4], P. J. SMITH[3];
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Abstract: Bmal1/Clock heterodimer in the SCN is a well-defined master trigger of the internal circadian clock, which synchronizes biological rhythms and behavioral activities in human and animals. To maintain the rhythmic cycles, there are multiple lines of negative biofeedback that help to terminate the circadian upward phase. Chemicals that affect the feedback lines, affect the features of circadian signals too. It is reported that longdaysin, a CK1 inhibitor, increased the length of circadian cycles. We observed effects of longdaysin on several behaviors under normal light dark conditions. We performed a series of behavioral tests on adult wildtype mice that were housed under controlled conditions. The light dark schedule was 12 hours light and 12 hours dark. Drug was administered through intracerebroventricular route with either 4 µl vehicle (12.5% DMSO) or longdaysin dissolved in vehicle and tested for forced swim test, tail suspension test, social interaction test and novel object recognition test. In order to see if longdaysin has anti-fatigue effects, a weight bearing (bearing a small weight on the back equal to ~7% body weight) swim test was also conducted. Due to a relative long period (25 min) of swim in this test, longdaysin (20 mg/kg) or vehicle was administered through intraperitoneal route after confirmation that the drug was able to cross blood-brain-barrier nicely. Surprisingly, results for immobility were different in forced swim test and in tail suspension test. Compared with the vehicle group, mice treated with longdaysin had significantly longer swim time in the forced swim test but had significantly longer immobile time in the tail suspension test. In novel object recognition test, mice in longdaysin group spent less time with the first object and more time in exploring the second object. In the social interaction test, group differences for the time spent with playmate 1 and playmate 2 were not significant in all measured variables. In weight bearing test, we measured the time to first immobile period (defined as equal to or longer than 3 seconds), the swim time to the second immobile period and the total swim time (i.e., to the third immobile period). Means of group differences were not significant in any of the measured variables. In conclusion, longdaysin treatment has an effect on immobility and novel object recognition but it does not have anti-fatigue effect. More test need to be done for further define the effect of CK1 inhibition on behavioral regulation.

Disclosures: **P. Feng:** None. **A. Akladious:** None. **Y. Hu:** None. **P.J. Smith:** None.

Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

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Topic: E.08. Biological Rhythms and Sleep

Support: DGAPA grant IN-220014-3

CONACYT grant 236908

Title: The expression of muscarinic receptor m1AChR of the suprachiasmatic nucleus varies throughout the day

Authors: E. VIEYRA[1], L. MENDOZA-GARCÉS[2], R. DOMINGUEZ[1,2], *M. B. CRUZ[1]; [1]Univ. Autonoma De México, Mexico DF, Mexico; [2]Dept. of Basic Res., Natl. Inst. of Geriatrics, Mexico City, Mexico

Abstract: The "central pacemaker" that orchestrates the circadian rhythms is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The central cholinergic system regulates the circadian system and modulates the activity of neurons in the SCN. In rats, the SCN receives cholinergic projections from the basal forebrain including the nucleus basalis magnocellularis and the brainstem from the pedunculopontine tegmental nucleus and laterodorsal tegmental nucleus. The role of the muscarinic receptors in the regulation of the SCN is unknown. We have previously shown that the unilateral blockade of muscarinic receptors of the right SCN at the day of proestrus by the microinjection of atropine, resulted in the blockade of ovulation at the next day. Because atropine blocks all the muscarinic receptors, the aims of present study were to analyze the distribution of the m1AChR receptors in the right or left SCN at different hours of the day, on each day of the estrous cycle. For this purpose, we analyzed the number of neurons positive to the m1AChR receptor in the left and right SCN on each day of the estrous cycle of rats sacrificed at 11.00 and 17.00 h. The presence of m1AChR receptors was demonstrated by immunocytochemistry. In animals sacrificed at 11.00 h the number of neurons expressing m1AChR receptors in the right and left SCN of was higher than those sacrificed at 17:00 h (right 133.7±29.5 vs. 44.7±14.6, $p<0.05$ Student's t test; 110.1±30.6 vs.42.6±17.5 n.s.). Present results suggest that expression of the muscarinic receptors m1AChR in the right or left SCN depends on the time of the day. Supported by DGAPA grant IN-220014-3 and CONACYT grant 236908

Disclosures: E. Vieyra: None. **L. Mendoza-Garcés:** None. **R. Dominguez:** None. **M.B. Cruz:** None.

Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

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Support: NIH Grant NS036607

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NIH Grant RR025440

NIH Grant RR023432

Title: Localization and circadian expression of GABA transporters in the suprachiasmatic nucleus

Authors: M. MOLDAVAN[1], O. CRAVETCHI[1], M. WILLIAMS[2], R. P. IRWIN[1], S. A. AICHER[2], *C. N. ALLEN[1];
[1]OR Inst. Occup. Hlth. Sci., [2]Depart Physiol & Pharm, Oregon Hlth. Sci. Univ., Portland, OR

Abstract: GABA is the principal neurotransmitter in the hypothalamic suprachiasmatic nucleus (SCN), the master circadian clock. Despite the importance of GABA uptake for GABAergic neurotransmission, the localization and function of GABA transporters (GATs) in the SCN has not been investigated. Western blot analysis, immunohistochemical, and electron microscopic studies demonstrated the presence of GABA transporter 1 (GAT1) and GABA transporter 3 (GAT3) in the SCN. Both GATs exhibited diurnal expression with a nadir at Zeitgeber time (ZT) 12 and peak at ZT 24. By light microscopy, GAT1 and GAT3 were co-localized and distributed throughout the SCN. GAT1 and GAT3 were not expressed in the perikarya of arginine vasopressin (AVP)- or vasoactive intestinal peptide (VIP)-immunoreactive neurons, nor in neuronal processes of the SCN neurons of adult rats labeled with the neuronal marker Neurofilament Heavy Chain. SCN neurons in cell culture (P8) also were immunonegative for GAT1. By electron microscopy, astrocytic glial processes immunoreactive (-ir) for GAT1 or GAT3 surrounded unlabeled neuronal perikarya and axons. GAT1- or GAT3-ir glial cells also enveloped symmetric and asymmetric axo-dendritic synapses. GFAP-immunopositive astrocytes grown in cell culture were immunoreactive for GAT1 and GAT3 - both GATs could be observed in the same cell. These data demonstrate that synapses in the SCN function as a “tripartite” synapse consisting of presynaptic axon terminals, postsynaptic membranes, and astrocytes. This

model suggests that astrocytes expressing both GATs may regulate the diurnal changes of extracellular GABA and modulate the activity of neuronal network in the SCN.

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Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH NS092545

NIH NS078220

Title: The influence of light on GABAA receptor expression in the suprachiasmatic nucleus of male Syrian hamsters

Authors: ***J. C. WALTON**, J. K. MCNEILL, IV, H. E. ALBERS;
Neurosci. Inst. and Ctr. for Behavioral Neurosci., Georgia State Univ., Atlanta, GA

Abstract: GABAA Receptors (GABAARs) are pentameric ligand gated ion channels and their subunit composition determines channel properties and location on the cell membrane. GABAARs containing the δ subunit are tonically active high-affinity non-desensitizing channels found at extrasynaptic locations, whereas classical phasic inhibition is mediated by synaptic GABAARs containing the γ2 subunit. Recent studies have revealed that expression levels of δ and γ2 subunits may regulate the balance between tonic and phasic inhibition in multiple brain regions. In addition, GABAAδ receptors also have a specific role in the regulation of photic input into the SCN in a circadian phase-specific manner and we have recently shown that the mRNA for these two receptors is expressed in an antiphase circadian rhythm. However, whether these transcriptional rhythms translate into rhythms of GABAAR protein expression in the SCN is unknown. Toward this end, we collected brains from male Syrian hamsters that were exposed to either a 14:10 L:D cycle or constant dark (D:D) at CT/ZT 1, 6, 13, and 19. We then assessed GABAAδ and γ2 expression in the SCN using immunohistochemistry. Both subunits are expressed in a 24h pattern under an L:D cycle, with peak immunoreactivity of both proteins occurring at night. However, when held in constant dark for 10 days this expression pattern was abolished for GABAAδ. Surprisingly, constant darkness inverted the expression pattern of

GABAAγ2, resulting in peak immunoreactivity occurring during the subjective day. Furthermore, GABAAγ2 immunoreactivity was higher during the subjective day under D:D than at night under L:D. Taken together, these results indicate that light may suppress protein expression of both GABAAδ and γ2 in the SCN resulting in a 24h pattern of expression, yet when released from photic inhibition in DD, a circadian rhythm for GABAAγ2 is unmasked.

Disclosures: J.C. Walton: None. **J.K. McNeill:** None. **H.E. Albers:** None.

Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

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Topic: E.08. Biological Rhythms and Sleep

Support: CIHR Grant MOP 114994

CIHR Grant MOP 13625

Title: eIF4E phosphorylation regulates mammalian circadian behavior via translational control of Period 1 and Period 2

Authors: *R. CAO[1], C. G. GKOGKAS[3], N. DE ZAVALIA[4], I. D. BLUM[2], K.-F. STORCH[2], S. AMIR[4], N. SONENBERG[1];
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Abstract: The circadian (~24 h) clock is continuously entrained (reset) by ambient light so that endogenous rhythms are synchronized with daily changes in the environment. Light-induced gene expression is thought to be the molecular mechanism underlying clock entrainment. mRNA translation is a key step of gene expression, but the manner in which clock entrainment is controlled at the level of mRNA translation is not well understood. We found that a light- and circadian clock-regulated MAPK/MNK pathway led to phosphorylation of the cap-binding protein eIF4E in the mouse suprachiasmatic nucleus of the hypothalamus, the locus of the master circadian clock in mammals. Phosphorylation of eIF4E specifically promoted translation of Period 1 (Per1) and Period 2 (Per2) mRNAs and increased the abundance of basal and inducible PER proteins, which facilitated circadian clock resetting and precise timekeeping. Together,

these results highlight a critical role for light-regulated translational control in the physiology of the circadian clock.

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Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

Location: Hall A

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Topic: E.08. Biological Rhythms and Sleep

Support: NHLBI R01-102758

NIAMS T32-AR007592

Title: β2-mediated inactivation drives the diurnal difference in BK current levels in suprachiasmatic nucleus and regulates circadian rhythmicity

Authors: *J. P. WHITT[1], J. R. MONTGOMERY[2], A. L. MEREDITH[3];
[1]Physiol., Univ. of Maryland Baltimore, Baltimore, MD; [2]Physiol., Univ. of Maryland Sch. of Med., Baltimore, ME; [3]Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Circadian patterning of neural activity in the suprachiasmatic nucleus (SCN) of the hypothalamus, the brain's circadian clock, is achieved through daily regulation of ionic currents, including the BK K^+ current. BK currents are larger at night compared to the day, and the diurnal variation in BK current regulates SCN action potential (AP) frequency and circuit rhythmicity. Of the two BK channel auxiliary subunits expressed in SCN, β2 and β4, we found that loss of β2 (β2 KO) decreased circadian circuit rhythmicity. To assess the influence of β2 subunits on BK current properties and AP activity in the SCN, current and voltage-clamp recordings were made from neurons in acute slices. We found that BK current size was inversely correlated with the number of BK currents showing inactivation. During the day when BK currents were smaller, 65% of neurons exhibited BK inactivation. In contrast, during the night only 35% of BK currents inactivated. β2 KO neurons had a complete loss of BK current inactivation, as well as a reduced daytime AP frequency (1.9 ± 0.2 Hz for WT and 0.5 ± 0.1 Hz for β2 KO). BK current inactivation was restored in SCN neurons through intracellular delivery of a soluble 45 amino acid inactivating "ball" peptide derived from the N-terminus of β2 (β2N). Rescue of inactivation by β2N reduced daytime BK current magnitude to WT levels (42 ± 9 pA/pF for β2 KO/β2N and

36 ± 7 pA/pF for WT) and increased daytime AP firing in β2 KO neurons to levels compared to WT (1.9 ± 0.1 Hz). This increase in firing due to restoration of inactivation was associated with a decrease in the amount of depolarization necessary to reach threshold (ΔV=[Threshold potential - baseline membrane potential]; 8.7 ± 0.8 mV for WT; 11.4 ± 0.3 mV for β2 KO; 8.4 ± 0.5 mV for β2 KO/β2N). These data demonstrate that β2-mediated inactivation of BK currents is required to facilitate higher frequency firing during the day and is necessary for normal SCN circuit rhythmicity.

Disclosures: J.P. Whitt: None. **J.R. Montgomery:** None. **A.L. Meredith:** None.

Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

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Topic: E.08. Biological Rhythms and Sleep

Support: NHLBI R01-102758

NIAMS T32-AR007592

Title: Diurnal regulation of BK-Ca^{2+} channel coupling in the mouse suprachiasmatic nucleus

Authors: *A. MEREDITH[1], J. WHITT[2];
[1]Dept. of Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Physiol., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: BK K^+ channels are regulated by membrane depolarization and increases in the local intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Across neurons and muscle, BK channels have been shown to differentially couple to voltage-gated Ca^{2+} channels of the L-, P-, Q-, and N-type , as well as to ryanodine receptor (RyR)- mediated release from intracellular Ca^{2+} stores. In the suprachiastmatic nucleus (SCN) of the hypothalamus, the brain's circadian clock, both intracellular free Ca^{2+} and voltage gated Ca^{2+} channel (VGCC) currents are regulated in a circadian manner, with greater $[Ca^{2+}]_i$ during the day. We used a pharmacological approach to identify the Ca^{2+} sources for BK current activation in day and night SCN neurons. We found a 69% reduction in BK current magnitude with nimodipine during the day, with a lesser effect at night (10% reduction), suggesting that L-type VGCCs are the primary Ca^{2+} source for BK activation during the day. Conversely at night, when VGCC currents are reduced, we found a significant decrease (76%) in BK current magnitude using dantrolene, which blocks Ca^{2+} release

from RyRs. Thapsigargin, which depletes Ca^{2+} from intracellular stores, caused a similar 79% reduction in BK current, demonstrating that nighttime BK activation is primarily driven by Ca^{2+} release from intracellular stores through RyRs. Consistent with functional coupling between BK channels and these Ca^{2+} sources, the voltage dependence of BK activation was shifted to more positive potentials with nimodipine during the day and both dantrolene and thapsigargin at night. Lastly, BK currents were reduced by ω-conotoxin MVIIC to a similar extent between day and night (40% and 45%, respectively), suggesting that a fraction of BK channels maintain stable Ca^{2+} channel coupling over the circadian cycle. These data demonstrate diurnal regulation of the coupling between BK channels and their Ca^{2+} sources in SCN and suggest that circadian changes in Ca^{2+} coupling could contribute to BK current regulation and its role in circadian rhythmicity.

Disclosures: **A. Meredith:** None. **J. Whitt:** None.

Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

Location: Hall A

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Topic: E.08. Biological Rhythms and Sleep

Support: GM096873

Title: Identification of firing patterns in SCN VIP neurons that promote VIP release

Authors: ***C. MAZUSKI**[1], E. D. HERZOG[2];
[1]Washington Univ. In St. Louis, Saint Louis, MO; [2]Washington Univ. in St. Louis, St. Louis, MO

Abstract: VIP (vasoactive intestinal polypeptide) expressing neurons are a functionally and anatomically distinct subset of circadian neurons in the suprachiasmatic nucleus (SCN). We hypothesize that distinct firing patterns from VIP neurons are responsible for the release of VIP, which synchronizes and entrains circadian rhythms in firing and gene expression in other SCN neurons. We have characterized spontaneous firing patterns of SCN neurons in response to VIP stimulation by "optically tagging" VIP neurons expressing Channelrhodopsin-2 (VIP-ChR2) following multiday recordings of extracellular electrical activity on multielectrode arrays (MEAs). Preliminary results from SCN neurons cultured on 3 individual MEAs yielded at least 2 distinct classes of neurons based on response to VIP stimulation. The first class, which constitutes approximately 6-10% of neurons, were VIP-ChR2 expressing, because they exhibited increases in firing probability above the 99% confidence interval within 10ms of a light flash for

the entire 20 min stimulation. The second class of SCN neurons changed firing rate and/or pattern during the stimulation but did not fit the temporal criteria for "optically tagged" VIP neurons. These included neurons that decreased firing probability between 10-50ms after a laser pulse in a phase locked manner, suggesting a response to GABA release. Other neurons from the second class rapidly increased or decreased firing rate 2-3 minutes into the stimulation - a change that persisted for the duration of the stimulation. Future analyses aim to identify characteristic spontaneous, circadian, and evoked firing patterns of VIP neurons that result in release of VIP and circadian rhythm entrainment.

Disclosures: C. Mazuski: None. **E.D. Herzog:** None.

Poster

168. Suprachiasmatic Nucleus Anatomy, Physiology, and Neurochemistry

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: E.08. Biological Rhythms and Sleep

Support: CHDI Grant A-7293

Title: The Q175 mouse model of Huntington's disease shows decline in circadian rhythms of SCN electrical activity

Authors: *T. KUDO, C. COLWELL;
Dept. of Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA

Abstract: Many patients with Huntington's disease (HD) exhibit disturbances in their daily cycle of sleep and wake as part of their symptoms. These patients have difficulty sleeping at night and staying awake during the day, which has a profound impact on the quality of life of the patients and their care-takers. For developing treatments of the human disease, knock-in (KI) models offer advantages of genetic precision and control of mutation copy number. Therefore, we used a relatively new model of HD with an expansion of the KI repeats (Q175). In the previous paper, we found Q175 showed circadian disruption in the behavior, but the SCN electric activity is not yet examined. Hence, we examined the SCN electric activity in pre-symptomatic (5 months of age) and symptomatic (7 months of age) Q175 mouse. In pre-symptomatic stage, day-night difference was lost in Q175 mouse, but there was no difference with wild-type mouse during day time. In symptomatic stage, day-night difference was lost and day time firing rates was significantly reduced in Q175 mouse during day time. Together, this data is consistent with the hypothesis that the HD mutations interfere with the expression of

robust circadian rhythms in behavior and physiology. The data raise the possibility that the electrical activity within the central clock itself may be altered in this disease.

Disclosures: **T. Kudo:** None. **C. Colwell:** None.

Poster

169. Perception and Imagery

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: The Royal Society e-Gap Grant 2006/R1

Intramural Research Program of the National Institute of Mental Health, NIH/DHHS

Title: Auditory function in humans with heterozygous FOXP2 mutation

Authors: D.-E. BAMIOU[1], L. M. LUXON[2], I. YASIN[3], T. BALDEWEG[4], S. BOYD[5], M. MISHKIN[7], *F. VARGHA-KHADEM[6];
[1]Neuro-otology, UCL Ear Inst. and Natl. Hosp. for Neurol. & Neurosurg., London, United Kingdom; [2]Neuro-otology, UCL Ear Inst. and Natl. Hosp. for Neurol. and Neurosurg., London, United Kingdom; [3]UCL Ear Inst., London, United Kingdom; [4]Section on Cognitive Neurosci. & Neuropsychiatry, [5]Section on Clin. Neurosciences, [6]UCL Inst. of Child Hlth., London, United Kingdom; [7]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Background An inherited speech and language disorder with broadly spared cognition but severe articulatory and language production impairments in half the members of the three-generational KE family, has been linked to a selective abnormality in the FOXP2 transcription factor gene (Lai et al, 2001; Vargha-Khadem et al, 2005). However, the orofacial motor control deficits do not sufficiently explain the wide ranging language impairments, suggesting the FOXP2 gene may affect diverse neural pathways underpinning not only speech production, but also language reception in general. In humans, the FOXP2 gene is expressed in brainstem, thalamic and neostriatal structures, while in mice with mutations in this gene, abnormal auditory brainstem evoked responses are reported (Kurt et al, PLoS One, 2012). We aimed to determine whether there are functional deficits in the subcortical auditory pathway by assessing low brain stem, neural and cochlear auditory function in affected individuals. Methods Seven affected members of the KE family (3 females, age range 24-62), were tested with pure tone audiometry, acoustic impedance tests (tympanometry/acoustic reflexes), transient evoked otoacoustic

emissions and auditory brainstem responses (ABR). Results Six of the seven participants had normal audiometric thresholds for their age range. Otoacoustic emissions were present in all and were normal in 11 ears; reductions were attributable to age in two and to noise exposure in one. Acoustic reflexes were normal in six and ABRs in all cases. Conclusions Subcortical auditory pathways are not involved at a clinically significant level in KE family members with a FOXP2 mutation, in contrast to the findings of a study in mice with this mutation (Kurt et al, 2012). References Lai et al, Nature 2001;413:519- 523. Vargha-Khadem et al, Nat Rev Neurosci 2005;6:131-138. Kurt S, Fisher SE, Ehret G. PLoS One 2012;7(3):e33130.

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Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Support: German Reseach Foundation DFG SFB936/A3/Z1

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EU ERC-2010-AdG-269716

German National Academic Foundation

Title: The role of intrinsic coupling modes for cognitive processing and cortical communication

Authors: *R. F. HELFRICH[1], H. KNEPPER[1], G. NOLTE[1], C. S. HERRMANN[2], T. R. SCHNEIDER[1], A. K. ENGEL[1];
[1]Univ. Med. Ctr. Hamburg, Hamburg, Germany; [2]Univ. of Oldenburg, Oldenburg, Germany

Abstract: Conscious perception might arise from the dynamic interplay of functionally specialized but widely distributed cortical areas. While previous research mainly focused on phase coupling as a correlate of cortical communication, more recent findings indicated that several different coupling modes might coexist and possibly subserve distinct functions. Here, we studied two coupling modes, namely phase and amplitude coupling, which might differ in their origins, putative functions and dynamics. In a first EEG experiment, participants performed a bistable motion task and we utilized source-space connectivity analysis techniques to study the

functional relevance of different coupling modes for conscious perception. Our results provide correlative evidence that gamma-band phase coupling in extrastriate visual cortex mediates the integration of visual tokens into a coherent percept during ambiguous visual stimulation, while long-range fronto-occipital gamma-band amplitude coupling sustains the horizontal percept during ambiguous motion perception. Additionally, our results suggest that local parieto-occipital alpha-band phase coupling controls the inter-hemispheric information transfer. In a second combined EEG-tACS (transcranial alternating current stimulation) study, we demonstrate the differential modulation of both coupling modes during selective entrainment of distinct spectral components. Our results reveal that entrainment of the low frequency component increased phase-amplitude coupling, where gamma power became preferentially locked to the trough of the alpha oscillation, while gamma-band entrainment reduced alpha power through enhanced amplitude coupling. These results provide causal evidence for the functional role of coupled alpha and gamma oscillations for visual processing and indicate that distinct coupling modes are involved in different cortical computations. The rich spatiotemporal correlation structure of the brain might constitute the functional architecture for cortical processing and specific multi-site communication.

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Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Support: NICHHD Grant T32HD071845

Title: A role for the occipital place area in navigating the local visual environment

Authors: ***F. S. KAMPS**, V. LALL, D. D. DILKS;
Emory Univ., Atlanta, GA

Abstract: Neuroimaging studies have identified multiple scene-selective regions in human cortex, but the precise role each region plays in scene processing is not yet clear. A recent hypothesis, with some empirical support, is that two scene-selective regions - the occipital place area (OPA) and the retrosplenial complex (RSC) - are involved in navigation, while another region - the parahippocampal place area (PPA) - is involved in scene categorization (i.e.,

recognizing a scene as a kitchen versus a beach). Here we test this two streams for scene processing hypothesis by measuring the magnitude of response in each of these regions to both dynamic scene stimuli (i.e., video clips of first-person perspective navigation through a scene) and static scene stimuli (i.e., still images taken from these same movies, rearranged such that motion through the scene cannot be inferred). If a region is involved in navigation, then it should respond more to dynamic than to static scene stimuli, since the dynamic stimuli mimic actual navigation through the local visual environment. By contrast, if a region is involved in categorization, then it should respond similarly to the dynamic and static stimuli, since dynamic information does not help to categorize a scene (e.g., a kitchen is a kitchen whether one is moving through it or standing still within it). Indeed, we found that OPA responded significantly more to dynamic than static stimuli, relative to PPA, consistent with OPA's role in navigation and PPA's role in categorization. However, RSC also responded less to dynamic than static stimuli, relative to OPA, suggesting a division of labor even within the navigation stream. Taken together, these findings i) are consistent with the two streams for scene processing hypothesis (i.e., navigation and categorization), and ii) further suggest a novel division of labor within the navigation stream, with one system (including OPA) particularly responsible for navigating the local, immediately visible environment.

Disclosures: **F.S. Kamps:** None. **V. Lall:** None. **D.D. Dilks:** None.

Poster

169. Perception and Imagery

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Program#/Poster#: 169.04/V38

Topic: F.01. Human Cognition and Behavior

Support: Kakenhi Grant-in-Aid for Scientific Research (B) #23300102

Kakenhi Grant-in-Aid for Challenging Exploratory Research #26540074

Title: Correlation of gamma-band brain activities with subjective confidence in the 3-D object perception from motion

Authors: *S. IWAKI;
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Abstract: Two-dimensional optic flow is a cue to perceive 3-D object embedded in the natural scene, (3-D structure from motion: 3DSFM) [1][2]. In this study, we manipulated parametrically

the coherence of randomly moving dots to create different levels of 3-D perception to study associated changes in perception and the brain activity. Specifically, we focused on the correlation between EEG activities and the subjective confidence of 3DSFM. Nineteen subjects participated in the study. All experimental procedures were in compliance with the iRB of AIST. Visual stimuli consisted of 1000 random dots inside a spherical area, which started to move 500 ms after the onset of presentation with various motion coherences. The coherence of the motion across dots varied from 0 to 100 %. A fully (100%) coherent stimulus had all the dots moving as if they belonged to a spherical surface rotating along a randomly tilted axis, and in the other (x % coherent) conditions, movement direction of (100 - x) % of dots were randomized. The subjects were required to reply the tilt angle of the rotation axis and to rate subjective confidence of the answer by a 0 to 10 scale. The stimulus related EEG epochs of 2 s including 0.5 s pre-stimulus baseline, were recorded with a sampling rate of 1 kHz. The results of the correlation analysis between the subjective rating of the confidence of the 3-D object perception and EEG time-frequency representation showed that there was significant positive correlation between fronto-parietal 40 Hz gamma-band power and the subjective confidence. It is reported in the previous studies that transient gamma-band responses are modulated by dopamine system in the human brain [3] which biases the subjective confidence in perceptual tasks [4]. The current results suggest that gamma-band synchrony in the fronto-parietal regions plays an important role in perceiving confidence during 3DSFM. References [1]Orban GA et al, Neuron 24: 929-940, 1999. [2]Iwaki S et al, J. Integr. Neurosci. 12: 355-367, 2013. [3]Ahveninen J et al, Neurosci Lett. 292:29-32., 2000. [4]Andreou C et al, Front. Psychol. 6:414, 2015.

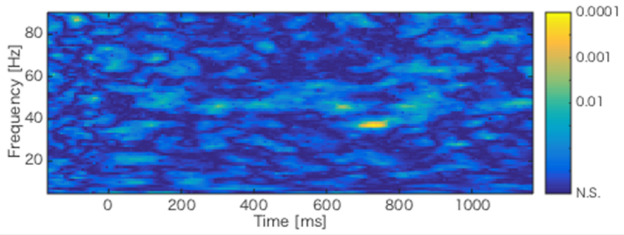

Fig.1: Results of the correlation analysis between the subjective confidence during 3DSFM task and the time-frequency representation of the event-related EEG data measure at the parietal EEG channel in the typical subject. The figure shows that there was significant correlation between the subjective confidence and 40-Hz gamma-band power.

Disclosures: S. Iwaki: None.

Poster

169. Perception and Imagery

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust 100227

Title: Magnetoencephalographic correlates of perceptual state during auditory bistability

Authors: *R. D. SANDERS[1], J. WINSTON[2], G. BARNES[2], G. REES[2];
[1]Dept of Anesthesiol., Madison, WI; [2]Univ. Col. London, London, United Kingdom

Abstract: Background: Bistable perception occurs when two alternative experiences can be derived from the same physical stimulus; the neural correlates of different subjective experiences can be correlated with observed electrophysiological differences between the alternative states. This may have practical application in studying perception during states of altered consciousness. Here we used a bistable auditory stimulus to determine whether the two perceptual states could be distinguished electrophysiologically at the individual participant level. Methods: Fourteen participants underwent magnetoencephalography (MEG) while reporting their perceptual experience resulting from listening to a continuous stream of auditory tones, a previously well-characterized bistable stimulus. MEG data were analyzed both in sensor space and after source reconstruction in unconstrained analyses (based on the five sources of maximum posterior variance) and within a priori identified regions of interest: right and left auditory cortex (rAC and lAC) and right posterior intraparietal sulcus (rIPS). Results: Participants reported bistability with a similar overall proportion of the two alternative percepts (52% v. 48%). In sensor space, electrophysiological discrimination between the percepts was possible in 11/14 participants (79%). Unconstrained source space analysis also showed significant differences between percepts in 11/14 participants, predominantly based on sources in the temporal lobe. Source space analysis restricted to a priori identified ROIs showed discrimination was possible in the rAC (9/14), lAC (2/14) and rIPS (1/14). Conclusions: MEG can be used to objectively define the neural correlates of consciousness for auditory experience at the single participant level.

Disclosures: R.D. Sanders: None. **J. Winston:** None. **G. Barnes:** None. **G. Rees:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Support: European Research Council (ERC) Consolidator grant nr. 614244 (acronym: P-CYCLES)

Title: 10 Hz perceptual echoes propagate as travelling waves in the human brain

Authors: *D. LOZANO-SOLDEVILLA, R. VANRULLEN;
Univ. Paul Sabatier, CNRS-UMR 5549, Toulouse Cedex 9, Ctr. de Recherche Cerveau et Cognition (CerCo), Toulouse Cedex, France

Abstract: Recent evidence suggests a key role for alpha oscillations (8-12Hz) in visual perception: both the amplitude and the phase of these oscillations can be related to perceptual outcome. It is still unknown, however, whether the alpha rhythm influences sensory processing uniformly, with the same phase across all affected locations, or whether its rhythmic influence propagates across the retinotopic space with systematic phase variations, i.e. like a travelling wave. We have previously revealed that alpha rhythmic activity produces a long-lasting perceptual "echo" of the visual stimulation (VanRullen & Macdonald, Curr Biol, 2012). Electroencephalography (EEG) was recorded from human observers while the luminance of a peripheral disc fluctuated randomly as a white noise sequence. Cross-correlation between the luminance sequence and the EEG time series revealed strong ~10Hz reverberations in occipito-parietal sensors that could last beyond lags of 1s. When two discs with random luminance sequences were simultaneously presented in the left and right visual hemifields, it was possible to extract two independent "echo" functions in response to each of the two stimulation sequences. Here, we re-analyzed the original dataset from VanRullen & Macdonald (2012) to probe the existence of systematic phase variations across visual and/or cortical space, i.e. the existence of travelling waves. In one analysis, we compared for each participant (n=10) the analytic phase of the alpha oscillatory echoes recorded in response to the left vs right-hemifield luminance sequences. Across participants, we found highly consistent phase differences, such that for parietal sensors, the ipsilateral sequence was always represented with a positive phase lag relative to the contralateral sequence. In another analysis, we mapped the analytic phase of the echo response to a given stimulus sequence across neighboring scalp (sensor) locations. Again, we found consistent phase differences, with ipsilateral parietal sensors presenting a positive phase lag relative to contralateral parietal sensors. In both analyses, the phase lag ranged from pi/4 to pi/2 radians, corresponding approximately to a 15-25ms temporal delay. In short, alpha perceptual echoes travel rapidly (within a quarter-cycle) from contralateral to ipsilateral visual representations. These findings represent, to our knowledge, the first evidence for alpha travelling waves across the cortical representation of retinotopic space in the human brain.

Disclosures: D. Lozano-Soldevilla: None. **R. VanRullen:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Support: NIH/NEI Grant R01EY024056

Title: The role of the perirhinal cortex in tactile perception and memory in the blind

Authors: *L. CACCIAMANI, L. T. LIKOVA;
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Abstract: The perirhinal cortex (PRC) is a medial temporal lobe structure that has been implicated in visual object processing during both perception and memory (e.g., Murray et al., 2007). For instance, prior research has found that the PRC is activated during the visual perception of novel objects, but reduces its activation in response to those objects if they are made experimentally familiar (Henson et al., 2003). Here, we investigated the PRC's role in tactile perception and memory in the absence of visual input. To do so, we used the Cognitive-Kinesthetic Drawing Method (Likova, 2012; 2014) to train 5 blind subjects on a memory-guided drawing task which has been shown to produce notable improvements in spatiocognitive performance and cortical reorganization in low-level "visual" regions. The tasks were (20s each): perception via tactile exploration/memorization (EM) of experimentally novel raised line drawings of objects and faces, tactile memory retrieval via drawing (MD), and a scribble motor control (S). FMRI was conducted during these tasks before and after a week of the drawing training. Blood oxygen level dependent (BOLD) activity in the PRC was assessed for task-dependent differences in activation from pre- to post-training, i.e., when the drawings were novel vs. familiar, respectively. In every subject, a suprathreshold ($z > 1.96$, $p < .05$) cluster of voxels in bilateral PRC was found during both EM and DM tasks in which activation was significantly ($p < .05$) reduced post-training vs. pre-training. No significant effects were observed in the PRC during the motor control task. These results indicate that BOLD activity was suppressed upon the novel line drawings becoming familiar via the training, suggesting that the PRC is involved in tactile object processing in the blind as it's involved in visual object processing in the sighted. Moreover, its involvement is evident during both perceptual (EM) and mnemonic (DM) tasks. This study sheds light on tactile object representations in a structure typically assessed in the visual modality, and suggests that the reorganization towards tactile representations that occurs at low levels in the blind brain extends to higher-level associative areas as well.

Disclosures: L. Cacciamani: None. **L.T. Likova:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH R01 EY021755

Title: Neural fusion of sensation and expectation

Authors: *M. F. PANICHELLO[1], N. B. TURK-BROWNE[2];
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Dept. of Psychology, Princeton Univ., Princeton, NJ

Abstract: Expected visual stimuli are perceived faster and more accurately, but how? Sensory cues and expectations may independently contribute information about perceptual features to decision-making processes. Alternatively, information provided by sensory cues and expectations might be integrated into a "fused" feature representation (in a process analogous to maximum likelihood estimation) prior to perceptual decision-making. In Experiment 1, we approached this question using a psychophysical approach in which we assessed the impact of a predictive tone and face stimulus on perceived gender. We first used a training task to map a continuous range of tone frequencies onto a distribution of face stimuli ranging in gender from strongly masculine to strongly feminine. We next tested for fusion by measuring the sensitivity of participants in a gender discrimination task as we varied the strength of sensory cues (i.e., faces of varying gender) and expectation cues (i.e., tones of varying frequencies). Differences in gender could be conveyed by differences in the visual stimuli (stimulus-alone condition), the predictive tones (expectation-alone condition), or both (congruent condition). The fusion model predicts that sensitivity in the congruent condition should exceed the quadratic sum of the sensitivity in the stimulus- and expectation-alone conditions. Furthermore, the performance of a fusion mechanism will suffer when sensory and expectation cues conflict (incongruent condition), while an independence mechanism will be unaffected. Consistent with a fusion mechanism, discrimination sensitivity exceeded quadratic summation in the congruent condition and incongruent cues impaired performance. In Experiment 2, we used fMRI to examine at which stage of processing such fusion may be occurring in the brain. We again trained participants to map tones onto gender and then showed them tone-face pairs. Rather than asking them to perform a gender discrimination task, however, we instead trained a linear classifier to decode gender based on local patterns of BOLD activity for each of our four trial types (stimulus-alone, expectation-alone, congruent, incongruent). A tentative whole-brain searchlight analysis revealed worse decoding for incongruent trials in cortical regions associated with visual and auditory processing and conflict monitoring, following one of the predictions of the fusion

account. These findings complement prior studies on cue combination, suggesting that expectation can be an important cue in guiding perception.

Disclosures: **M.F. Panichello:** None. **N.B. Turk-Browne:** None.

Poster

169. Perception and Imagery

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Support: Finnish Academy grant #277655

Finnish Academy grant #127401

Title: Evidence for genetic regulation of the human parieto-occipital 10 Hz rhythmic activity

Authors: ***H. M. RENVALL**[1], E. SALMELA[2], J. KUJALA[1], O. HAKOSALO[2], J. KERE[2,3], R. SALMELIN[1];
[1]Aalto Univ., Espoo, Finland; [2]Univ. of Helsinki, Helsinki, Finland; [3]Karolinska Institutet, Stockholm, Sweden

Abstract: Human cerebral cortex shows several intrinsic oscillations that can be characterized with noninvasive neuroimaging methods such as magnetoencephalography (MEG) and electroencephalography (EEG). The most prominent of them is the 10-Hz "alpha" rhythm recorded over the parietal and occipital cortices. The cortical sources of alpha activity in humans have been located around the parieto-occipital sulcus, and intracortical recordings in dogs have revealed simultaneous activity in the thalamic nuclei, suggestive of involvement of the two brain regions in the rhythm generation. The rhythm is strongly attenuated by opening of the eyes, and it has important functional roles e.g. in visual attention and imagery. Its reactivity has been widely used to probe cortical functions both in healthy and clinical populations. Several EEG studies have demonstrated the high heritability of the rhythm, but little is known about its underlying genetic determinants. To uncover the possible genetic determinants of the parieto-occipital 10-Hz rhythm in a normal population, we measured spontaneous brain activity with MEG in 210 individuals (from 100 families) while the subjects had their eyes closed and open. The cortical activity was recorded with 306-channel Elekta Neuromag neuromagnetometer, and amplitude spectra at each channel were calculated using FFT. DNA was extracted from blood samples and genotyped with Affymetrix 250K array. In the analyses we used genotypes for more

than 28000 markers. Brain activity was quantified from the difference spectra between eyes-closed and eyes-open conditions. Width of the main spectral peak at ~10 Hz, peak frequency, and peak strength were measured at the maximum channels over the left, middle and right parieto-occipital cortices. In accordance with earlier EEG studies, peak strengths of the rhythm were highly heritable ($h2 > 0.75$). Variance component-based analysis of the genomic markers revealed linkage for both the strength and the width of the spectral peak. The strongest linkage was detected for the width of the spectral peak over the left parieto-occipital cortex on chromosome 10[q23.2] (LOD = 2.814, nominal $p < 0.03$). This genomic region contains several functionally plausible genes, including GRID1 and ATAD1 that regulate glutamate receptor channels mediating synaptic transmission, NRG3 with functions in brain development, and HRT7 involved in serotonergic system, circadian rhythm, and sleep. Overall, our results demonstrate the potential of genetic analysis in linking macroscopic cortical phenotypes with the molecular level through association with specific genes.

Disclosures: **H.M. Renvall:** None. **E. Salmela:** None. **J. Kujala:** None. **O. Hakosalo:** None. **J. Kere:** None. **R. Salmelin:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Support: Swiss National Science Foundation (n° 51AU40_125759)

Title: Insula mediates suppression of synchronous cardio-visual stimuli from awareness- a high resolution fMRI study

Authors: ***R. SALOMON**[1], R. RONCHI[1], J. DÖNZ[1], J. BELLO-RUIZ[1], B. HERBELIN[1], R. MARTET[1], N. FAIVRE[1], K. SCHALLER[2], O. BLANKE[1];
[1]EPFL SV BMI LNCO, EPFL, Lausanne, Switzerland; [2]Neurosurg., HUG, Geneva, Switzerland

Abstract: Interoceptive signals conveying information regarding the state of the body are processed in the insular cortex and this has been suggested to underlie self-awareness. However it is not known whether interoception impacts also visual awareness and whether this also involves the insular cortex. Here, in a series of 10 experiments including 170 participants we show that the relative timing of visual stimuli with respect to the heartbeat modulates visual awareness and that this is related to insular activity. Our psychophysical results showed that

awareness for visual stimuli synchronous to participants' heartbeat was suppressed compared to the same stimuli presented asynchronously to their heartbeat. Two high resolution fMRI experiments showed that the insular cortex was sensitive to both visible and unseen cardio-visual stimulation, showing reduced activation for synchronous cardio-visual targets. Finally, neuropsychological data from a patient with insular damage showed that heartbeat-related suppression of visual awareness was abolished by insular damage. Our results show that interoceptive insular processing impacts visual awareness, demonstrating the role of the insula in bridging interoceptive and exteroceptive awareness. Our data support an account based on predictive coding in which the sensory consequences of self -generated interoceptive signals are suppressed in the insular cortex.

Disclosures: **R. Salomon:** None. **R. Ronchi:** None. **J. Dönz:** None. **J. Bello-Ruiz:** None. **B. Herbelin:** None. **R. Martet:** None. **N. Faivre:** None. **K. Schaller:** None. **O. Blanke:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Title: Human perception of statistical significance and effect size

Authors: *B. R. SHETH[1], J. S. PATEL[2];
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Abstract: Statistics are ubiquitous in our Big Data age. Today, statistical literacy is a requirement for all. Most judgments are fast and based on perception, however. Therefore, we asked: How sensitive is human perception of statistics? In experiments, we displayed scatter plots of dots chosen from two Gaussian probability distributions that had different means or variances (but not both). 1) Across trials, effect size (mean1 - mean2/√variance) and statistical significance (p-value from KS test) were co-varied. We asked if naïve observers (Os) were sensitive to this combined statistical manipulation. The two dot clusters were displayed in the same figure (fig.) in red and blue colors. The means or variances of the underlying distributions varied (as did the difference between them, hence the effect size and p-value measured using Kolmogorov-Smirnoff test varied as well) across trials. Os (N=12-15) judged the two clusters as "different" on >30% of trials when the effect size was zero and the p-value insignificant on all trials (false positives), but "same" on >50% of trials when the effect size was moderate and the p-value significant on 100% of trials (misses). The proportion of "different" perceptual

judgments increased with difference in mean or variance of the two distributions but the slope of increase was significantly flatter as compared to that for "different" statistical judgments. Thus, response bias cannot account for the results (also, confidence ratings were typically flat with change in mean or variance). When the clusters were displayed in two different figs side by side, the correspondence between human data and statistics improved little. 2) Effect size and statistical significance were independently varied. In two separate figs presented side-by-side, red and blue clusters were shown in each fig. One of the two figs had more dots than the other (numbers of red and blue dots displayed in each fig. were same). In one fig. compared to the other, the difference between the two clusters it contained was more significant (due to more dots) despite a smaller effect size of the difference (due to smaller difference in means or variances of the two distributions). Os had to choose the fig. for which red and blue dot clusters differed more. If effect size (statistical significance) drives perception of difference, Os will overwhelmingly choose the larger effect size (smaller p-value) fig. Instead, Os (N=25) chose the larger effect size fig. on 33-51% (over a range of differences in effect size) of trials. Our work shows we are largely insensitive to statistical significance and effect size. We will test if learning can cause long-term improvements in sensitivity to statistics.

Disclosures: B.R. Sheth: None. **J.S. Patel:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH R01 EY012440

Title: Synesthetes are more sensitive to cross-modal correspondences than non-synesthetes

Authors: M. MARTINEZ, *S. A. LACEY, K. MCCORMICK, K. SATHIAN;
Emory Univ., Atlanta, GA

Abstract: Synesthesia is a phenomenon in which experiences in one sensory or cognitive domain are consistently associated with automatic, involuntary experiences in a second domain. The most common form is grapheme-color synesthesia which involves consistently experiencing a specific color when seeing a particular letter or number. These synesthetic associations are idiosyncratic, i.e., different synesthetes may experience different colors in relation to the same letter. However, synesthetic associations are not totally random: similar graphemes tend to evoke

similar colors. Since non-synesthetes may also exhibit similar, albeit weaker, associations, synesthesia may be at one end of a spectrum. Cross-modal correspondences refer to the tendency of a stimulus in one modality to be linked with a seemingly unrelated stimulus in another modality. For example, people commonly associate high (low) pitch with high (low) spatial elevation, or with small (large) size. Similarly, people associate certain pseudowords (e.g., "lomo") with rounded shapes and others (e.g., "keke") with spiky shapes. Since synesthesia also involves associations between two different domains or modalities, we hypothesized that synesthetes are more sensitive to cross-modal correspondences than non-synesthetes. We tested this hypothesis using the implicit association test (IAT) to examine pitch-elevation, pitch-size, and pseudoword-shape correspondences in the visual and auditory modalities. In the IAT, participants learn to associate two responses with the same response key, with specific pairings rotating across blocks of trials. Pairings are either congruent (e.g., high pitch/small size) or incongruent (high pitch/large size). On each trial, a single stimulus is presented in one modality, and participants make speeded responses, which are faster when the key associations are congruent and slower when incongruent, i.e., there is a congruency effect for cross-modal correspondences (Parise & Spence, Exp Brain Res 2012). Participants (n=27) completed the online Synesthesia Battery (SB: Eagleman et al., J Neurosci Meth, 2007); 12 were classified as synesthetes, with various kinds of synesthesia, and 15 as non-synesthetes. Synesthetes had larger congruency effects than non-synesthetes for all three correspondences in both modalities. Interestingly, the strength of synesthetic associations, as measured by the SB, was uncorrelated with congruency effects on the IAT. Consistent with our hypothesis, synesthetes are more sensitive than non-synesthetes to cross-modal correspondences, supporting the notion of quantitative differences along a spectrum.

Disclosures: **M. Martinez:** None. **S.A. Lacey:** None. **K. McCormick:** None. **K. Sathian:** None.

Poster

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Title: Stimulating Associations

Authors: *E. M. AMINOFF[1], Y. LI[2], J. A. PYLES[1], M. J. WARD[3], G. GHEARING[3], R. M. RICHARDSON[3,4], A. S. GHUMAN[3,4];
[1]Dept. of Psychology & Ctr. for the Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA; [2]Program in Neural Computation, Carnegie Mellon Univ. and Univ. of Pittsburgh, Pittsburgh, PA; [3]Univ. of Pittsburgh, Pittsburgh, PA; [4]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Interaction with the environment involves the perception of sensory stimuli (e.g., object recognition, scene perception) and relating these percepts to an existing knowledgebase as well as past experiences. For example, it is not useful to recognize a kitchen without having the associated knowledge that this is where people cook and eat; that where there is an oven, a refrigerator will likely be nearby; and within the refrigerator there is food. Previous studies have demonstrated that objects with strong contextual associations (e.g., an oven) activated regions of the ventral medial temporal cortex (VTC) - mainly within regions of the parahippocampal region spreading into lingual and fusiform areas - along with other regions in the retrosplenial complex and transverse occipital place area, to a greater extent and magnitude than objects with weak contextual associations (e.g., folding chair). This difference in activation can occur automatically during object recognition and may provide feedback in support of the recognition process. Understanding this brain activity within a framework of associative processing may provide a bridge for explaining why both high level visual processes and episodic memory activate the same regions of the VTC. However, the causal link between episodic, associative, and high level visual processes has not been established. In this study, we used intracranial electrocorticography (ECoG) in a patient who suffers from intractable epilepsy to investigate the characteristics of associative processing of objects in regions of the VTC. Here, we report that implanted electrodes along the medial left fusiform demonstrated a significant differential signal when recognizing objects with strong contextual associations compared with recognizing objects with weak contextual associations (peak broadband gamma difference $p < 0.05$). To test the nature of this effect, direct electrical stimulation was applied to this region. The stimulation resulted in the patient experiencing hallucination-like episodes that occurred during word and object recognition. When asked to describe what the patient had experienced he said things like “It reminded me of something from my past, like I went through a bunch of pictures in my mind”; and “It was...trying to connect to a different picture that was like it.” These results demonstrate that VTC plays a causal role in visual associative and episodic processes the can emerge during object recognition. In addition, these results may suggest a role of associative processing in the nature of hallucinations.

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Poster

169. Perception and Imagery

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Support: 2012CB825500

91137302

Title: Early responses discriminate global differences revealed by ecog

Authors: *R. WU;
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Abstract: Our previous studies have demonstrated that the visual system is sensitive to topological changes, such as the appearance and disappearance of holes in a figure. Accumulating results based on fMRI indicate that anterior temporal areas may underscore the advantages in perception of global properties such as topological differences. However, the lack of high-resolution timing information in fMRI makes the argument unsettled. Traditional ERP/EEG has high temporal resolution, but its spatial resolution is poor. To understand the neural mechanism of topological processing with a high temporal-spatial resolution, we use ECoG (electrocorticogram) from intracranial electrodes while epilepsy patients are monitored for seizure detection. Epilepsy patients implanted with intracranial electrodes for seizure detection purpose were invited to participant this study. There are 284 electrodes in the temporal lobe we recorded. ECoG was recorded by a MicroMed SD128 system at a sampling rate of 512 Hz. Stimuli were 37 pairs of figures (composed from 22 figures) randomly presented for 200 ms each with an interval of 600 - 1200 ms between pairs. Subjects were required to attend to one side of the visual field cued by the arrow at the center of the screen and press a button whenever they found the current figure was the same shape as the previous one. Neural responses as ERPs for different figure pairs were aligned to the onset of the second figure and averaged after notch filtering, baseline correction and artifact reduction. A moving-window t-test was applied to test the difference between figure groups. Neural responses in anterior temporal areas can discriminate global differences as early as about 50 ms after stimulus onset. More electrodes showed shorter latency for topological changes, and the pattern to some degree agreed with the functional hierarchy of the geometrical figures. The distribution of the first 40 electrodes with shortest latency showed different patterns for topological changes, compared with affine or

Euclidean changes ($P < .005$ and $P < .05$, respectively, rank sum test). Shortest responses of topological changes were not modulated by attention.

Disclosures: R. Wu: None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Title: What does functional brain imaging reveal?

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Abstract: Introduction: Modern functional brain imaging studies have been dominated by hemodynamics-based approaches. A huge amount of effort and energy has been spent in watching local blood events in attempts to find a correlation with neural impulse events and further with a thing commonly believed as brain function such as perception, awareness or cognition. Paradoxically, this chain of inferences may be much weaker than what most investigators would admit. We argue with our data: this is indeed the case. Method: We used our adapted diffusion tensor imaging approach (aDTI) [1] to image chronic pain in chronic migraine (CM, 20 patients) and fibomyalgia (FMS, 20 patients) as well as matched healthy controls. As aDTI did not use the blood flow surrogate, we removed the first uncertainty - neurovascular coupling in the inference chain. Thus, we gained the basis to ask what neural impulse events detected in our patients really represented. Result: Both the CM and FMS groups in comparing with the control group had abnormal baseline neural impulse loci in cerebral white matter fibers in the central pain matrix (a popular term that lumped a few activation loci together). The FMS group had additional loci ($p < .001$) in the fiber tracts of the orbitofrontal cortex and brainstem. Also, in the brainstem of the CM group, additional multiple loci of abnormal baseline activity ($p < 0.001$) were found in the cerebral peduncle, in the middle cerebellar peduncle, in the midline midpons, and in the upper medulla. Conclusion: Our pain study aims at exploring the mechanisms of perception, awareness and cognition as most published functional brain studies do explicitly or implicitly. We all resort to a similar inference chain. Although aDTI discards the blood-flow surrogate, fundamental questions remain: What is exactly brain function? What does functional brain imaging reveals? Our Human Brain Mapping Metamodel [2] helps in starting

addressing these questions, as summarized in the figure. There is no direct information passage from impulses to senses.

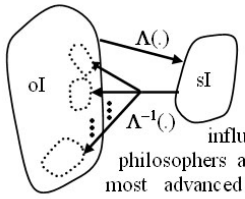

Fig. Human Brain Mapping Metamodel [2] that helps to draw the following conclusion. We take it as granted that the brain is the seat for perception, awareness and cognition, under the overwhelming influence of generations of psychologists and philosophers as well as the modern explorers with the most advanced tools such as MRI. This brain seat "intuitive" has been leading us seeking centers, streams, grandma cells, or arbitrators in the frontal cortex. That is, we are looking for brains in the brain while ignoring understanding a highly distributed system in the correct way. Psychoactive agents, chronic pain medicine, or a great part of neuroscience drugs share a rather peculiar common feature: multiple indication and limited efficacy on a particular. DBS or rTMS shares the same features. Stimulation or disruption? Current theories cannot settle the debate. As a result, even the groundless Freudian "unconscious" is still taken as a basis for certain therapy. Our metamodel hints that a collective of distributed extracerebral actuation may give rise to perception, awareness and cognition. As such, if we do a brain parenchyma transplant (not whole head), hypothetically, the one with the new brain would see self and others as strangers and the world very distorted at best. It is time to question our inherited intuitive that does not apply beyond the fact that the brain is just a wiring center.

Reference: 1. Leith et al., Soc Neurosci 2011 2. Leith et al., Soc Neurosci 2014

Disclosures: C.C. Leith: None. **L. Robbins:** None. **L. Siegel:** None. **S. Ouyang:** None. **H. Sun:** None.

Poster

169. Perception and Imagery

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: Personal Grant for Elisa Ruohonen from Finnish Cultural Foundation

Project Grant for PA from The Academy of Finland project nro. 140126

Title: Pre-attentive change detection of sound intensity is affected in first episode depression but not in recurrent depression

Authors: *E. RUOHONEN, J. L. O. KURKELA, P. ASTIKAINEN;
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Abstract: Depression is associated with abnormal cognitive processing of emotional information but recent research suggests that this bias exists also in the level of basic physical feature processing. In the auditory modality, there is some evidence that dysfunction in brain's protecting mechanism for loud sounds would be associated with depression. However it is not clear whether processing of sound intensity as such or change detection mechanism is affected in depression. In order to test this, we studied auditory evoked potentials (AEPs) in patients diagnosed with either first episode (n=17) or recurrent depression (n=25) and compared their brain responses to healthy controls' (n=21) data. In the experimental design frequently presented low intensity sounds (60 dB) were rarely and randomly replaced by high intensity sounds (80 dB). In the other stimulus block the intensities for the frequent and rare sounds were reversed. The sounds were 100 ms in duration and 1000 Hz in frequency and were presented with randomly varying onset to onset interval of either 450, 500 or 550 ms. AEPs were extracted from two time windows: at 90-140 ms (N1) and at 150-200 ms (mismatch negativity, MMN) post-stimulus interval. N1 was larger in amplitude to rare high intensity, but not to rare low intensity sound, compared to the frequent sound. N1 amplitude was also different between the groups. First episode depression group showed larger N1 responses to rare sounds, irrespective of their intensity, compared to recurrent depression group and control group. MMN, which is a correlate of automatic change detection, was larger in amplitude for both, low and high intensity, rare sounds. There were no group differences in the MMN response, however. Results suggest that central auditory processing of sound intensity, especially change detection mechanism, is affected in first episode depression. This dysfunction is however recovered in those who have recurrent depression. This finding, although based on cross-sectional data, may indicate that compensatory mechanisms can normalize depression-related perceptual dysfunction in the course of time.

Disclosures: **E. Ruohonen:** None. **J.L.O. Kurkela:** None. **P. Astikainen:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Support: KAKEN25700015

KAKEN22240026

Title: Effect of tDCS on rubber hand illusion in relation to schizotypal parsonality

Authors: *N. YODA, T. IGARASHI, S. SHIMADA;
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Abstract: The rubber hand illusion (RHI) is an illusion in the integration of visual and tactile stimuli of the self-body. It is known that the temporo-parietal junction (TPJ) is crucially involved in self-body representation and the occurrence of RHI. In this study, we applied transcranial direct current stimulation (tDCS) to left TPJ to investigate whether it modifies the quality of RHI in relation to the degree of schizotypal personality, which is characterized as a predisposition to schizophrenia. Thirty right-handed healthy subjects (7 female, aged 21.0 ± 1.7) participated in the experiment. Subject's level of schizotypal personality was examined by means of the Japanese version of the Schizotypal Personality Questionnaire (SPQ). The subjects put their right hand on the table, and the rubber hand was placed 15 cm leftward from their hand. The subjects watched delayed image (100, 300, or 500 ms) of the rubber hand that was filmed using a video camera. Two paintbrushes were used to simultaneously stroke the index finger of the subject's own hand and that of the rubber hand at approximately 0.5-1 Hz. The stimulation period was 3 min. After each session, subjects completed RHI questionnaire, which in 9 items, identical to that used in the previous study (Botvinick & Cohen, 1998). The subject first underwent a RHI session for each delay condition (pre-stimulation sessions), followed by 10 min tDCS stimulation (1 mA). The subject then underwent the second RHI session for each delay condition while applying tDCS throughout the session (post-stimulation sessions). Subjects were randomly assigned to the anodal, cathodal, or control "sham" groups. The stimulation was induced with a pair of saline-soaked surface sponge electrodes ($35cm^2$) and delivered by a battery-driven constant current stimulator. For the stimulation of the left TPJ, the anodal or cathodal electrode was placed over a point midway between T3 and P3 (international 10/20 system). The reference electrode was placed over left prefrontal cortex (a point midway between F3 and FP1). The result on questionnaire data showed that differential (pre - post-stimulation) RHI score (item 2) showed a significant difference between the anodal and cathodal groups in the 500ms delay condition ($t(18) = 2.28$, $p < 0.05$). This indicates that the subject in the anodal group felt more RHI after stimulation. In addition, we found positive correlations between the RHI score (item 2) and a SPQ subscale score ("No Close Friends") in the anodal group in 500ms delay condition ($r = 0.75$, $p < 0.05$). These results suggest that tDCS to left TPJ can alter the experience of RHI, which can be further modulated by schizotypal personality.

Disclosures: **N. Yoda:** None. **T. Igarashi:** None. **S. Shimada:** None.

Poster

169. Perception and Imagery

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ANR-10-LABX-0087 IEC

ANR-10-IDEX-0001-02 PSL*

Title: Repetition probability effects on neural repetition suppression are dependent on context predictability

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Abstract: Several recent functional Magnetic Resonance Imaging (fMRI) studies have shown that the probability of stimulus repetitions, P(rep), influences the degree of Repetition Suppression (RS): the neural response to repeated stimuli is decreased to a greater extent in high compared to low P(rep) blocks. In line with predictive coding theories, this has been interpreted as a modulation of high-order contextual expectations: when repetitions are more likely, brain responses to repeated stimuli are suppressed as a result of expectation suppression. An alternative account of those results would rely on the existence of a constant 'default' repetition prior in the brain, rooting in the relative stability of our visual environment, which precision would vary depending on the overall predictability of the context. In all studies of P(rep) effects, context predictability covaries with repetition probability: in alternation trials, the first stimulus can be followed by any other, hence the identity of the second stimulus of a pair is globally harder to predict in low P(rep) blocks. As a consequence, the 'default' repetition prior would be applied with lower precision, resulting in less suppression for repeated stimuli. We posit that if alternations become predictable, that is, if a stimulus A can either be repeated (25%) or followed by a given stimulus B (75%) instead of any other exemplar, stimulus repetitions would lead to RS to a similar extent as in high P(rep) blocks. We investigated this hypothesis with fMRI, using face stimuli and focusing our analyses on the fusiform face area (FFA). On each trial, participants were presented with either the same face twice or two different faces, in 3 experimental contexts: high P(rep) (75%), low P(rep) (25%) & unpredictable alternations, and low P(rep) (25%) & predictable alternations. The latter were learnt during a behavioral training session. We used long Inter-Stimulus Intervals (7 seconds, jittered) in order to dissociate brain responses to the first and second face of each pair using a General Linear Model, and computed RS as the difference between responses to the first and second face of repeated trials.

Consistently with previous studies, RS was strong in 'high P(rep)' blocks, while it was decreased in 'low P(rep) & unpredictable alternations' blocks. Crucially, RS was present in 'low P(rep) & predictable alternations' blocks to a similar extent as in 'high P(rep)' blocks. This supports the hypothesis of a 'default' repetition prior that is applied with variable strength depending on the overall predictive context of the block, rather than a modulation of the prior itself as a function of P(rep).

Disclosures: **A. Pajani:** None. **V. De Gardelle:** None. **S. Kouider:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Support: ERC-YSt-263584

ANR- JCJC-1904

Title: Decoding perceived duration in various sensory modalities from MEG-EEG data

Authors: ***T. W. KONONOWICZ**, V. VAN WASSENHOVE;
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Abstract: The sensory modality affects duration perception (Penney, Gibbon, and Meck, 2000). For instance, for the same physical durations, auditory stimuli have often been reported to be perceived as longer and more accurately than visual stimuli (Penney et al., 2000; Van Wassenhove, Buonomano, Shimojo, Shams, 2008). In the context of internal clock, which integrates temporal pulses to provide an estimate of elapsed time, auditory dominance is conceived to result from the enhanced pacemaker rate. The premise of the internal clock model is that there is a common mechanism involved in timing irrespective of sensory modality. On the contrary, modality specific models propose specific mechanisms for each modality. This problem has frequently been tackled by using perceptual learning approach where subjects are extensively trained on one modality and tested other modality in order to check whether training improves performance on tested modality. Behavioral data have not provided any evidence for transfer from the visual to the auditory modality (Lapid, Ulrich, & Rammsayer, 2009; Bratzke, Seifried, & Ulrich, 2012). Yet, transfer from the auditory to the visual modality has been found (Bratzke et al. 2012), suggesting auditory dominance in timing. However, psychophysical

methods are not sufficient to reveal neural underpinnings of such intersensory transfer. Therefore, we asked participants to discriminate time intervals demarcated by auditory, visual, or audiovisual markers while recording brain signals using EEG and MEG. On each and every trial the standard duration (700ms) was presented, followed by the presentation of the comparison interval (630, 700, 770ms; 10%, 80%, 10% of all trials respectively). We used support vector machines algorithm (Pedregosa et al., 2011) to decode MEG activity to discriminate the subjectively perceived 'short' and 'long' durations. Preliminary data show that sensory-specific responses predict subjective perception of duration when training and testing the algorithm is performed within the same modality. Specifically, above chance level classification has been found around 100ms after the duration onset and suggesting that the early components contribute to the duration perception. Importantly, to evaluate predictions of the internal clock model and modality specific models we will train classifier on one modality and assess classification accuracy in the other modality to assess whether early and late components contribute to transfer of timing mechanisms between modalities.

Disclosures: T.W. Kononowicz: None. **V. van Wassenhove:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Title: Gist perception in the presence of information overflow

Authors: *K. MOGI;
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Abstract: One of the salient constraints on the cognitive processes in the brain is that there is an overflow of information (e.g. Lau & Rosenthal 2011, Block 2011), so that only a subset can be represented in the cognitive system. Seen from the volume of information processed, higher cognitive processes in the brain handle gradually less information. In the visual system, it is estimated that about 10^10 bits/sec of information is registered at the retina, about 6x10^6 bits/sec of information leaves the retina, and about 10^4 bits/sec of information reaches the layer IV of area V1 (Raichle 2010). In visual perception, there is empirical evidence to suggest that not all the information present in the visual field is processed in the cognitive system. In change blindness (Simons and Levin 1997), the subject is unaware of large changes in the visual field. In inattentional blindness (Mack and Rock 1998), the subject fails to register a salient object in the

scene. Given these constraints, in order to adapt to the various relevant elements in the environment, the brain needs to encode and react to the overflow of information in an efficient way. Barlow (2001) argues that redundancy reduction is one of the fundamental principles behind cognition. Friston (2009, 2010) proposes the free energy principle to understand how the cognitive systems are organized and function. There is evidence that the visual system is able to support gist perception (Oliva 2005, Cohen et al. 2011), while discarding the details. Here I present a model to account for the nature of "gist" in the cognitive processes in the presence of information overflow. The gist is formulated as an effective minimization of Bayesian "surprise" (Friston 2009) in the processing of information. Effective gist perception is formulated as one of the most significant selection pressure on consciousness. I argue that sensory qualia evolved to give a repertoire of gist representation, the combination of which would give an effective picture of the environment even when a majority of information coming from the environment is lost through the adaptation to the information overflow.

Disclosures: K. Mogi: None.

Poster

169. Perception and Imagery

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Program#/Poster#: 169.21/W7

Topic: F.01. Human Cognition and Behavior

Support: Air Force Research Laboratory Core Funds

Title: Applications of transcranial direct current stimulation for the military: An overview

Authors: *A. MCKINLEY[1], L. K. MCINTIRE[2], J. NELSON[2];
[1]Air Force Res. Lab., New Carlisle, OH; [2]Infoscitex, Dayton, OH

Abstract: With increases in reliance on remotely piloted vehicles and automation, a growing number of military jobs are beginning to resemble traditional office settings and require a new, nontraditional skill set. In the Air Force, much of the mission emphasis has shifted to surveillance, intelligence, and reconnaissance (largely conducted by remotely piloted aircraft) due to the seemingly insatiable demand for such sorties. Remotely piloted aircraft operators and the supporting image analysts have long shifts (up to 12 hours a day, 6 days a week) to try to keep up with demand. Looking for targets over a long period of time is both tedious and effortful requiring intense sustained attention/vigilance. Unfortunately, the ability to find such targets degrades over time, a phenomenon known as the vigilance decrement. Likewise, acute and

chronic fatigue stress can further reduce performance. Given that misidentifying or not detecting the targets can potentially put lives at risk, investigating new methods of reducing the performance declines has been an area of interest for the Air Force. Over the past several years, we have examined the efficacy of transcranial direct current stimulation (tDCS) to modulate attention, accelerate learning, and mitigate the effects of fatigue. We have discovered that tDCS has a large effect on target detection accuracy in a variety of vigilance tasks and this effect is repeatable and not dependent on the particular vigilance task. Specifically, tDCS extends vigilance performance by at least two fold. Likewise, we have found that small changes in the position of the anode did not significantly influence the performance outcomes. We have also found that tDCS significantly reduces the effects of fatigue on vigilance performance and that the effect lasts three times longer than caffeine. Likewise, there are beneficial effects on training, specifically for image analysts. Analysts receiving tDCS during training performed 25% better on their tests than analysts receiving sham tDCS. This effect has since been replicated at two different laboratories and appears to be robust and reliable. It is likely these effects may have been caused by improvements in attention. Greater attention during training often leads to improved retention following training. Taken together, tDCS has shown great promise for improving aspects of cognition that are important for existing and emerging military jobs. Determining the longevity of the effect and the effects of chronic stimulation remain critical research questions to tackle before such an intervention can be considered for deployment.

Disclosures: **A. McKinley:** None. **L.K. McIntire:** None. **J. Nelson:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Title: fMRI time-course analysis of the immediate post-orgasmic phase in men

Authors: ***K. ALLEN**[1,2], W. BIRBANO[2], N. WISE[2], E. FRANGOS[2], B. KOMISARUK[2]; [1]PWP, Princeton Univ., Princeton, NJ; [2]Psychology, Rutgers Univ., Newark, NJ

Abstract: Few neuroimaging studies have investigated the “refractory period” in men, which is the epoch after orgasm/ejaculation during which most men do not respond to genital stimulation with a second orgasm. In the present study, we report evidence of two distinct epochs within this refractory period. Using functional Magnetic Resonance Imaging (fMRI), we collected data as research participants self-stimulated to orgasm. They specified the start and end of orgasm using

a button press and were asked to press the button again to indicate when they felt that they had "recovered" from orgasm. Our time-course analysis indicates that this initial refractory period begins as a distinct pattern of distributed activity at the end of orgasm and persists for a period of a few seconds to several minutes. Its initiation is characterized by a rapid attenuation of orgasm-related neural activity across many brain areas that include amygdala, hippocampus, insula, anterior cingulate cortex, anterior hypothalamus, paracentral lobule and S II. The end of this initial period is characterized by a brief spike in neural activity across similar areas. Analysis of neural activity in men during the refractory period may provide insight into the mechanism(s) of anorgasmia.

Disclosures: K. Allen: None. **W. Birbano:** None. **N. Wise:** None. **E. Frangos:** None. **B. Komisaruk:** None.

Poster

169. Perception and Imagery

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Program#/Poster#: 169.23/W9

Topic: F.01. Human Cognition and Behavior

Title: Manipulating gamma oscillations in the human visual cortex with MEG based neurofeedback

Authors: N. MERKEL[1], G. BLAND[1], *W. SINGER[1], M. WIBRAL[2];
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Abstract: The brain can learn to modify ongoing activity through neurofeedback (NFB). Since the development of EEG based NFB methods in the late 1960s (Kamiya et al., 1669), an increasing number of studies were devoted to explore this phenomenon both in human subjects and animals. These studies indicate that subjects can learn to deliberately influence the hemodynamic response in specific brain structures (Caria et al., 2010; Sulzer et al., 2013), the power of oscillatory activity in selected frequency bands (Gruzelier, J. 2014) and neuronal discharge rates (Fetz, E. 1969, Clancy et al., 2014). Often NFB training was associated with changes in cognitive functions (Gruzelier, J. 2014). However, little is known about the specificity of these NFB effects in the spatio-temporal domain and on the dynamic mechanisms mediating them. We designed a NFB training protocol for healthy human subjects using magnetoencephalography (MEG). We attempted to restrict training effects to a small volume of visual cortex and to a narrow frequency band to test the specificity of changes and the NFB

training related network dynamics. We used online beamforming techniques to extract the feedback signal in source space. The pitch of a continuous tone feedback signaled the increase of narrow band gamma oscillations relative to oscillations in neighboring frequencies within a selected voxel of the visual cortex. The pitch of the tone increased with gamma-power and was updated every second. Subjects were asked to increase or decrease this pitch in a controlled way. So far, we studied 10 subjects who underwent 10 training sessions each. The NFB training with online beamforming substantially increased the power in the frequency band selected for training in 4 out of 10 subjects. Subjects were able to enhance narrow band gamma activity in early visual cortex in a circumscribed region located within 2.5 cm from the target voxel. As performance improved, beta oscillations became more prominent and synchronized across the right midfrontal and left parietal cortex, presumably reflecting enhanced top-down control by the visual attention network (Buschman & Miller, 2007; Bastos et al., 2014). It is still an open question how different areas interact to form attentional networks (Gross et al., 2004). We expect that this NFB approach will contribute to a better understanding of the mechanisms supporting top-down control of visual gamma oscillations. We further hope to provide tools to selectively tune pathological brain oscillations towards a more homeostatic set-point and thereby balance out disturbed network dynamics, which could underlie certain diseases (Ros et al., 2014).

Disclosures: **N. Merkel:** None. **G. Bland:** None. **W. Singer:** None. **M. Wibral:** None.

Poster

169. Perception and Imagery

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Program#/Poster#: 169.24/W10

Topic: F.01. Human Cognition and Behavior

Title: Neural systems selectively involved in navigation and categorization of scenes

Authors: *A. PERSICHETTI[1], D. D. DILKS[2];
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Abstract: Previous neuroimaging studies have identified three cortical regions involved in human visual scene processing: the parahippocampal place area (PPA), retrosplenial complex (RSC), and occipital place area (OPA). While the exact function each of these regions plays in scene processing remains unknown, it is currently believed that the scene processing system as a whole is a monolithic system in the service of navigation. However, we recently found that while RSC and OPA encode ‘sense’ (left-right) and ‘egocentric distance’ (close-far) information, which are both critical for navigation, PPA does not, challenging the pervasive theory that all of

these cortical regions serve the purpose of navigation. Instead, we propose that scene processing is comprised of two distinct pathways: one responsible for scene categorization (e.g., recognizing a scene as a kitchen, a beach, city hall), including PPA, and the other responsible for navigation, including RSC and OPA. If scene processing is comprised of a categorization stream (including PPA) and a navigation stream (including RSC and OPA), then it should be possible to selectively modulate the two streams by task demands. Specifically, using fMRI, we asked whether PPA would respond more to an image of a scene when a viewer is performing a 'categorization task' on the image than when the same viewer is performing a 'navigation task' on the exact same image, while RSC and OPA would show the opposite pattern. Indeed, preliminary data show a significantly higher BOLD response in PPA during the categorization task than to the navigation task, relative to OPA and RSC. By contrast, OPA responded significantly more during the navigation task than the categorization task relative to PPA and RSC. Such a double dissociation strongly suggests distinct cognitive and neural systems that are selectively involved in navigation and categorization of scenes.

Disclosures: **A. Persichetti:** None. **D.D. dilks:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Support: General Researcher Program (#2013058415) of National Research Foundation of Korea

Title: Correlation between illusory bistable perception and motion perception under noisy condition

Authors: ***W. CHOI**, M. HWANG, S.-B. PAIK;
KAIST, Daejeon, Korea, Republic of

Abstract: When visual system receives an ambiguous signal such as Necker cube, perceived state switches periodically between two interpretations. This phenomenon is called bistable perception, and has been studied extensively to investigate the working mechanism of sensory perception. Importantly, the reversal time - switching interval between two states - seems to be an important factor to understanding underlying mechanism, but its detailed dynamics is still elusive. In this psychophysics study, we investigated the correlation between the reversal time in

bistable perception of illusory motion and the performance in real motion perception within a subject. Our hypothesis is that the reversal time reveals an intrinsic time scale of a neural circuit for a certain type of functions, such as motion detection and it must be correlated with the information processing time for similar type of tasks. For example, reversal time in bistable illusory motion perception is defined as switching time between the two illusory motion directions, and this process parallels similar tasks, such as motion detection under noisy condition. If fast reversal time means fast processing for a given task, we expect that fast reversal time would be correlated with a better perceptual performance in general. To confirm this idea, we designed human psychophysics experiment using "racetrack" stimulus [1], which can provide both bistable illusory motion perception and real motion perception under noisy conditions. We varied a coherence parameter, c, of the racetrack stimulus so that the experiment condition can be either bistable illusory motion (c=0) or real motion with different amount of noise (c>0). We then examined the relationship between the reversal time in illusory motion condition (c=0) and the correctness in coherent motion condition (c>0). As we expected, subjects who showed fast reversal time for c=0 showed higher correctness for c>0 than those who showed slow reversal time. (N=5, R=-0.9, p=0.03, Pearson's correlation coefficient). This result suggests that the reversal time in bistable perception reveals an intrinsic time scale of a perceptual process, and might be a crucial factor to understand the mechanism of information processing in the brain.
Reference 1. Jain, Siddharth. "Performance characterization of Watson Ahumada motion detector using random dot rotary motion stimuli." PloS one 4.2 (2009): e4536.

Disclosures: **W. Choi:** None. **M. Hwang:** None. **S. Paik:** None.

Poster

169. Perception and Imagery

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Topic: F.01. Human Cognition and Behavior

Support: Alfred P. Sloan Foundation

Title: What type of stimulus location information is represented in human hippocampus?

Authors: *A. SHAFER-SKELTON, J. D. GOLOMB;
Dept. of Psychology, The Ohio State Univ., Columbus, OH

Abstract: The hippocampus is thought to be involved in diverse types of spatial processing--researchers have reported spatial maps of an animal's external environment, memory for spatial

relations, and view-invariant representations of scenes. However, there has been little research in hippocampus investigating how the locations of visual stimuli are represented--for example, in eye- or head-centered reference frames. Golomb & Kanwisher (2012) found that throughout visual cortex, stimulus locations are represented in retinotopic (eye-centered) coordinates across fixations. Here we used their paradigm to look in human hippocampus. In two experiments, we used fMRI multi-voxel pattern analysis (MVPA) to compare patterns of hippocampal activity for stimuli presented in different locations. First, we compared within-fixation information: whether there was more information for pairs of stimuli with 1) the same fixation and the same stimulus location, vs 2) same fixation and different stimulus location. Next, we measured across-fixation location information for conditions with different fixation locations, comparing: 3) same retinotopic (eye-centered) stimulus location and 4) same spatiotopic (world-centered) stimulus location vs. 5) different retinotopic and spatiotopic locations. In each block, stimuli were either objects or scenes. Participants pressed a button when the same image repeated twice in a row. Stimuli were arranged horizontally in Experiment 1 and vertically in Experiment 2. We used correlation-based MVPA to compare information present for the 5 pairs of location conditions. Preliminary results suggest that for both experiments, hippocampus may contain information about stimulus location when fixation is held constant, but that this information is only tolerant of changes in fixation for the horizontal stimulus arrangement. Furthermore, when there is across-fixation location information, it seems to be retinotopic. These findings have important implications for our understanding of hippocampus and its role in the processing of visual location information.

Disclosures: A. Shafer-Skelton: None. **J.D. Golomb:** None.

Poster

169. Perception and Imagery

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 169.27/W13

Topic: F.01. Human Cognition and Behavior

Support: HFSP Grant RGP0054

Title: An attempt to physiologically measure human magnetoreception

Authors: *A. MATANI[1], S. SHIMOJO[2], J. L. KIRSCHVINK[2];
[1]Grad. Sch. of Information Sci. and Technology, the Univ. of Tokyo, Tokyo, Japan; [2]Caltech, Pasadena, CA

Abstract: Humans have magnetite (Kirshvink et al., 1992) and cryptochromes (Foley et al., 2011), known as magnetic receptors, in their heads. It would not be surprised if they have an unconscious potential for magnetoreception. We thus attempted to physiologically measure human magnetoreception with a precise experiment on the basis of animal behavioral studies and communication theories. We built two Meritt coils and a Helmholtz coil in a Faraday shielded room (Kirschvink, 1992). Appropriate groundings allowed us for applying almost purely magnetic stimuli by reducing capacitive and inductive couplings between the coils and subjects. Subjects were placed in the supine position and their heads were located in the center of the coils. The polar coordinate system was set such that their head and feet pointed north and south, respectively. Note that this setting was free from the geographic north and south; we applied currents to the coils to cancel the geographic magnetic field. In addition to the baseline nulling currents, we applied two kinds of currents to the coils to produce rotating magnetic stimuli: inclination of ±50-degree and declination of ±45-degree with a constant magnitude that was the same as the geographic magnetic field at the experiment site. Thus, subjects were exposed to an up-and-down magnetic north change in the first experiment and a left-and-right magnetic north change in the second experiment. These settings were based on animal behavioral studies; migratory birds seem to respond to inclination magnetic fields (Wiltschko et al., 2005), and rats to declination (Marhold et al., 1997). These two magnetic field changes were coded with a two-valued pseudo random sequence used for a multiple communication. 31-ch electroencephalograms (EEGs) were recorded during the exposures. With mathematical characteristics of the sequence, EEGs were decomposed into 16,383 linear and nonlinear components of an EEG generation model and hence permutation tests were performed by ranking the magnitudes of the components of interest. Three subjects participated in this study. The study received approval from the Ethics Committee of the University of Tokyo and Caltech. All the subjects showed a significant spike-like response at a latency of 0.2 s and one subject showed a significant alpha-ringing for a latency duration of 0.5-0.9 s only for the declination magnetic change in the most fundamental EEG component of interest. With such a nonnegative result, we continue our efforts to detect evidence of human magnetoreception with psychophysiological and neuroengineering approaches.

Disclosures: **A. Matani:** None. **S. Shimojo:** None. **J.L. Kirschvink:** None.

Poster

169. Perception and Imagery

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 169.28/W14

Topic: F.01. Human Cognition and Behavior

Support: MH095984 to Bradley R. Postle.

Title: The speed of posterior alpha-band oscillations predicts the speed of visual perception

Authors: *J. SAMAHA, K. MARISKA, S. CIMAROLI, B. R. POSTLE;
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Abstract: In animal studies, scalp electroencephalographic (EEG) recordings of neural oscillations in the alpha-band (8-12 Hz) have been shown to reflect phasic activity of thalamocortical projections from the lateral geniculate nucleus to primary visual cortex. Experiments in humans have found that the phase of ongoing alpha oscillations predicts visuocortical excitability, conscious perception, and the magnitude of visually evoked responses. This has led to the suggestion that the alpha rhythm reflects the "open" and "closed" cycling of discreet windows of excitability that dictate the speed at which visual information can be sampled. We directly tested this hypothesis by measuring eyes-closed individual alpha frequency in 20 healthy young adults, and then regressing this against an estimate of the "speed" of their perception, derived from a flicker fusion paradigm. Flicker-fusion thresholds were estimated by fitting a psychometric function to the discrimination of single or double flashes at 5 levels of inter-flash interval (10, 20, 30, 40, or 50 ms). Results revealed a strong negative correlation between individual peak alpha frequencies and flicker-fusion thresholds, such that subjects with faster alpha oscillations displayed lower thresholds, indicating faster perception. This effect was specific to alpha-band oscillations measured at posterior electrodes, and to the threshold parameter, not the slope parameter, of the psychometric fits. These results provide support for the idea that thalamocortical oscillation in the alpha-band may pace the phasic updating of visual information.

Disclosures: **J. Samaha:** None. **K. Mariska:** None. **S. Cimaroli:** None. **B.R. Postle:** None.

Poster

169. Perception and Imagery

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 169.29/W15

Topic: F.01. Human Cognition and Behavior

Support: NMRC/STaR/015/2013

Title: Repetition enhances the distinctiveness of neural activation patterns

Authors: *J. H. POH[1,2], M. W. L. CHEE[1];
[1]Ctr. for Cognitive Neurosci., Duke-NUS Grad. Med. Sch., Singapore, Singapore; [2]NUS Grad. Sch. for Integrative Sci. and Engin., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Behavioral priming results in speedier performance when a stimulus is repeated and is accompanied by repetition suppression (RS) of sensory responses. While it has been suggested that RS influences information processing by sharpening neural representations, there is presently no direct empirical support for this. Here we hypothesized that repetition will accentuate the unique features that distinguish different visual stimuli, and that this would be evidenced by enhanced pattern distinctiveness to individual pictures. Participants classified a mixture of once repeated indoor or outdoor scenes while undergoing fMRI. The neural representation of each stimulus was computed from voxels within functionally defined PPA. Distinctiveness of each stimulus was defined as 1-r, where r was the mean correlation between that stimulus and every other stimulus (Fig 1a). Repeated scenes elicited faster RT, reduced activation, and an increase in distinctiveness within the PPA (Fig 1b). Critically, repetition suppression was highly correlated with the change in pattern distinctiveness (mean r= .72, p< .001). We also observed strong anti-correlation of signal amplitude and pattern distinctiveness (mean r= - .75, p< .001), suggesting that higher activation relates to neural activation that is common across multiple scenes. As change in distinctiveness might be driven by task demands, we computed distinctiveness separately within- & between- categories. However, this was obviated by the finding of higher correlation for within-category than between- category comparisons (t¬¬¬10 = 14.33, p< .001). Our findings demonstrate that repetition enhances the distinctiveness of activation patterns to repeated scenes while reducing the mean BOLD response, consistent with the sharpening hypothesis where repetition 'prunes' non-specific information from neural responses (Lee & Mumford, 2003).

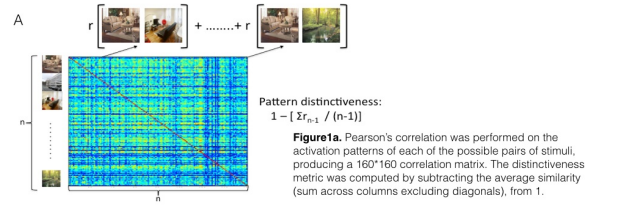

Figure1a. Pearson's correlation was performed on the activation patterns of each of the possible pairs of stimuli, producing a 160*160 correlation matrix. The distinctiveness metric was computed by subtracting the average similarity (sum across columns excluding diagonals), from 1.

Figure1b. In line with previous findings, we observed significant reduction in RT (t_{10} = 5.70 p< .001), and a significant reduction in amplitude of PPA activation (t_{10} =9.88 p< .001). To perform statistical analysis on the distinctiveness scores, all values were z-transformed. As hypothesized, Presentation 2 showed significantly greater distinctiveness than Presentation 1 (t_{10} =6.34 p< .001).

Disclosures: J.H. Poh: None. **M.W.L. Chee:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.01/W16

Topic: F.01. Human Cognition and Behavior

Title: Emotional face processing evokes physiological high frequency oscillations (80-500 Hz) in localized regions of the human brain

Authors: W. CALIBOSO[1], J. J. LIN[2], *B. A. LOPOUR[1];
[1]Biomed. Engin., [2]Neurol., Univ. of California, Irvine, CA

Abstract: Over a decade of neuroimaging work has characterized the neural basis of face perception and identified brain regions that preferentially respond to faces over other stimuli (Collins 2014), including the fusiform face area, occipital face area, posterior temporal sulcus, ventral anterior temporal lobe, and amygdala (Haxby 2000). Electrophysiological studies have found face-specific event-related potentials (ERPs) after the onset of a face stimulus (Allison 1994) and face-induced spectral perturbations in the gamma band (Lachaux 2005). While ERPs have provided information about localized information processing in the brain, this signal-averaging approach may discard oscillatory potentials that are not phase-locked across trials (Engell 2011). Physiological oscillations extending beyond the gamma frequency range have been demonstrated, but their function in cognitive processing has not been fully elucidated (Kucewicz 2014). These transient high frequency oscillations (Ripples: 80-250 Hz; Fast Ripples: 250-500 Hz) have been found to be potentially important tools in the study of cognition and epilepsy (Le Van Quyen 2012). Here we investigate high frequency oscillations evoked during face perception in humans. Twelve subjects undergoing clinical evaluation for epilepsy surgery passively viewed a paradigm to activate face-sensitive regions in the brain (Schacher 2006). The paradigm consisted of alternating 24-second blocks of baseline video clips (serene domestic landscapes) and activation video clips (faces of actors expressing fear with high intensity) for a total of 16 blocks. Target locations for the implanted grid, strip, and depth electrodes were determined solely for the purpose of seizure localization. We analyzed data from a total of 465 bipolar signals, after excluding electrodes within the seizure onset zone and anatomical defects and those having excessive noise. Using an automatic HFO detector (Staba 2002), we compared the rate of ripples and fast ripples during viewing of landscapes versus viewing of fearful faces. In 14 sites across four subjects, we found a significant increase in the rate of ripples during viewing of fearful faces. One site showed a significant increase in the rate of fast ripples during face viewing. Several of these sites corresponded to regions previously associated with face processing, and the additional sites were located in frontal and orbitofrontal regions. These

findings may provide important information about the activity of brain regions during face processing that cannot be inferred from phase-locked ERPs.

Disclosures: W. Caliboso: None. **J.J. Lin:** None. **B.A. Lopour:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.02/W17

Topic: F.01. Human Cognition and Behavior

Title: Emotional interference of baby vs adult faces on the automatic attention in parenthood

Authors: *V. OLIVEIRA[1], M. GOULART[2], M. K. LUCION[3], P. P. SILVEIRA[4], L. BIZARRO[1];
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Abstract: Protecting and nurturing offspring have been crucial behaviors to human evolution. Babies are, hence, salient emotional stimuli to human beings. The study of automatic attention to baby faces is a new research field with potential clinical relevance. The current study compared the emotional interference of baby faces and threat stimuli (adult fearful faces) on the automatic attention in parents and non-parents. Participants were 61 men and women aged 20 to 35 years. Parents (n=33) had a single child aged up to 2 years old. Images of baby and adult faces with different emotional expressions were used in a Go/No-Go task adapted from a paradigm (Pearson, 2010. Psychol Med 40) that assesses attentional bias. In the Go/No-Go task 30 images of 10 different babies with either distress, neutral or happy expressions and 30 images of 10 different adults with fearful, neutral or happy faces were employed as emotional stimuli. Trials sequence started with a screen with an initial fixation cross (750ms) followed by either Go (green cross) or No-Go (red cross) screen with emotional stimuli as background and vertical and horizontal peripheral bars as targets (240ms). A blank screen remained until response was given. Participants were instructed to press spacebar for No-Go trials and answer location of vertical bar pressing “right arrow” or “left arrow” in the keyboard for Go trials. E-prime software controlled trials display and recorded reaction time. Attentional bias indexes (ABI) were calculated for biases towards baby distress, baby vs adult faces (nurturing biases) and adult fear (protective bias). Each ABI was inserted in an univariate ANOVA with variables sex and parental status. Parents showed a higher attentional bias to baby vs adult faces in comparison to non-parents

(mean difference=26.77 ms, F(1)=5.392, p=0.024, eta sq=0.08). No significant effect of sex or parental status was found in the attentional bias to baby distress or in the bias to adult fear. This task was sensitive to parental status influence on attentional bias to babies even in a nonclinical sample. Men and women showed no difference in the preferential processing of baby faces possibly due to sample characteristics. The current outcome contributes to the reduced literature on the implicit processes involved in the care provided to babies by men and women, parents and non-parents. The results might be relevant also to future clinical studies by both indicating new points of enquiry and proposing a new research tool.

Disclosures: V. Oliveira: None. **M. Goulart:** None. **M.K. Lucion:** None. **P.P. Silveira:** None. **L. Bizarro:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.03/W18

Topic: F.01. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number 26750194

Title: Right hemispheric superiority in the conscious face processing: A high-density ERP study with hemifield stimulation

Authors: *N. TAKAMIYA[1,2], T. MAEKAWA[2], K. OGATA[2], T. YAMASAKI[2], E. YAMADA[2], M. TANAKA[2], S. TOBIMATSU[2];
[1]Prefectural Univ. of Hiroshima, Mihara-Shi, Japan; [2]Grad. Sch. of Med. Sci., Kyushu Univ., Fukuoka, Japan

Abstract: [Background] The face recognition is specialized for the right hemisphere in the literature. Our aim was to clarify the right hemispheric superiority in the unconscious and conscious face processing by using high-density ERPs with hemifield stimulation. [Methods] Eighteen right-handed healthy volunteers (9 female, 21-27 years old) were examined. Two types of stimuli (faces and objects) were presented to left or right hemifield in a random fashion. The visual stimuli were presented either in the subliminal (17-ms duration) or supraliminal (300-ms duration) condition using a backward masking paradigm. ERPs were recorded by a 128-ch EEG machine and a repeated measures ANOVA was applied to the latencies and amplitudes of the occipital P100 and occipito-temporal N170. [Results] In the subliminal condition, P100 but not N170 was robustly elicited. There were no significant effects of the stimulus type, presentation

side and hemisphere on the P100 amplitude and latency. In the supraliminal condition, a significant main effect of the presentation side was evident also on the P100 amplitude ($p<.0001$) but not on its latency irrespective of the stimulus type. We also found significant main effects of the stimulus visual field ($p=0.0020$), hemisphere ($p=0.0113$), and a significant interaction on the stimulus visual field and hemisphere ($p<.0001$) for the N170 amplitudes to the face stimuli but not on its latencies.. [Discussion] It is well established that the P100 reflects the function of the primary visual cortex (V1) while N170 is originated from the fusiform face area (FFA). The P100 amplitudes were significantly larger over the contralateral hemisphere regardless of the stimulus type and presentation side in the supraliminal condition but not in the subliminal condition. Thus, the P100 simply reflects the retinotopic organization in the conscious visual processing. Interestingly, the N170 appeared only in the supraliminal condition with the selectivity for face stimulus and right hemispheric superiority. Therefore, right FFA plays an important role for the conscious face processing irrespective of the visual hemifield.

Disclosures: N. Takamiya: None. **T. Maekawa:** None. **K. Ogata:** None. **T. Yamasaki:** None. **E. Yamada:** None. **M. Tanaka:** None. **S. Tobimatsu:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.04/W19

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH05286

Title: vATL contributes to biographical knowledge of faces via feedback to FFA

Authors: Z. SHEHZAD[1], J. TAYLOR[1], *G. MCCARTHY[2];
[1]Psychology, [2]Yale Univ., New Haven, CT

Abstract: As we learn to recognize a person's face, it's natural to also learn biographical facts about that person such as their name or occupation. In neural models of face processing, linking such person-specific semantic knowledge with perceptual representations of an individual's face is represented in the ventral anterior temporal lobe (vATL). However, some work has also implicated the fusiform face area (FFA) in processing biographical information for a face (e.g., as a feature in face space). Since the FFA and vATL are part of an extended 'face network', it is possible that responses in the FFA to biographical information may reflect bi-directional communication between the FFA and vATL. To disentangle the roles of the vATL and FFA in

face processing, we conducted an fMRI study comparing brain responses to viewing faces for whom participants had learned biographical facts (name, location, occupation) or physical facts (eye color, gender, race). When viewing each face, participant's were asked to recall the facts associated with that face and either made no response (passive viewing task) or responded to knowing a probe fact (question task). To focus our analyses, we selected regions-of-interest in the occipital face area (OFA), FFA, and vATL based on local maxima from a probabilistic atlas of face-selective responses (Engell & McCarthy, 2013). Across both passive viewing and question tasks, we found significantly greater activity in the left vATL (but not in the FFA) for viewing faces associated with biographical facts (biographical condition) versus faces associated with physical facts (physical condition). Increased (undirected) connectivity was observed for the biographical versus physical condition between the R OFA - R vATL, R FFA - R vATL, and L FFA - L vATL, but only for the questions task. Finally, in a directed connectivity analysis based on a linear non-gaussian acyclic model (LiNGAM; Ramsey et al., 2014), we found that feedback signals from left vATL to left FFA significantly increased for the biographical versus physical condition while feedforward signals from left OFA to left FFA and lateral signals from left FFA to right FFA significantly increased for the physical versus biographical condition. Our findings suggest that responses to biographical knowledge of faces is lateralized to the left hemisphere and selective to the vATL. We propose that the vATL represents biographical knowledge of faces through feedforward signals from the FFA to the vATL and feedback signals from the vATL to the FFA contribute to representations of biographical knowledge as features in a face space.

Disclosures: Z. Shehzad: None. **J. Taylor:** None. **G. McCarthy:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.05/W20

Topic: F.01. Human Cognition and Behavior

Support: NSERC 346135-2012

EGP #451681-13

Title: Face and body recognition in dancers and non-dancers

Authors: L. VINGILIS-JAREMKO[1], V. GUIDA[2], S. E. MAGUIRE[3], *J. F. DESOUZA[4]; [1]Psychology, York Univ., Toronto, ON, Canada; [2]Psychology, Ctr. for Vision Res., Toronto,

ON, Canada; [3]Psychology, Univ. of Toronto, Toronto, ON, Canada; [4]Psychology & Biol., York Ctr. For Vision Res., Toronto, ON, Canada

Abstract: The ability to recognize others is critical to our everyday social interactions. Although extensive research has explored the role of the face for person recognition, little has explored the role of the body, which may be used for recognition at a distance. Because bodies may be processed similarly to faces (Rhodes, Jeffery, Boeing, & Calder, 2013; Robbins, Coltheart, 2010; Robbins, Coltheart, 2012), we explored whether body recognition abilities are influenced by visual experience, as are face recognition abilities. We tested two groups with different types of visual experience with bodies: dancers (n=29), who spend much of their time observing and comparing bodies in form fitting clothing to achieve a physical aesthetic, and non-dancers (n=37), who tend to see bodies in more obstructive clothing and spend much of their time in front of computers. Participants viewed images of bodies wearing identical clothing, and after a short break, selected which body from a pair of bodies they had seen before. Participants completed the same task with faces in a separate, counterbalanced block. We hypothesized that dancers would have better accuracy at recognizing bodies, but perform similarly to non-dancers at recognizing faces. First, we found that participants recognized faces better than bodies ($p<0.001$), consistent with previous research (Burton, Wilson, Cowan, & Bruce, 1999). Most importantly, we found that dancers recognized faces and bodies better than non-dancers (main effect of participant group, $p=0.043$). These results suggest that dancers are more accurate at recognizing identity using the body than non-dancers, putatively due to their extensive visual experience with bodies. More accurate face recognition abilities among dancers could result from facilitation effects across brain networks, as bodies and faces are typically seen together.

Disclosures: L. Vingilis-Jaremko: None. **V. Guida:** None. **S.E. Maguire:** None. **J.F. DeSouza:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.06/W21

Topic: F.01. Human Cognition and Behavior

Support: ARRS grant P3-0171

Title: Neural correlates of dance imagery induced by music

Authors: *M. BRESJANAC[1], V. POLITAKIS[2], B. PANTIć[3], A. VOVK[1], G. REPOVŠ[2];
[1]Univ. Ljubljana Med. faculty, Ljubljana, Slovenia; [2]Univ. of Ljubljana Fac. of Arts, Ljubljana, Slovenia; [3]Gibor, Ljubljana, Slovenia

Abstract: Neural correlates of movement, movement perception and mental imagery of movement have been shown to extensively overlap. The aim of our pilot exploration was to employ music-induced dance imagery to study dance responses to music. Healthy, young adult volunteers (18 non-dancers (7 male and 11 female) and 17 dancers (9 male, 8 female)) were recruited for the fMRI-based study using a 3T scanner. Resting state BOLD signal was recorder first, followed by BOLD signal recordings during blocks of listening to music with instructions on how to respond to it with mental dance imagery. Clips of groovy (dance music) and non-groovy (foreign national anthems) instrumental music were used as stimuli. Immediate feed-back was obtained from the subjects on the extent of experienced mental imagery of dance as well as on their emotional engagement while listening to the music. The subjects' heart rate was simultaneously recorded to provide a physiological measure of arousal. To obtain insight into neural activity underlying mental imagery of movement, movement observation and emotional engagement (without musical stimuli) in the same subjects, BOLD signal was also recorded while participants were instructed to imagine swimming, observing a swimmer, experiencing a passionate kiss, etc. The results revealed interesting behavioural as well as neural activation responses that point to expected commonalities, but also to some less expected differences between dancers and non-dancers in their responses to groovy and non-groovy music.

Disclosures: M. Bresjanac: None. **V. Politakis:** None. **B. Pantić:** None. **A. Vovk:** None. **G. Repovš:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.07/W22

Topic: F.01. Human Cognition and Behavior

Title: Do mental rotation and manual tracking share an underlying mechanism?

Authors: *U. B. NUNES[1], R. RUSSELL[2], M. SCHLESINGER[2];
[1]Psychology, Southern Illinois Univ. Carbondale, Carbondale, IL; [2]Psychology, Souther Illinois Univ. Carbondale, Carbondale, IL

Abstract: Mental imagery is a complex human ability that is used in everyday life such as in sports, driving and reading. An important question concerns the specific imaging strategies used during image-based neurocognitive tasks, such as mental rotation. To investigate this question, we measured performance on three tasks that ostensibly recruit different areas of the brain and diverse forms of mental imagery: (1) the standard mental rotation task, (2) the Vividness of Visual Imagery Questionnaire (VVIQ), and (3) a manual-tracking task that includes both visible and occluded moving targets. Our core prediction was that scores on the mental rotation task would be positively correlated with tracking accuracy during both visible and occluded tracking, but that mental rotation performance would not be correlated with VVIQ scores. Our preliminary results confirmed these predictions. Ongoing analyses of these data are directed toward diagnosing distinct imaging strategies during mental rotation and relating these strategies to performance during manual tracking and individual differences on the VVIQ.

Disclosures: **U.B. Nunes:** None. **R. Russell:** None. **M. Schlesinger:** None.

Poster

170. Face, Body, and Action

Location: Hall A

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Program#/Poster#: 170.08/W23

Topic: F.01. Human Cognition and Behavior

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The Israel Science Foundation (grants No. 1771/13 and 2043/13)

Human Frontiers Science Project (HFSP) Career Development Award (CDA00078/2011-C)

Title: The neural signature of self: recognition of self-generated handwriting within the action observation network

Authors: *R. GILRON, R. MUKAMEL;
Tel Aviv Univ., Tel Aviv, Israel

Abstract: Mirror neurons are nerve cells that are active both during action execution and action observation. This neurons are present in a network of distributed areas associated with motor function, including the primary and pre-motor cortices, inferior parietal regions and the supplementary motor area (SMA). This network is often termed the Action Observation Network

(AON). Previous research demonstrates differential activation within the AON when experts (e.g. basketball players, dancers, musicians) observe the performance of actions within their domain of expertise. It has been suggested that actions that are within one's motor repertoire resonate more strongly in the AON. We chose hand writing as a model to examine this issue since each individual is an expert in generating and perceiving his own spatiotemporal dynamics during handwriting. We recorded whole-brain fMRI signals from eight subjects while they viewed pre-recorded dynamic traces of their own and others' handwriting. Using whole brain search light multi-voxel pattern analysis we were able to detect the identity of the observed hand-writing trace, with above 75% classification in a network of sensorimotor regions including left primary motor cortex (subjects were right handed), posterior STS and inferior parietal lobule (IPL). These regions are commonly associated with the AON. Our results, that neural activity within this network during passive observation may play an important role in simulating observed actions. Moreover, they demonstrate that observing the sensory consequences of an action (dynamic hand-writing trace) without the actual agent or effector (a view of the physical hand writing) is sufficient to evoke activity in the action observation network.

Disclosures: R. Gilron: None. **R. Mukamel:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.09/W24

Topic: F.01. Human Cognition and Behavior

Title: Defying the laws of gravity: Motor Imagery of rotation along body axes

Authors: *M. KALICINSKI[1], O. BOCK[1], N. SCHOTT[2];
[1]German Sport Univ. Cologne, Cologne, Germany; [2]Dept. of Sport and Exercise Sci., Univ. of Stuttgart, Stuttgart, Germany

Abstract: Motor Imagery (MI) is described as an active cognitive process during which the representation of a specific action is internally reproduced in working memory from a first-person perspective, without any overt motor output (Decety & Grèzes, 1999). When performing MI tasks with rotation along roll or pitch body axis, one has to imagine movements which are physically impossible on earth as they would defy gravity. The purpose of the present study is to find out whether young adults are able to produce accurate MI of such movements. 68 adult (23.94 ± 3.07 years; male n = 33) participants completed a modified version of the CMI test (Schott, 2013). With eyes closed, subjects were asked to imagine moving their body according to

six consecutive verbal instructions. After the sixth instruction, participants opened their eyes and arranged a flexible doll into the assumed final body configuration. Each shown element of the final body configuration was scored with 1 point when correct. The time in seconds needed to assume the final configuration (response time) was measured. In a first condition, participants were instructed to imagine themselves standing on the ground and received only instructions for moving body segments (CMIground). In a second condition, participants were instructed to imagine themselves floating above ground and received instructions for moving body segments and either one (Group: CMIfloat1) or two (Group: CMIfloat2) full body rotations around the three body axes (CMIfloat). After each condition, participants were asked to rate their subjective visual and kinesthetic vividness of MI. For the mean scores significant condition differences (CMIground = 45.7 ± 4.1; CMIfloat = 41.2 ± 5.3; $F (1, 66) = 62.596$; $p < .001$ $\eta^2 = .467$) emerged, as well as a Group X Condition interaction ($F (1, 66) = 7.506$; $p = .007$; $\eta^2 = .102$): differences between CMIground and CMIfloat were more pronounced in group CMIfloat2. Condition differences could be also shown for response time (CMIground = 10.9 ± 3.8; CMIfloat = 17.0 ± 8.1; $F (1, 66) = 56.694$; $p < .001$ $\eta^2 = .462$) and for vividness ratings ($F (1, 66) = 50.929$; $p < .001$ $\eta^2 = .436$). Our data suggest that it is possible to mentally imagine movements that cannot be executed on earth, but this imagery is more error-prone, especially with increasing complexity due to the amount of body rotations.

Disclosures: **M. Kalicinski:** None. **O. Bock:** None. **N. Schott:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.10/W25

Topic: F.01. Human Cognition and Behavior

Title: Brain mechanisms of exteroceptive and interoceptive integration in bodily self-consciousness

Authors: *F. BERNASCONI[1,2], R. RONCHI[1,2], J. BELLO-RUIZ[1,2], C. PFEIFFER[1,2], O. BLANKE[1,3,2];
[1]EPFL, Lausanne, Switzerland; [2]Lab. of Cognitive Neurosci., Lausanne, Switzerland; [3]Ctr. for Neuroprosthetics, Lausanne, Switzerland

Abstract: Bodily self-consciousness (BSC) refers to the conscious experience of the self within the body. Different bodily information (e.g., vision and touch) are integrated to generate BSC: recently, researchers focused their attention on the signals arising from inside the body

(interoceptive, e.g. heartbeat), and the integration of exteroceptive and interoceptive signals. Despite the importance of these processes, limited data are available about how and where interoceptive (e.g., heart) and exteroceptive (e.g., vision) signals for BSC are integrated in the brain. In this study we have investigated the spatiotemporal brain dynamics underpinning cardio-visual integration in 14 healthy subjects. We recorded visual evoked potentials with electroencephalography (EEG) during the perception of a body or a scrambled-body picture appearing (or not) at the same frequency of participants' heartbeat, and we analysed the brain responses linked to the visual processing. Results showed a global increase in brain response (i.e., global field power) specific for body stimuli presented in synchrony with the heart, occurring at about 200ms after the stimulus onset. No modulation in topographies configuration was observed across conditions. These results suggest that the cardio-visual integration results in an enhanced brain response of similar intracranial generators. Source estimation revealed enhanced activity within the left parietal lobe. In conclusion, these findings indicate that cardio-visual processing leads to an increased integration of external (i.e., picture or a body) and internal (i.e., own heart beat) bodily features that might be relevant for BSC.

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Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.11/W26

Topic: F.01. Human Cognition and Behavior

Title: The integration of gravity in implicit and explicit motor representations

Authors: *F. LEBON[1,2], E. TRAVERSE[1,2], L. FADIGA[3,4], T. POZZO[1,2,3], C. PAPAXANTHIS[1,2];
[1]INSERM U1093, DIJON, France; [2]Univ. of Burgundy, Dijon, France; [3]RBCS Dept, Inst. Italiano di Tecnologia, Genova, Italy; [4]Section of Human Physiol., Univ. of Ferrara, Ferrara, Italy

Abstract: Mental representation of movement is an important high-level process in motor control. By using transcranial magnetic stimulation (TMS) technique, previous studies have found an increase of corticospinal excitability when imagining (explicit representation, Fadiga et al. 1999; Jeannerod, 2001) or observing (implicit representation, Fadiga et al., 1995) an action. This facilitation suggests the involvement of the primary motor cortex in the mental

representation of the action. The aim of this study was to test whether gravity representation is explicitly (during motor imagery) or implicitly (during action observation) expressed in mental actions. Ten participants were instructed to rest, to actually perform, to imagine, or to observe repetitive flexion/extension of the right forearm in a vertical or horizontal plan in time with a 0.5 Hz metronome. TMS was triggered over the arm representation of the left primary motor cortex when the elbow was at 90°. Motor-evoked potentials (MEPs) were recorded from biceps brachialis muscle of the right arm and their amplitude was considered a marker of corticospinal excitability. We hypothesized that if gravity is integrated in action observation and motor imagery then MEPs should increase during flexion against gravity (concentric contraction), extension with gravity (eccentric contraction), and flexion against inertia in the horizontal plane. We found that MEPs were facilitated in all conditions in comparison to rest (all, $p<0.05$), confirming the facilitation of the motor cortex during motor imagery and action observation. During actual execution trials, MEPs were greater during flexion than that during extension for both horizontal and vertical movements. The amplitude was the greatest while flexing on the vertical plan, showing specific patterns of activation while moving against gravity. During imagined trials, MEPs were greater during flexion than during extension for horizontal movements, but did not differ for vertical movements. This indicates that mental simulation dissociates movements with and without gravity. During observation trials, MEPs were similar during flexion and extension for both horizontal and vertical movements, indicating the absence of muscle specificity related to the movement direction and thus gravity force. Overall, these results show for the first time that gravity force is integrated in the construction of explicit (motor imagery) motor representations but not in implicit (action observation) ones.

Disclosures: F. Lebon: None. **E. Traverse:** None. **L. Fadiga:** None. **T. Pozzo:** None. **C. Papaxanthis:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.12/W27

Topic: F.01. Human Cognition and Behavior

Title: Evidence for predictive coding in the human motor system during action observation

Authors: *M. HANSEL-LESMY[1], A. GOLDSTEIN[1], J. M. KILNER[2];
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Abstract: Previous studies have shown that neurons in the macaque monkey's premotor cortex are active during action observation. These neurons discharge more during observation of transitive action than for intransitive actions and are even activated by partially occluded movement. In concordance with the direct recordings of these 'mirror neurons' non-invasive human neurophysiological and neuroimaging studies have demonstrated that similar areas are active during action observation. Despite much research little is known about the functional role, if any, of the activity in motor regions during action observation. One major theory proposes that our motor systems are active during action observation as they are the best model we have to enable us to predict the sensory consequences of any observed movements. The aim of this study was to test this predictive coding hypothesis by studying the modulation of the motor cortex activity when observing actions that were either occluded or in full view. Suppression of beta oscillations (15-30Hz) have been associated with an increase in activity of the human motor cortex. We used MEG to measure beta oscillations while participants passively observed video clips showing either reaching and grasping of a ball, or miming this same action. Clips started with a 1000ms static frame, showing a hand on the left of the screen either with or without a ball present on the right of the screen. After 1000ms the hand moved to either grasp the ball if it was present or to mime grasping if it was absent. In half of the clips a screen appeared after the static phase and occluded the grasp. Participants knew whether the action would be occluded prior to the trial starting. Here we found that beta oscillations were suppressed both for full viewed movements and partially occluded movements. No significant differences were found between the suppression for transitive and intransitive actions. Surprisingly, although before the hand crossed the occluder the movement was identical for full-viewed and partially occluded actions, beta power was more suppressed when the movement was partially occluded. That is, the same stimulus elicited different modulations of beta power. After the hand crossed the occluder, no significant difference was found between full viewed conditions and partially occluded conditions. Our results indicate that when participants knew that in the future the hand would cross the occluder, they attended more to the visible part of the movement. Our results support the view that during action observation, the motor system activity is not only modulated by the kinematic of the movement but also by the expectations of the observer.

Disclosures: **M. Hansel-Lesmy:** None. **A. Goldstein:** None. **J.M. Kilner:** None.

Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.13/W28

Topic: F.01. Human Cognition and Behavior

Title: The spatial extent of action sensitive perceptual channels decrease with visual eccentricity

Authors: *L. FADEMRECHT[1], I. BÜLTHOFF[1], N. E. BARRACLOUGH[2], S. DE LA ROSA[1]; [1]Human perception, cognition and action, Max Planck Intitute for Biol. Cybernetics, Tuebingen, Germany; [2]Dept. of Psychology, Univ. of York, York, United Kingdom

Abstract: Actions often occur in the visual periphery. Here we measured the spatial extent of action sensitive perceptual channels across the visual field using a behavioral action adaptation paradigm. Participants viewed an action (punch or handshake) for a prolonged amount of time (adaptor) and subsequently categorized an ambiguous test action as either 'punch' or 'handshake'. The adaptation effect refers to the biased perception of the test stimulus due to the prolonged viewing of the adaptor and the resulting loss of sensitivity to that stimulus. Therefore the more a channel responds to a specific stimulus the higher is the adaptation effect for that certain channel. We measured the size of the adaptation effect as a function of the spatial distance between adaptor and test stimuli in order to determine if actions can be processed in spatially distinct channels. Specifically, we adapted participants at 0° (fixation), 20° and 40° eccentricity in three separate conditions to measure the putative spatial extent of action channels at these positions. In each condition, we measured the size of the adaptation effect at -60°,-40°,-20°, 0°,20°,40°,60° of eccentricity. We fitted Gaussian functions to describe the channel response of each condition and used the full width at half maximum (FWHM) of the Gaussians as a measure of the spatial extent of the action channels. In contrast to previous reports of an increase of midget ganglion cell dendritic field size with eccentricity (Dacey, 1993), our results showed that FWHM decreased with eccentricity (FWHM at 0°: 56°, FWHM at 20°: 29, FWHM at 40°: 26). We then asked whether the response of these action sensitive perceptual channels can be used to predict average recognition performance (d') of social actions across the visual field obtained in a previous study (Fademrecht et al. 2014). We used G(x) - the summed response of all three channels at eccentricity x, to predict recognition performance at eccentricity x. A simple linear transformation of the summed channel response of the form a+b*G(x) was able to predict 95.5% of the variation in the recognition performance. Taken together these results demonstrate that actions can be processed in separate spatially distinct perceptual channels, their FWHM decreases with eccentricity and can be used to predict action recognition performance in the visual periphery.

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Poster

170. Face, Body, and Action

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Title: Perception through action: Where and When. Differentiation of mirror regions using intracranial recordings

Authors: *A. PERRY[1,2], J. STISO[2], E. F. CHANG[3], J. J. LIN[4], J. PARVIZI[5,6], R. T. KNIGHT[1,2];
[1]Psychology, [2]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; [3]Dept. of Neurolog. Surgery and Physiol., Univ. of California, San Francisco, San Francisco, CA; [4]Comprehensive Epilepsy Program, Univ. of California, Irvine, Irvine, CA; [5]Stanford Human Intracranial Cognitive Electrophysiology Program (SHICEP), [6]Dept. of Neurol. and Neurolog. Sci., Stanford Univ. Med. Ctr., Stanford, CA

Abstract: Embodied theories of cognition emphasize the central role of sensorimotor transformations, a correspondence between sensory and motor domains, in the representation and understanding of actions and emotions of others. These theories assume that automatic simulation is required to understand others' actions, and that such simulation may also serve as the basis for imitation. Support for this theory has been provided by reports of mirror neurons in non-human primates. Neuroimaging techniques have been used to study a putative mirror neuron system in humans, and have provided evidence for frontal, parietal and sensorimotor regions that are activated for both performing and viewing goal directed actions. However, most of these studies were limited in either their temporal or spatial resolutions, and so the exact timing of these activations, and how much they actually overlap remain unknown. To address these questions, we utilized the spatial and temporal advantages of intracranial cortical recordings (electrocorticography, ECoG) to test whether neuronal populations in mirror neuron regions are activated during viewing and imitating goal directed actions. We tested 6 participants with intractable epilepsy, who had been implanted with electrode grids over the right or left frontal, parietal and sensorimotor cortexes for pre-operative monitoring. Patients viewed grasping actions towards different objects, and following a rest period of 2 seconds, had to perform the same actions. Increases in spectral power in the high gamma range (70-150 Hz) were used to index cortical activation. Our results show robust high gamma activation in sensorimotor,

premotor IFG and IPL during performance and viewing of motor actions. These activations showed complex temporal patterns: while some sites were active throughout the whole viewing and action conditions, some were active only at the initiation of movement towards the object, and some only for the actual grasp. These results support the role of the existence of a distributed neural network engaged in the perception of others' actions and provide temporal differentiation between different stages of the perception of action.

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Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: Center for ADvanced Brain Imaging Seed grant, Atlanta

Title: The role of action context on the neural substrates underlying gesture recognition

Authors: ***N. NATRAJ**[1], S. BASUNIA[1], J. MIZELLE[1,2], L. WHEATON[1];
[1]SCHOOL OF APPLIED PHYSIOLOGY, COLLEGE OF SCIENCES, GEORGIA INSTITUTE OF TECHNOLOGY, Atlanta, GA; [2]Atlanta Veteran Affairs, Atlanta, GA

Abstract: Prior research has shown diffuse representations in the brain for the processing of transitive (tool-based), intransitive (communicative) and meaningless gestures. Specifically, transitive, intransitive and meaningless gestures elicited activations over bilateral posterior parietal cortex, superior temporal cortex, occipitotemporal regions and right pre-supplementary areas. In particular, intransitive gestures elicited greater activations than transitive gestures over the left inferior frontal gyrus. An important confound however, is that the kinematics of the gesture was not controlled in the aforementioned study. For example, the kinematics of waving good bye (intransitive gesture) and wiping a window (transitive gesture) are similar and it is only the action context that discriminates the two gestures. In addition, it is possible that controlling action kinematics can better identify the differences between meaningless and contextual gestures. Thus, overall, it is unclear to what extent the neural substrates underlying gesture recognition are influenced by action context alone. To address this issue, an event-related fMRI study was designed and data was collected from 19 right handed, 20-30 year old subjects across three runs as they viewed static images of an actor performing a right handed gesture. The first

run consisted of neutral gestures devoid of any context at all. In the second run, the exact same gestures were placed in a transitive context. In the third run, the exact same gestures were placed in an intransitive context. fMRI data was analyzed with the FSL software package in Linux. All three gestures elicited activity over occipitotemporal areas, parietal cortex, premotor cortex and supplementary motor areas. However, activations over parietofrontal regions tended to be more left hemispheric lateralized. Kinematically similar gestures in transitive and Intransitive contexts both elicited greater activity over bilateral occipitotemporal and left parietal regions when compared to neutral gestures while neutral gestures elicited greater activity over frontal regions, ostensibly to try and fill in context. Importantly, when contrasting kinematically similar transitive and intransitive gestures directly, results showed greater left posterior parietal cortex activity for transitive context over intransitive context and greater bilateral frontal activity for intransitive context over transitive context. Results here show the importance of action context on the neural substrates underlying gesture recognition.

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Poster

170. Face, Body, and Action

Location: Hall A

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Program#/Poster#: 170.16/W31

Topic: F.01. Human Cognition and Behavior

Support: JSPS KAKENHI 26750242

JSPS KAKENHI 26242065

Title: Motor imagery beyond the motor repertoire: V1 activity predicts capability of kinesthetic motor imagery of complex whole body movements

Authors: ***N. MIZUGUCHI**[1], H. NAKATA[2], K. KANOSUE[1];
[1]Waseda Univ., Tokorozawa, Japan; [2]Nara Women's Univ., Nara, Japan

Abstract: To elucidate the neural substrate associated with capabilities for kinesthetic motor imagery of complex whole-body movements, we measured brain activity during a trial involving both kinesthetic motor imagery and action observation as well as during a trial with action observation alone. Brain activity was assessed with functional magnetic resonance imaging (fMRI). Nineteen novices imagined three types of whole-body movement with the horizontal bar: the Giant Swing, Kip, and Chin-Up. No participant had tried to perform the Giant Swing.

The vividness of kinesthetic motor imagery as assessed by questionnaire was highest for the Chin-Up, less for the Kip and lowest for the Giant Swing. The primary visual cortex (V1) was significantly more activated during kinesthetic motor imagery of the least-vivid Giant Swing than the most-vivid Chin-Up within participants. Across participants, V1 activity during Kip imagery was negatively correlated with vividness of Kip imagery. These results suggest that activity in V1 is dependent upon the capability of kinesthetic motor imagery for complex whole-body movements. Since V1 activity is likely related to the creation of a visual image, we speculate that visual motor imagery unintentionally substitutes for the less vivid kinesthetic motor imagery which occurs with difficult, complex whole-body movements.

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Poster

170. Face, Body, and Action

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Topic: F.01. Human Cognition and Behavior

Support: University of Ribeirão Preto

Title: Overestimation of body size is not exclusively related to one's own body

Authors: ***M. F. LAUS**[1], B. M. ALVES[2], R. C. M. MOREIRA[2], S. S. ALMEIDA[1], T. BRAGA COSTA[2];
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Abstract: It has been suggested that people exhibit greater size overestimation for themselves as compared to mannequins or neutral objects because: (1) perceptual accuracy of body image is primarily predicted by biopsychosocial influences (including one's weight); and (2) judgments of the own size, but not the size of objects, are generally performed by recall-size estimation tasks. These tasks involve a memory judgment rather than visual information, which includes cognitive knowledge and beliefs about body size and shape that may affect perception. Even though Figure Rating Scales (FRS) are extensively used to evaluate accuracy of body size estimation and results have shown a remarkable rate of overestimation among men and women, research using this instrument to evaluate the accuracy in estimating the size of other people has never been conducted. If one or both hypotheses above are true, we expect that individuals will present a more accurate perception when estimating the size of other people compared to the size of their

own body. Using a FRS with 15 drawings of each gender from leaner to heavier bodies, 200 college students (100 men) with different body weights and levels of prescreened body size distortion estimated the size of 4 individuals from the same gender, representing different categories of nutritional status. Results demonstrated that, regardless the size of the stimulus, only 10.75% of the judgments were accurate, 13.25% were underestimated and 76.0% were overestimated. Accuracy of participants' own estimation did not influence the judgments about the size of others ($p > .05$) and participants' own weight also did not influence their judgments about the size of other people ($p > .05$). Overestimation was significantly higher than underestimation or accurate perception for the overweight ($p < .001$) and obese stimuli ($p < .001$), while the underweight stimulus was underestimated by more than 60.0% of men ($p < .001$) and overestimated by only 8.0% of them ($p < .001$). Among women, however, almost 100% of judgments were overestimated for the normal weight, overweight and obese stimuli ($p < .001$), and more than 70% for the underweight stimulus ($p < .001$). In sum, participants' weight and perceived body size were not associated to the degree of inaccuracy of stimuli's sizes, and overestimation occurred even when judgments were performed with visual information available. We conclude that perception of body size may involve unknown factors that lead people to impressively overestimate the size of others. Taken together, our results suggest that overestimation of body size is not exclusively related to one's own body.

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Poster

170. Face, Body, and Action

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 170.18/W33

Topic: F.01. Human Cognition and Behavior

Title: Influence of attention and stance on postural control stochastic dynamics

Authors: *G. CHAPARRO[1], Y. MOON[2], D. WAJDA[2], J. SOSNOFF[2], M. HERNANDEZ[2]; [2]Kinesiology, [1]Univ. of Illinois at Urbana Champaign, Urbana, IL

Abstract: As attentional demands increase while maintaining an upright stance, the risk of falling increases, particularly for older adults with neurological conditions. Traditionally, research examining the effects of attention on postural control has focused in deterministic features of postural sway such as the root mean square error and velocity of the center of pressure (COP) in a quiet stance. However, these measures do not capture the changes that may

arise in postural control stochastic dynamics due to the relocation of attention to concurrent cognitive tasks. Furthermore, it is of importance to capture these changes in varying stances to ascertain the interaction between postural control and attentional demands. We expand upon a novel method for evaluating postural control stochastic dynamics, the center of pressure velocity autocorrelation function (COP-VAF), by examining the effects of attention and stance on the COP-VAF. Participants were instructed to 'stand as still as possible', while performing single or dual tasks (i.e., concurrent motor and cognitive tasks) in a bilateral or split stance. Cognitive tasks consisted of recitation of alternating letters of the alphabet in a backwards order. Dual tasks led to increased initial COP-VAF values, decreased minimum values, and increased low frequency processes, consistent with the use of increased compensatory postural responses, COP velocities, and neural control. Thus, the COP-VAF may further our understanding of the underlying mechanisms behind the maintenance of balance in human upright stance.

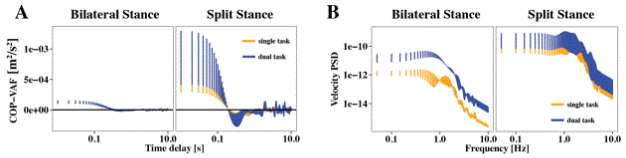

Figure 1. A.) Mean (Standard Error) COP Velocity Autocorrelation function (COP-VAF) trajectories for healthy young adults (N=2, 2 females) in bilateral and split stance under single or dual task conditions. B.) Mean (Standard Error) COP-VAF Power Spectrum Density (PSD) trajectories for healthy young adults in varying task and stance conditions.

Disclosures: G. Chaparro: None. **Y. Moon:** None. **D. Wajda:** None. **J. Sosnoff:** None. **M. Hernandez:** None.

Poster

171. Human Memory Encoding Processes

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 171.01/W34

Topic: F.01. Human Cognition and Behavior

Title: The effects of semantic relatedness on long term survival memory processing

Authors: *S. MATTINGLY, J. PAYNE;
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Abstract: Memory may have evolved, at least partially, to preferentially retain and apply information relevant to problems faced by early humans during the Pleistocene era, such as procuring food, shelter, and protection from predators. Several studies have shown that stimuli encoded in a survival mindset (i.e. "survival processing") are better remembered after a brief

delay than a variety of other encoding strategies (such as intentional learning). In order to be useful, however, memory must be applied to situations temporally distinct from encoding. While the underlying proximate memory processes remain equivocal, one theory suggests that the observed memory benefit conferred by survival processing is related to a congruency effect in which items that are semantically related to the encoding condition (e.g. sword in survival encoding condition vs. box in moving control condition) are remembered better. However, it is possible that survival memory relies more on schematic processing than other forms of encoding. In an attempt to test if survival processing relies more on schema and semantic relatedness between items across a long delay, we utilized the survival processing experimental procedure in which participants are instructed to imagine themselves in a grasslands survival scenario (experimental, n=15) or in a moving to a foreign land scenario (control, n=15), and then to encode a list of 30 words by rating them in terms of usefulness to the scenario. 12 hours later, participants completed free recall and recognition memory tests of the 30 original words, along with 15 semantically related foils and 15 semantically unrelated foils. Additionally, participants rated words at recognition on scales of valence and arousal. With the current sample size, we were not able to replicate the generally reported main finding of better memory for 'survival' at 12 hours previously reported, but data collection is still in progress. However, we found a significant group effect: those who encoded stimuli in the survival condition had significantly more false alarms to semantically related compared to semantically unrelated lures ($t(15) = 3.174$, $p<.01$). Within the 'moving' group, there were no differences between false alarm rates of related and unrelated lures. No additional effects were observed between groups on the ratings of utility, valence, or arousal, nor were there any memory effects for ratings of utility, valence, or arousal. This suggests that survival memory over longer time delays may rely more on semantic processing compared to moving condition.

Disclosures: **S. Mattingly:** None. **J. Payne:** None.

Poster

171. Human Memory Encoding Processes

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 171.02/W35

Topic: F.01. Human Cognition and Behavior

Support: PSI2013-46057-P

Title: Spatiotemporal pattern similarity during the encoding of context boundaries supports memory for sequential order of events

Authors: I. SOLS[1,2], L. DAVACHI[3], *L. FUENTEMILLA[1,2];
[1]Inst. of Biomedicine Res. of Bellvitge (IDIBELL), Barcelona, Spain; [2]Dept. of Basic Psychology, Univ. of Barcelona, Barcelona, Spain; [3]Dept. of Psychology, New York Univ., New York, NY

Abstract: Given that experience unfolds in a continuous manner over time, how does the experiencing become discretized into cohesive memory representations available at long term? A current proposal involves the detection of contextual shifts or boundaries, which help us form bound memory representations from encoded sequences of events. However, because context boundaries cannot be anticipated during sequence encoding, the neural mechanism supporting the memory formation for sequences of events may take place retrospectively during the detection of contextual boundaries. Here, we asked whether similar spatiotemporal patterns of electrophysiological (EEG) activity elicited during sequential encoding could be detected during the detection of a contextual shift. Participants were enrolled in an episodic sequence task in which series of alternated sequences of object and face pictures were presented, thus using picture category as a contextual boundary during sequential encoding. Behaviorally, we found that memory for the order of events was enhanced within, rather than across context shifts, thus suggesting that boundaries successfully parcel out sequences of events into separate memory representations. Using similar representational analysis on EEG features from distributed electrodes and extended time windows, we found that neural activity elicited during the encoding of sequences of items within a context were identified on neural responses elicited by the encoding of a boundary item. These findings were specific to whether boundary items were preceded by sequences of different items of the same category and not by the mere presentation of repeated items within a sequence, thereby suggesting the results cannot be explained only by neural mechanisms underlying contextual shifts. Current results support the notion that a rapid memory reinstatement of the preceding encoded sequence of events is elicited during context shifts to promote the formation of a bound memory representation.

Disclosures: I. Sols: None. **L. Davachi:** None. **L. Fuentemilla:** None.

Poster

171. Human Memory Encoding Processes

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Support: ERC Grant R0001075

Title: The neural dynamics of updating and accumulating linguistic conceptual knowledge

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Abstract: Conceptual knowledge contains principles of how related perceptual features share meaning. This knowledge is acquired by generalization across repeated learning experiences, which is brought to bear on future experiences. Previous studies tracked brain activity with fMRI during the acquisition of conceptual knowledge, but the neural processes instrumental in updating this knowledge are unclear. Here, we systematically tracked conceptual knowledge formation based on an artificial language that was structured by a set of hierarchical associative rules, which were not known to participants beforehand. Specifically, participants learned shape/non-word combinations in an hour-long, scanned learning session (3T MRI). The shapes were simple geometric figures with a specific colour and movement across the screen (three features), and the words consisted of three syllables. For example, a blue circle with and upward moving trajectory was paired with the word: 'NI-JO-TO'. In each trial, first the shape was presented (the cue phase), then participants were asked to select the corresponding tri-syllabic word (the response phase), and lastly the correct non-word was presented (feedback phase). Participants were asked to select the correct syllables for each given shape out of three response options. Across learning, 27 participants learned the associations up till ceiling-level performance. Explicit knowledge of the associative rules was confirmed by a debriefing questionnaire and a test of memory transfer a day later. Next, we modeled the acquisition of conceptual knowledge using a State-Space model, which provided us with trial-by-trial estimates of the current state of knowledge as well as the change of knowledge with respect to the previous trial. We used these two estimates as parametric modulators of BOLD-activity during the cue-response period. In line with previous studies, activity in medial prefrontal cortex, angular gyrus, orbitofrontal cortex and posterior cingulate cortex was modulated by the state of knowledge. Updating of knowledge, on the other hand, modulated activity in a selective region in the right caudate nucleus. Psychophysiological interaction analysis shows that connectivity of this region fluctuated along with these learning parameters with regions in the medial prefrontal cortex and left angular gyrus. This pattern of results is in line with a model where the caudate nucleus is instrumental in driving changes in conceptual knowledge by updating representations in medial prefrontal and angular regions where knowledge is accumulated. These results provide new insight into the dynamics of conceptual knowledge acquisition.

Disclosures: **R. Berkers:** None. **M. van der Linden:** None. **D. Neville:** None. **M. van Kesteren:** None. **R. Morris:** None. **J. Murre:** None. **G. Fernandez:** None.

Poster

171. Human Memory Encoding Processes

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 171.04/W37

Topic: F.01. Human Cognition and Behavior

Support: ERC Grant R0001075

Title: Durable memory through coherent activation patterns and hippocampal-neocortical interactions

Authors: *I. WAGNER, M. VAN BUUREN, L. BOVY, G. FERNÁNDEZ;
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Abstract: Memory durability, or how persistent a memory trace will be, is partly determined while we initially encounter information. During encoding, a network of brain regions that includes medial temporal, lateral prefrontal and ventral visual structures is assumed to show stronger activation for visually presented material that will later be remembered rather than forgotten. Additionally, successful encoding is known to involve enhanced hippocampal-neocortical coupling. Such 'subsequent memory analysis' is often based on memory tests administered briefly after study - which makes claims about the durability of successfully formed memories impossible. In the present study, we aimed at expanding current knowledge by predicting the durability of memories. Thirty-four subjects studied unique picture-location associations inside the MR-scanner and performed a cued-recall test immediately after study, as well as 48 hours later. We defined associations as 'weak' if memory was preserved only during immediate retrieval. 'Durable' memories on the other hand, should persist also 48 hours later. Initial results indicate increased activation in medial temporal lobe, lateral prefrontal, and ventral visual cortex during encoding of durable as compared to weak or forgotten memories. Additionally, using psychophysiological interaction analyses, we found enhanced hippocampal-neocortical coupling during encoding of durable relative to weak memories. On a more fine-grained level, neural patterns within the hippocampus showed significant similarity across trials for both the weak and durable, but not for forgotten associations. We suggest that memory formation for weak and durable memories is supported by increased hippocampal pattern similarity across unique encoding events. Moreover, the formation of durable memories requires enhanced activation and functional coupling within a specific network. These results might indicate the necessity of coherent hippocampal processes that enable initial memory formation, while the formation of durable memories additionally requires embedding of the hippocampus into a more extended network.

Disclosures: **I. Wagner:** None. **M. van Buuren:** None. **L. Bovy:** None. **G. Fernández:** None.

Poster

171. Human Memory Encoding Processes

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Program#/Poster#: 171.05/W38

Topic: F.01. Human Cognition and Behavior

Title: Morris water maze as a translational paradigm for investigating spatial memory

Authors: ***N. C. MÜLLER**[1,2], L. GENZEL[3], S. CAMPBELL[3], M. NONAKA[3], B. KONRAD[1,2], M. CZISCH[4], G. FERNÁNDEZ[1,2], M. DRESLER[1,2], R. MORRIS[3];
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Abstract: Introduction: Cognitive neuroscience crucially depends on both human and animal research, as the former targets the main explanandum while the latter provides unique means of investigation. However, few cognitive tasks can be used across species. By utilizing the ratio of the second to the fourth digit (2D4D) as an established anatomical marker of navigational strategy and spatial memory traits, we here show that a virtual reality (VR) version of the Morris water maze (MWM) task is an appropriate human analogue of the classical version used in rodents [1]. Method: We tested 22 male rats using MWM, and 40 male humans using the VR-MWM. For each individual we measured the 2D4D ratio, and for humans we additionally acquired a structural and functional resting state MRI brain scan. We then associated MWM performance to 2D4D ratio, and in humans investigated structural and functional neuronal correlates of it using voxel-based morphometry and seed-based correlational analysis. The resting state analysis used the results from the structural analysis as seed. Results: We found that 2D4D ratio significantly predicted performance for both species in the MWM task. In humans, we found grey matter volume in the right insula to be significantly positively correlated with 2D4D ratio. We further found functional connectivity between this region and the dorsolateral pre-frontal cortex to be significantly positively correlated with MWM performance. Conclusion: Our study demonstrates that the MWM can be utilized across species, as demonstrated by a similar predictive value of the 2D4D ratio for performance in rats and humans. We further provide evidence that 2D4D ratio as a marker of spatial memory performance extents to other animals beyond humans. Finally, we showed that in humans, functional connectivity between the right insula and the dorsolateral pre-frontal cortex links 2D4D ratio and MWM performance at

the neural level. References: [1] Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. Journal of neuroscience methods, 11(1), 47-60.

Disclosures: **N.C. Müller:** None. **L. Genzel:** None. **S. Campbell:** None. **M. Nonaka:** None. **B. Konrad:** None. **M. Czisch:** None. **G. Fernández:** None. **M. Dresler:** None. **R. Morris:** None.

Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Support: NEXT program (LZ001)

JSPS KAKENHI Grant Number 25245069

The Naito Foundation

Title: Competing against a familiar friend: Interactive mechanism of the temporo-parietal junction with the reward-related regions during successful encoding

Authors: *H. SUGIMOTO, Y. SHIGEMUNE, T. TSUKIURA;
Kyoto Univ., Kyoto, Japan

Abstract: Learning is enhanced by competitive interactions with others. Previous studies have reported that three processes of social motivation, mentalizing, and reward anticipation are important in the competition such as learning situations. However, little is known about the neural mechanisms how these processes are modulated by social relationships with opponents during encoding of episodic memories. To investigate this issue, we conducted a hybrid-designed fMRI experiment for healthy young adults. In the present study, 24 pairs of familiar friends participated. During encoding with fMRI, participants learned target words in three encoding conditions of Friend, Other, and Self. In Friend, participants encoded words by competing against their friend, in which they were instructed to do their best to learn more words than their friend participating together in the experiment. In Other, participants encoded words by competing against an unfamiliar person, and in Self, participants encoded words without competing against others. During retrieval without fMRI, participants were presented with target and distracter words one by one, and made old/new judgments for the words in high and low confidence. Encoding trials in each encoding condition were categorized into subsequent hits (Hit) and misses (Miss), which were decided by the subsequent memory paradigm. Thus, we

prepared six conditions of Friend-Hit, Friend-Miss, Other-Hit, Other-Miss, Self-Hit, and Self-Miss. In addition, participants rated their subjective feelings of motivation (MO), confidence (CO), mentalizing (ME), pleasantness (PL), and mental distance (MD) in each encoding condition by a visual analogue scale. Three major findings emerged from this study. First, in a 3 (encoding condition: Friend, Other, Self) x 2 (subsequent memory: Hit, Miss) ANOVA, right temporo-parietal junction (rTPJ) activations were significantly greater in Friend than in Other and Self. In a regression analysis for a contrast of Friend-Hit vs. Other-Hit, rTPJ activations were significantly correlated with Friend-Other differences of MO scores. Second, putamen and amygdala activations were significantly correlated with PL scores. Third, functional connectivity between the rTPJ and reward-related regions including the putamen and substantia nigra was significantly higher in Friend than in Other and Self during successful encoding. These findings suggest that interactions between the rTPJ related to the social motivation and the reward-related regions could contribute to the competition during episodic encoding, and that the interactive mechanisms could be modulated by social relationships with opponents.

Disclosures: H. Sugimoto: None. **Y. Shigemune:** None. **T. Tsukiura:** None.

Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Support: NSF CAREER (BCS 1056019)

Title: Individual differences in the motivational modulation of memory are reflected in neural representations of reward context

Authors: *B. D. GELMAN[1], D. ZEITHAMOVA[2,1], A. R. PRESTON[1];
[1]Univ. of Texas At Austin, Austin, TX; [2]Univ. of Oregon, Eugene, OR

Abstract: Memory is influenced by motivation, such as when remembering an event leads to a monetary reward. Prior research suggests that better memory for highly rewarding events may result from the incorporation of motivational context into stored memory representations. However, individuals differ in the degree to which motivational factors, such as reward, influence the ability to remember. One possibility is that individual differences in motivational modulation of memory stem from variability of reward representation within hippocampus and reward centers including midbrain and ventromedial prefrontal cortex (vmPFC). To elucidate the

behavioral and neural sources of individual differences in reward modulation of memory, participants performed a motivated encoding task during fMRI scanning. Participants studied object pairs, each preceded by a reward cue indicating the monetary reward for successfully remembering the object pair. Reward cues varied on the level of reward (penny, dime, and dollar) and the visual form of the cue (picture or word). After scanning, participants performed a cued recall test for their memory of the object pairs, along with a surprise source memory test for the value and visual form of the reward cue associated with each pair. While memory was enhanced for pairs associated with the highest reward value across the group, participants differed in the degree to which object pair memory was influenced by reward. Participants were also able to identify the reward value, but not the visual cue form, associated with individual pairs. Notably, source memory accuracy was correlated with reward modulation of cued recall, consistent with the idea that reward modulation of memory results from enhanced binding of events to their motivational context. Reward sensitivity, as measured by a personality inventory, was highly correlated with reward modulation of cued recall and source memory accuracy, suggesting personality factors may have an important influence on memory processes. Moreover, we found that reward value (irrespective of cue form) could be decoded using multivoxel pattern analysis from encoding patterns in hippocampus, midbrain, and vmPFC, but only for those participants who showed better memory for the high value pairs. These results indicate that individual differences in memory modulation are predicted by both neural representations of motivational information formed during encoding and individual differences in reward sensitivity. Participants who were more sensitive to reward formed distinct memory representations that incorporated the motivational salience of events, resulting in enhanced memory for event details.

Disclosures: **B.D. Gelman:** None. **D. Zeithamova:** None. **A.R. Preston:** None.

Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Support: NIMH Grant R01MH080833 awarded to EAK

NSF GRFP Fellowship DGE1258923 awarded to SMK

Title: Increases in arousal influence encoding processes in valence-specific ways

Authors: *S. M. KARK, E. KENSINGER;
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Abstract: The amygdala has consistently been associated with arousal-related memory effects (Murty, Ritchey, Adcock, & LaBar, 2011). Prior work has suggested that the effects of arousal on neural processes may depend on valence. For instance, increased connectivity between the amygdala and middle occipital gyrus occurred during encoding of high arousal negative visual stimuli compared to low arousal negative stimuli, while increased arousal was associated with decreased coupling between these regions during encoding of positive visual stimuli (Mickley Steinmetz, Addis, & Kensinger, 2010). In the present functional magnetic resonance imaging (fMRI) study, we extended this research by examining how amygdala connectivity tracked with self-reported arousal ratings during encoding of negative and positive visual stimuli. During fMRI, seventeen participants (aged 19-35) studied line-drawing outlines of International Affective Picture System photos, followed by the complete colorful photo. After a 20-minute delay, participants were shown outlines of the previously studied and new photos and asked to make an old-new recognition judgment. Parametric connectivity analyses examined how amygdala connectivity varied as a function of trial-level post-scan ratings of arousal during successful encoding of negative and positive visual stimuli. For negative stimuli, arousal was associated with strengthened connectivity between the bilateral amygdala and the lateral occipital cortex (BA37); this effect was specific to negative valence. For positive stimuli, arousal was not associated with strengthened amygdala connectivity but instead was associated with weakened connectivity between the bilateral amygdala and the lateral occipital cortex (BA18/19). These findings suggest the strength of coupling between the amygdala and lateral occipital cortex increases linearly with subjective arousal during encoding of negative visual stimuli, while the opposite pattern is true for positive visual stimuli. These results extend prior work that suggests the effect of arousal on emotional memory depends on valence and implicate increased affective-sensory processing during encoding of negative information.

Disclosures: S.M. Kark: None. **E. Kensinger:** None.

Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Support: NSF Early Career Development Award (DS)

NSF GRFP (EKB)

Title: Both rewards and losses retroactively enhance memory for preceding neutral events

Authors: *E. K. BRAUN[1], B. VAIL[1], G. E. WIMMER[2], D. SHOHAMY[1];
[1]Psychology, Columbia Univ., New York, NY; [2]NeuroImage Nord Univ. Clin. Hamburg-Eppendorf, Hamburg, Germany

Abstract: For memory to be adaptive, it is essential to prioritize memory for events that are important for future behavior. However, events that are initially neutral can often lead to important outcomes, raising questions about the cognitive and neural mechanisms by which salient events can retroactively enhance memory. Moreover, it is unknown whether rewarding and aversive outcomes influence memory via similar mechanisms. To test whether behaviorally relevant outcomes retroactively modulate episodic memories, we developed a maze exploration paradigm. Human participants navigated through a series of grid mazes on a computer screen, one maze at a time. One group searched the mazes to find hidden "gold coins" ($1 gain), while a separate group tried to avoid hidden "land mines" ($1 loss). For both groups, the gain or loss outcomes terminated half of the mazes. While navigating through the mazes, participants incidentally encountered images of neutral trial-unique objects. 24-hours after encoding, memory for these objects was tested with a surprise recognition memory test. We found that participants' memory for the objects was retroactively modulated by both monetary gains and losses. In both the gain and the loss conditions, we found an interaction such that participants' memory was enhanced for objects closest to the gain or loss outcome compared to the neutral outcome, with no significant differences between the gain and loss conditions. These findings demonstrate that salient events retroactively enhance memory for the preceding neutral events. The similar effect of both reward and loss on memory raises important questions regarding the shared versus distinct neural mechanisms underlying these phenomena.

Disclosures: E.K. Braun: None. **B. Vail:** None. **G.E. Wimmer:** None. **D. Shohamy:** None.

Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Title: Validity of cues predicting reward delivery influences eyegaze behavior and recognition memory for associated novel scenes

Authors: *N. J. CLEMENT, R. ADCOCK;
Duke Univ., Durham, NC

Abstract: In order to make optimal choices, humans must be able to learn whether, and how strongly, stimuli in their environments predict motivationally significant outcomes. Associative models of learning (e.g., Pearce, Kaye, & Hall, 1982) make specific predictions regarding the roles a person's experience of reward and uncertainty play in driving salience and learning. Data from animals (Lee et al., 2006; Esber et al., 2012; Roesch et al., 2012) and humans (Li et al., 2011; Boll et al., 2013) suggest that interactions between regions in the dopaminergic midbrain, striatum, and amygdala support the development of predictive learning over time, and shifts in attention in response to violations of those predictions. The extent to which these systems may interact with hippocampally-mediated activity supporting exploratory behavior and memory remains incompletely characterized. We probed the relationship between cue predictive strength, uncertainty salience, and learning by investigating the encoding of stimuli paired with predictive reward cues that differed in their level of certainty, while collecting eyetracking and BOLD fMRI. One cue consistently predicted the presence of reward on that trial, one predicted absence of reward, and three cues were unreliable predictors of reward. Of the unreliable cues, one cue predicted reward delivery with a constant validity of 50%, one varied in its predictive power over blocks, and one varied in its reward-predictive power over blocks while consistently being paired with a repeated image. The remaining four cues were each paired with trial-unique images. Memory for the images was tested following a twenty-four hour delay. Participants' eyegaze behavior during learning differed as a function of both cue predictive strength and stimulus novelty: participants showed a greater preference to attend to predictive (vs. non-predictive) cues, while also attending less to familiar (vs. novel) images. Gaze behavior was also driven by reward history and expectation-violations: gaze to cues varied as a function of outcome certainty and recent uncertainty Additionally, gaze behavior predicted subsequent memory for incidentally presented images and BOLD data from the hippocampus, both in predicting subsequent memory and in response to novel vs. familiar images. These data suggest a dynamic relationship between the neural and cognitive mechanisms supporting reward learning, uncertainty, and exploration behaviors in support of learning.

Disclosures: N.J. Clement: None. **R. Adcock:** None.

Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

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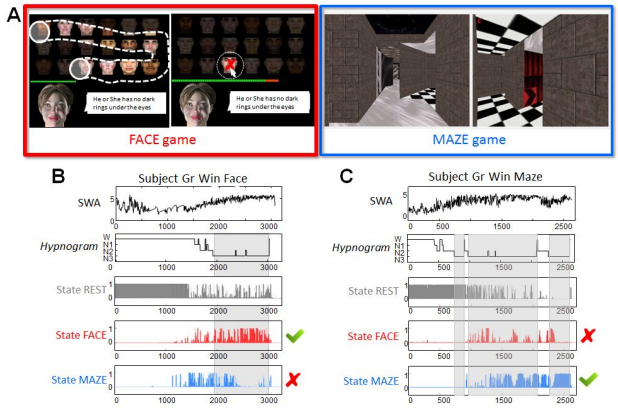
Swiss National Science Foundation (No 51NF40-104897)

Title: Victory triggers memory reactivation during sleep

Authors: *V. STERPENICH[1], H.-D. YANG[2], M. CATSIYANNIS[1], A. RAMYEAD[1], M. VAN SCHIE[1], S. PERRIG[3], D. VAN DE VILLE[4,5], S. SCHWARTZ[1];
[1]Lab. Nic, Univ. of Geneva, Genève, Switzerland; [2]Sch. of Computer Engin., Chosun Univ., Chosun, Korea, Republic of; [3]Sleep Laboratory, Dept. of Psychiatry and Mental Hlth., Geneva Univ. Hosp., Geneva, Switzerland; [4]Dept. of Radiology and Med. Informatics, Univ. of Geneva, Geneva, Switzerland; [5]Inst. of Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Abstract: Recent research provides evidence for memory reactivation and consolidation during sleep. We hypothesized that information with a high relevance for future behavior is favored in the competition for reactivation. To test this hypothesis, we manipulated the outcome of two game-like tasks and used a combination of EEG, functional MRI (fMRI) measurements, and neural decoding to analyze task-related activity and sleep data. We could thus track, during distinct stages of human sleep, the spontaneous reemergence of patterns of neural activity corresponding to the games that yielded a successful (or unsuccessful) outcome. Participants were first scanned while they played two games in alternation (a face and a maze game). We programmed the games so that only one of the 2 games would be won (randomized across participants). Then, participants slept in the MRI. A pattern classifier was trained on the data collected during game performance with three main states (face game, maze game, rest). When applied to the independent sleep data, the classification revealed a selective neural replay of the successful game during sleep stage N3. Next, we entered the time-course of the probability of each state as a regressor in a GLM applied to the sleep data. We found that each state, FACE or MAZE, activated regions involved in each task at wake, thus confirming the functional selectivity of the reactivation. Our findings provide a direct demonstration that neural events associated with a favorable behavioral outcome are prioritized for offline memory reprocessing during slow-wave sleep. Figure 1: A. Example of the face and maze games that are manipulated in order to make one won and one lost. B: Sleep data of one subject winning at the face game. The state identified by the classifier and related to the won game is more reactivated during deep sleep. The time course of the slow wave activity (SWA) is correlated to the state related to the won game. C. Sleep data of one subject wining at the maze game.



Disclosures: **V. Sterpenich:** None. **H. Yang:** None. **M. Catsiyannis:** None. **A. Ramyead:** None. **M. van Schie:** None. **S. Perrig:** None. **D. van de Ville:** None. **S. Schwartz:** None.

Poster

171. Human Memory Encoding Processes

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Support: NSF CAREER 1056019 (A.R.P.)

F32 MH094085 (D.Z.)

Title: Human hippocampus forms abstract, pattern separated representations of motivational context during encoding

Authors: ***D. ZEITHAMOVA**[1], B. D. GELMAN[2], A. R. PRESTON[2];
[1]Univ. of Oregon, Eugene, OR; [2]Univ. of Texas, Austin, TX

Abstract: Memory is influenced by motivation, such as a promise of future monetary reward for successfully remembering an event. Monetary reward cues preceding or immediately following an event have been shown to enhance activation within dopaminergic midbrain and the hippocampus, resulting in better memory for the associated event. Furthermore, distributed hippocampal patterns differentiate between events encoded in contexts with different reward cues, supporting the view that information about reward context is incorporated into stored memory representations. However, prior studies addressing this topic have confounded the reward value with the visual appearance of reward cues, leaving unanswered whether

hippocampal responses reflect the visual features of the reward cue or an abstract representation of reward value. Here, we employed a novel reward manipulation to dissociate between these accounts. Participants underwent functional MRI while encoding pairs of objects, with each pair being preceded by a cue indicating the value of the monetary reward the participant would receive if they successfully remembered the pair. Reward cues represented one of three values (dollar, dime, penny), each presented in one of two visual forms (word, picture) across trials. After scanning, participants were given a cued recall test for the associations. Behaviorally, participants remembered pairs associated with the highest reward (dollar) more often than pairs associated with lower rewards (dime or penny), irrespective of the visual form of the reward cue. We then tested which aspects of the cue (value or visual form) were represented in the hippocampus using pattern similarity analysis. Hippocampal encoding patterns were more similar for pairs of trials cued by the same reward value than those cued by different values, even when the visual form of the cue differed. Strikingly, and consistent with pattern separation accounts of hippocampal representations, the encoding patterns were least similar for trials cued by different values when the cues shared the same visual form. This pattern was most pronounced in participants who showed the greatest behavioral sensitivity to reward_better memory for high-value than low-value trials. These results provide a novel demonstration of abstract value representation within human hippocampus, one in which representations of visually similar cues are made distinct to reflect differences between the behavioral relevance of individual events. More broadly, our data illustrate that contextual representations within hippocampus go beyond space and time to include information about the motivational salience of events.

Disclosures: **D. Zeithamova:** None. **B.D. Gelman:** None. **A.R. Preston:** None.

Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Support: Gustavus and Louise Pfeiffer Research Foundation

Title: Single-trial learning of novel visual stimuli by individual neurons in the human substantia nigra

Authors: *J. KAMINSKI[1], A. MAMELAK[1], K. BIRCH[1], M. TAGLIATI[2], U. RUTISHAUSER[1,2,3];
[1]Neurosurg., [2]Neurol., [3]Biomed. Sci., Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: The ability to distinguish novel from familiar stimuli is essential for learning. Novelty-triggered release of dopamine in the temporal-and frontal lobes by neurons in the substantia nigra is thought to be crucial for the formation of new memories. We investigated this hypothesis by recording individual neurons in the human substantia nigra with microelectrodes during surgery for implantation of deep brain stimulation devices. In 8 patients (11 sessions), we presented a sequence of images and asked them to classify each as novel or familiar. Simultaneously, we recorded single unit neuronal activity in the substantia nigra using microelectrode and intracranial EEG from cortex surface using ECOG strip. The average firing rate of multi-unit activity recorded in the substantia nigra was 23.2 Hz. We isolated in total 39 single neurons, out of which 12 (31 %) showed a visually-triggered response. The majority (8/12, 66 %) decreased their firing rate in response to the onset of the visual stimulus. Further analysis of single unit responses showed that ten units (26 %) responded significantly different to novel compared to familiar pictures, with the majority (70 %) exhibiting higher firing rates to novel stimuli. Additionally we quantified the Spike Field Coherence (SFC) between units in the substantia nigra and intracranial EEG recorded from frontal cortex. We compared novel images as a function of whether they were later remembered or forgotten. This analysis showed that pictures that were later remembered were accompanied by larger SFC in the theta-frequency range relative to images later forgotten. Together, these results show the first evidence from human intracranial recordings that neurons in the substantia nigra are involved in the detection of novel visual stimuli. Additionally, we showed that successful memory formation is correlated with coordination of spike timing of substantia nigra neurons with cortical theta oscillations. These results indicate the important role of the human substantia nigra in the learning of visual stimuli.

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Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Title: Knowledge accumulation improves memory and reorganizes hippocampal - neocortical interactions

Authors: *G. BROD, Y. SHING;
Max Planck Inst. For Human Develop., Berlin, Germany

Abstract: Introduction The better new information can be connected to existing knowledge, the easier it is to learn and, later, remember it. This is assumed to be the case because neocortical schemas can incorporate the new, related information easily, which speeds up binding processes in the hippocampus (HC). An increase in knowledge should thus lead to an enhanced memory for schema-related information, and decreased HC recruitment for memory formation. We tested these notions in a real-world setting using repeated fMRI in a sample of medical students who prepared for their final state examination using an e-learning platform, which gave us detailed information about their knowledge increase. Methods 35 medical students were scanned three months prior to and immediately after the state examination during encoding of face-diagnosis (schema-related) or face-name (schema-unrelated) pairs. Subsequent memory effects were determined using a forced-choice associative recognition task. We tested for changes in brain activation that were larger for the diagnoses as compared to the names, and vice versa (Memory x Time x Condition Interaction). In addition, we tested for changes in coupling between the anterior HC and semantic processing regions using PPI analysis. Results We observed a selective enhancement in memory for schema-related information that was paralleled by a decrease in anterior HC activation (see Figure 1). The HC decrease was related to between-person differences in knowledge increase (as measured by the learning platform). Furthermore, we observed an increase in connectivity between the anterior HC and the left posterior Middle Temporal Gyrus, a brain area that is key to semantic processing. Our results suggest that increased knowledge facilitates binding in the anterior HC, presumably through an enhanced crosstalk with semantic processing areas. Furthermore, they demonstrate a close link between accumulation of knowledge due to intensive learning in a real-world educational setting and changes in brain activation as observed in a laboratory memory paradigm.

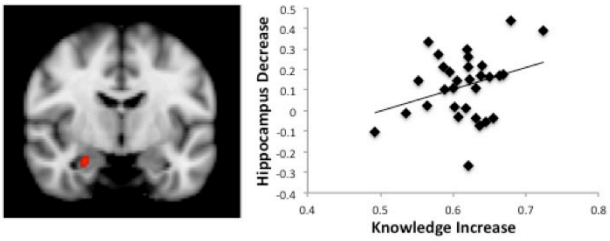

Figure 1. Memory x Time x Condition interaction in the right anterior hippocampus (peak voxel: 26, -8, -20). The HC decrease for the diagnosis condition was furthermore related to between-person differences in knowledge increase (r=.32, p=.04).

Disclosures: G. Brod: None. **Y. Shing:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: DARPA N66001-14-C-4016

Title: Nonlinear dynamical model of human hippocampal CA3-CA1 spike transformation as the computational basis of memory prostheses

Authors: *D. SONG[1], R. E. HAMPSON[2], B. S. ROBINSON[1], V. Z. MARMARELIS[1], S. A. DEADWYLER[2], T. W. BERGER[1];
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Abstract: We formulate hippocampal prosthesis as a closed-loop system that bi-directionally interacts with the hippocampus to restore memory functions. Different from sensory or motor prostheses, the hippocampal prosthesis uses internal brain signals, e.g., spike trains, as inputs and outputs. In order to bypass a damaged hippocampal region to restore its function, the prosthesis should be able to mimic the input-output transformations performed by that region, so that the lost output signals in the downstream hippocampal region (e.g., CA1) can be reinstated based on the input signals recorded from the upstream hippocampal region (e.g., CA3). In this study, we build multi-input, multi-output (MIMO) nonlinear dynamical hippocampal models to replicates the spike-train-to-spike-train transformational properties of the hippocampus. Spike trains are recorded from the hippocampal CA3 and CA1 regions of epileptic patients (n = 10) performing a memory-dependent delayed match-to-sample task. Using CA3 and CA1 spike trains as inputs and outputs respectively, second-order sparse generalized Laguerre-Volterra models are estimated with group lasso and local coordinate descent methods. These models allow parsimonious and accurate representations of the hippocampal input-output functions. Model sparsities are optimized with a 5-fold cross-validation method. Model goodness of fit are evaluated with a Kolmogorov-Smirnov test based on the time-rescaling theorem. Results show that these MIMO models can accurately predict the CA1 spatio-temporal patterns of spikes based on the ongoing CA3 spatio-temporal patterns of spikes on a single-trial basis. These results indicate the strong causal relations between hippocampal CA3 and CA1 activities and that it is possible to use the MIMO models as the computational basis of hippocampal memory prostheses.

Disclosures: **D. Song:** None. **R.E. Hampson:** None. **B.S. Robinson:** None. **V.Z. Marmarelis:** None. **S.A. Deadwyler:** None. **T.W. Berger:** None.

Poster

171. Human Memory Encoding Processes

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 171.16/X1

Topic: F.01. Human Cognition and Behavior

Support: DARPA N66001-14-C-4016

NIH U01 GM104604.

Title: Nonlinear dynamical identification of a functional spike-timing-dependent plasticity rule from ensemble hippocampal spiking activities in rats learning a delayed nonmatch-to-sample task

Authors: *B. S. ROBINSON[1], D. SONG[1], R. E. HAMPSON[2], S. A. DEADWYLER[2], T. W. BERGER[1];
[1]Biomed. Engin., USC, Los Angeles, CA; [2]Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: The computational basis of a hippocampal memory prosthesis can be formed with a nonlinear multi-input, multi-output (MIMO) dynamical model that characterizes the input-output spike train transformation in the hippocampus. In creating such a MIMO model, it is important to consider the effects of activity-dependent plasticity. Here, we augment previous MIMO model identification to include estimation of a pair-wise spike-timing-dependent plasticity (STDP) rule. Model estimation is performed with multi-unit spiking data recorded in-vivo from CA3 and CA1 rat hippocampal sub-regions during the learning of a delayed-nonmatch-to-sample (DNMS) memory task across several sessions. As part of the MIMO model identification, nonlinear feedforward functions are identified for each input that characterize the conditional firing probability of output spiking given that input's spiking events. For the STDP model, a separate set of functions are estimated for how the relative timing of input-output spike pairs change the strength of feedforward connections and the time course of plasticity induction. All model functions are estimated with Volterra kernels, basis function expansion and generalized linear model techniques. The inclusion of the identified STDP rules enhances prediction of CA1 output spiking from CA3 input spiking for certain behavioral sessions. We also demonstrate how several sets of assumptions regarding the structure and stability of the STDP rule vary in ability to capture the dynamics of the observed spiking activity. Furthermore, the identified plasticity

rules provide insights into how a pair-based STDP rule may lead to fluctuations in functional connectivity observed during learning.

Disclosures: **B.S. Robinson:** None. **D. Song:** None. **R.E. Hampson:** None. **S.A. Deadwyler:** None. **T.W. Berger:** None.

Poster

171. Human Memory Encoding Processes

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Program#/Poster#: 171.17/X2

Topic: F.01. Human Cognition and Behavior

Support: DARPA N66001-14-C-4016

WFBMC Dept. of Neurosurgery

Title: Restoring active memory via CA3/CA1 recording and MIMO model-derived CA1 stimulation in human hippocampus

Authors: *R. E. HAMPSON[1], D. SONG[6], B. S. ROBINSON[6], M. R. WITCHER[2], D. E. COUTURE[2], A. M. LAXTON[2], G. POPLI[3], M. J. SOLLMAN[3], D. FETTERHOFF[4], C. A. SEXTON[5], V. Z. MARMARELIS[6], T. W. BERGER[6], S. A. DEADWYLER[1]; [2]Neurosurg., [3]Neurol., [4]Neurosci., [5]Biomed. Engin., [1]Wake Forest Sch. of Med., Winston Salem, NC; [6]USC, Los Angeles, CA

Abstract: We have recently demonstrated that patterned CA1 stimulation based on a multi-input, multi-output (MIMO) model of hippocampal CA3-to-CA1 neural firing facilitated behavioral performance in both rodents (Berger et al., 2011) and nonhuman primates (Hampson et al., 2012). Here we extend this work to humans performing a similar working memory task. Patients who undergo intracranial monitoring with implanted electrodes for epileptic seizure localization provide an excellent model to understand discharge patterns of hippocampal neurons during learning and memory tasks (Suthana and Fried 2012). Since most patients can form and retain working memories even when the hippocampus is altered by epilepsy (Suthana, Haneef et al. 2012), hippocampal neural recordings under these conditions provide a valuable test-bed for studies leading to prosthetics to restore memory encoding and formation in humans. Adult subjects underwent surgical implantation of FDA-approved "macro-micro" hippocampal electrodes (capable of EEG, field potential and single-neuron recording; Ad-Tech Medical Instrumentation Corporation, Racine, WI) for localization of seizures. Inclusion in this study was

voluntary and consented separately from the surgical procedure and all study participants underwent appropriate clinical epilepsy screening evaluations. Electrode localization was confirmed using intraoperative stereotaxic placement and postoperative MRI. Single unit neural activity was isolated and recorded using Blackrock Cervello electrophysiological recording systems; multichannel microstimulation was provided by a Blackrock CereStim R96. Patients performed a visual object and position-oriented Delayed-Match-to-Sample on a touchscreen computer. Patients were tested in one session strictly for CA3 & CA1 neuron recording, and a second session that also included MIMO model-derived CA1 stimulation during the mnemonic encoding phase of the task. Neural and behavioral recordings have yielded a task-related MIMO model of CA3-to-CA1 mnemonic processing (Song et al. 2015 - adjacent poster), similar to that used for the successful tests of MIMO-derived stimulation in rodents and NHPs. These results suggest that this approach provides an appropriate model for development and testing of a neural prosthetic for human memory.

Disclosures: **R.E. Hampson:** None. **D. Song:** None. **B.S. Robinson:** None. **M.R. Witcher:** None. **D.E. Couture:** None. **A.M. Laxton:** None. **G. Popli:** None. **M.J. Sollman:** None. **D. Fetterhoff:** None. **C.A. Sexton:** None. **V.Z. Marmarelis:** None. **T.W. Berger:** None. **S.A. Deadwyler:** None.

Poster

171. Human Memory Encoding Processes

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 171.18/X3

Topic: F.01. Human Cognition and Behavior

Title: Inter-subject correlation in theta and gamma spectral power predicts memory for details of scientific talks

Authors: E. R. WEICHART, D. M. NIELSON, *P. B. SEDERBERG;
Psychology, The Ohio State Univ., Columbus, OH

Abstract: Previous work has shown that neural synchrony in fMRI activity across participants while viewing naturalistic stimuli predicts subsequent memory performance. In the current study, we sought to extend this finding to electroencephalography (EEG) and into the domain of memory for scientific talks. We collected EEG data while participants viewed three novel videos of informational science talks. After viewing all three talks, participants were asked to answer short answer questions related to content-driven details of the talks. Each question corresponded to a 3- to 15-second portion of the video, during which a specific answer was provided. We

calculated inter-subject correlation (ISC) in spectral power for 6 frequency bands during each question-specific time range to predict the proportion of participants who would correctly answer the individual question later. After first identifying the components comprising weighted combinations of electrodes and frequency bands that were most predictive of ISC, we sought to determine which components and first-level component interactions explained a significant amount of variance in group memory performance on each question. Different combinations of positive and negative ISC across frequency bands and electrodes correlated with subsequent memory performance. For example, one component positively predicted successful memory when gamma ISC was negative and theta ISC was simultaneously positive. Contrastingly, another component showed that memory performance was positively predicted by high gamma ISC localized to the posterior region of the scalp along with simultaneous theta ISC in the occipital region. In still another component, successful memory performance was predicted by negative alpha ISC in the posterior region with simultaneous positive posterior gamma ISC and negative gamma ISC in auditory regions. In future work, this approach will allow us to investigate the aspects of informative video presentations that promote memory, which will be increasingly important as e-learning programs become more common.

Disclosures: **E.R. Weichart:** None. **D.M. Nielson:** None. **P.B. Sederberg:** None.

Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Support: NSC 103-2420-H-002-MY3

MOE 103R8921~103R892103

Title: A time-dependent effect of post-trial intervention on consolidation of pair association memory

Authors: ***K. LIANG**[1,2], F.-Y. CHENG[2], P.-Y. CHEN[2], T.-L. CHOU[2];
[2]Psychology, [1]Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Memory consolidation is a process that a memory trace becomes stable and resists to disruption over time, which is supported by the evidence that a period of rest without interference after learning would enhance retention of learned materials and the closer in time the

interference to the original learning, the poorer the subsequent memory. However, whether a memory trace in humans becomes more resistant to post-learning interference along with time has been questioned. To address this issue, the present study was designed to examine the time-dependent influence of post-trial intervention on consolidation of associative memory for figure-noun pairs. Two groups of participants were randomly assigned into an immediately intervention group (II, N=15) or a delayed intervention group (DI, N=15) on day 1. In the DI group, participants learned the target list, followed by a one-hour interval of music listening, and then learned an intervention list. In contrast, participants in the II group learned the target list, followed immediately by learning the intervention list, and finally listened to music for one hour. In learning the target and intervention lists, participants were instructed to memorize two different sets of figure-noun association pairs. Retention of the target list was assessed by recognition on two time points: Right after the learning phrase on day 1 to serve as a baseline, and 24 hours later on day 2 to probe memory that has already undergone consolidation. For each participant, memory performance was assessed by recognizing the target pairs from foil pairs (rearrangement of the stimulus and response pairing from the list and not learned on day 1) and indexed by the sensitivity (d-prime) of signal detection analysis. The results showed that the II group showed a greater reduction of the d-prime score for detecting the target pairs from day 1 to day 2 than the DI group. Moreover, participants in the II group showed more false alarms on target recognition after 24-hour consolidation than those in the DI group. Our results indicate that learning an intervention list immediately after the target list produced more interruption of the 1-day memory than listening to music. These findings suggest that consolidation of human figure-noun associative memory is impaired in a time-dependent manner by processing materials similar to and shortly after learning the target ones.

Disclosures: **K. Liang:** None. **F. Cheng:** None. **P. Chen:** None. **T. Chou:** None.

Poster

171. Human Memory Encoding Processes

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 171.20/X5

Topic: F.01. Human Cognition and Behavior

Support: NIH Intramural Research Program

Title: Dynamic phase-amplitude coupling in episodic memory

Authors: A. VAZ[1], R. YAFFE[2], S. K. INATI[2], *K. A. ZAGHLOUL[2];
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Abstract: Phase-amplitude coupling (PAC) in intracranial EEG has been proposed as a communication mechanism that reflects coordination of local neuronal populations. This neural phenomenon has been shown to exist ubiquitously in human neocortex, and recent studies have shown that hippocampal PAC plays a role in working and episodic memory as well as in associative learning. However, a functional, task-dependent role of PAC has not been established in human neocortex. Here, we used intracranial recordings to examine PAC for encoding and retrieval periods as 33 participants with electrodes placed for seizure monitoring engaged in a paired associates episodic verbal memory task. We found that memory encoding dynamically modulates PAC in human neocortex. Correct and incorrect encoding exhibited significant differences in PAC in individual electrodes, and in different brain regions when aggregated across subjects. Furthermore, we observed that PAC is modulated in time and across different epochs of the experimental task, and that the extent of PAC is highly correlated with the power of the modulating frequency band. Our data suggest that PAC is a dynamic and task-specific neural correlate of episodic memory in human neocortex.

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Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Support: German National Academic Foundation

Title: Iron accumulation and loss of myelin relates to memory performance in the aging brain

Authors: *T. K. STEIGER[1,2], C. ECKART[2], N. BUNZECK[1,2];
[1]Dept. of Psychology, Univ. of Luebeck, Luebeck, Germany; [2]Dept. of Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: In healthy humans, age related structural changes have been demonstrated in a variety of cortical and subcortical brain regions. However, the underlying microstructural mechanisms, e.g. in myelin or iron, and their behavioral consequences remain poorly understood. In this study, we acquired multi-parameter images (MRI) of a group of young (18-32 years) and healthy elderly subjects (55-79 years) to investigate age related structural changes of the dopaminergic mesolimbic system and their relationship to learning and memory performance. Data were analyzed by using voxel-based morphometry (VBM) on grey matter volume, and voxel-based

quantification (VBQ) on magnetization transfer (MT, resembling myelin) and R2* (resembling iron content). Outside the MRI scanner, the elderly subjects performed the 'verbal learning memory test' (VLMT) to assess memory performance. As expected, we observed age-related differences in grey matter volume, MT and R2* within widespread brain regions, including the basal ganglia. Importantly, within the hippocampus there was a positive correlation between MT and R2* in younger and elderly subjects. This relationship was reversed in the nucleus accumbens of the elderly, accompanied by an accumulation of iron. Finally, microstructural changes of MT and R2* within the basal ganglia correlated with learning and memory performance in the elderly in a whole-brain analyses. Our results provide evidence for the notion that iron (R2*) drives myelination (MT) in young subjects, while it leads to oxidative stress and myelin loss in the elderly. Importantly, these microstructural changes relate to learning and memory performance, indicating a close link between the integrity of the dopaminergic mesolimbic system and learning and memory.

Disclosures: **T.K. Steiger:** None. **C. Eckart:** None. **N. Bunzeck:** None.

Poster

171. Human Memory Encoding Processes

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: Institutional Funding (Wayne State University)

Title: Spatial-temporal dynamics of memory formation in children: An electrocorticography (ECoG) investigation

Authors: ***A. T. SHAFER**, R. SCHWARZLOSE, E. ASANO, N. OFEN;
Wayne State Univ., Detroit, MI

Abstract: Episodic memory formation involves the medial temporal lobe (MTL) and lateral prefrontal cortex (PFC). Research using ECoG to examine memory-related neural activity in these regions in adults has shown increases in power for two frequency bands [theta (4-7 Hz) and gamma (30-100 Hz)]. Evidence from non-invasive neuroimaging methods such as functional MRI has shown protracted functional development of PFC, compared to MTL in support of memory formation. However, how memory development is related to local neural activity as can be assessed in ECoG measures by spectral perturbation remains unknown. Here, we examined changes in theta and gamma power for subsequently remembered vs. forgotten scenes in surface

electrodes placed on MTL and lateral PFC in children and adolescents. Data were collected from 8 epilepsy participants who were undergoing extraoperative ECoG as part of clinical management (aged 10-17). Participants studied pictures of indoor and outdoor scenes, and were informed that their memory of the scenes would be tested in a following recognition test. Data were recorded using a 192-channel digital system with platinum grid and strip electrodes (diameter = 4 mm, intercontact distance = 10 mm). Electrode location was determined using FreeSurfer software in conjunction with planar x-ray images and a T1-weigthed MR image. Event-related spectral changes were determined through wavelet time-frequency decomposition for -500 ms pre- to 3000 ms post-stimulus onset. To examine changes over time, standardized power values were binned into 100 ms time windows. Preliminary analysis focused on data sampled from 0 to 1000 ms post-stimulus onset within the left MTL and lateral PFC. Successful memory formation was associated with an early decrease followed by later increase in theta in both MTL and lateral PFC. The later increase in theta differed across regions such that it occurred earlier in the MTL (~200 ms) compared to the lateral PFC (~500 ms). Similar to the results for theta, differences in gamma power (50-150 Hz) for remembered vs. forgotten scenes showed early decreases followed by later increases in both MTL and lateral PFC. However, unlike with theta, the earlier gamma decrease for remembered scenes was sustained for longer in the lateral PFC (400ms, PFC vs. 100ms, MTL), while the later increase was sustained longer in the MTL (300ms, MTL vs. 100ms, PFC). These preliminary findings aid in characterizing neural activity in key brain regions that support successful memory formation in children and adolescents.

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Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number 26540069

Title: Predictability of subsequent retrieval after natural reading of literature: A scalp electroencephalogram study

Authors: *N. SATO;
Future Univ. Hakodate, Hakodate-Shi, Hokkaido, Japan

Abstract: The author recently demonstrated that memory that is formed during the natural reading of literature can be predicted by a scalp electroencephalogram (EEG) analysis. In agreement with previous studies of subsequent memory effects of words and pictures, the increases in theta-band EEG power and decreases in alpha-band EEG power during reading significantly correlated with the subjects' subsequent retrieval. The natural reading of literature is highly associated with daily behavior. Therefore, evaluations of its predictability are of interest for creating EEG applications to monitor reading. In this study, the predictability of EEG data that were recorded during reading of subjects' subsequent retrieval was evaluated. Eight participants (seven males; mean age, 21.1) read four short scientific essays (3,000 characters each) at a natural pace and wrote summary reports of the contents after about 30 min. During the reading, binocular eye movements were recorded, and 26-channel scalp EEG and 4-channel electro-oculogram recordings were made. Artifact-corrected EEG signals were segmented according to fixation onset, and the evoked power was analyzed according to the similarity in a probabilistic language model of the read texts and the summary reports. The recall rate of each word was predicted with EEG signals at a central electrode (Cz), and predictability was tested for each essay with a cross-validation procedure. The cross-validation analysis showed that the retrieval rate that was predicted by the EEG theta and alpha powers was significantly correlated with the observed retrieval rate (mean correlation coefficient, 0.131; range, -0.08 to 0.28; $p = 0.001$, permutation test), and three of the eight subjects showed a significant correlation in the individual analyses. The resulting correlation coefficients were similar to those for previously observed subsequent memory effects for simple memory tasks. These findings confirm the generality of EEG phenomena for subsequent retrieval. For the EEG application perspective, however, the number of subjects with significant correlations in the individual analyses was small. However, when predictions were made by either EEG theta or alpha power, the predictions were worse than those above (mean correlation coefficient, 0.09 for each condition; $p = 0.001$, permutation test). Interestingly, in this analysis, EEG theta and alpha predictability varied in each subject. These results suggest the importance of cross-spectral analyses of EEG data in characterizing subjects' subsequent retrieval and improving predictability.

Disclosures: N. Sato: None.

Poster

171. Human Memory Encoding Processes

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: NSF Grant BCS1461088

Title: Can auditory oscillations enhance memory performance by entraining neural oscillations of the same frequency?

Authors: *J. D. CREERY, K. A. PALLER;
Northwestern Univ., Evanston, IL

Abstract: Memory processing depends on neural communication across brain regions. This communication has been associated with neural oscillations at various frequencies. For example, efforts have been made to link memory formation to specific oscillatory activity in scalp and intracranial EEG recordings. Possible causal roles of oscillatory activity in memory functioning have been investigated in humans using oscillatory stimulation in the form of transcranial direct current stimulation (tDCS) and repetitive transcranial magnetic stimulation (rTMS). An alternative strategy is to use oscillatory auditory stimulation instead of oscillatory electromagnetic stimulation. In the current study, we presented pink noise in conjunction with pictures of visual objects in a spatial memory task, and we systematically modulated sound amplitude to include frequency-specific signals. We predicted that auditory oscillations would entrain brain activity in ways that would influence subsequent memory accuracy. We chose to use oscillations at theta and beta frequencies because (a) previous studies showed that these frequencies are associated with memory formation and (b) auditory oscillations have been shown to influence neural oscillations at both of these frequencies. Participants learned 60 object-location associations on a grid background. Objects were randomly presented with one of three types of auditory stimulus: 6-Hz theta oscillations (20 objects), 15-Hz beta oscillations (20 objects), or static noise (20 objects). Each association was learned to a criterion, and after a 15-min break, participants were tested for object-location recall accuracy. Participants were significantly more accurate for objects linked with beta stimulation than those linked with static noise. Intermediate results were found with theta stimulation. These findings support previous evidence suggesting that beta oscillations foster long-term potentiation. Further tests are in progress to isolate the source of these effects (e.g., learning, test, or both) and to examine neural oscillations in concurrent EEG recordings. Overall, these findings demonstrate that memory can be improved using oscillating auditory stimulation during learning and test.

Disclosures: **J.D. Creery:** None. **K.A. Paller:** None.

Poster

171. Human Memory Encoding Processes

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Topic: F.01. Human Cognition and Behavior

Support: NIMH Grant MH094263

NICDH Grant HD055352

Grant from the Center for Nutrition, Learning, and Memory

Title: Macular lutein is associated with cognitive performance in preadolescent children

Authors: *K. M. HASSEVOORT[1,2], S. E. ZOLA[3], S. M. MCCORKLE[4], L. B. RAINE[5], N. A. KAHN[4], A. F. KRAMER[1,3], C. H. HILLMAN[1,5], N. J. COHEN[1,3];
[1]The Beckman Inst. of Sci. and Technol., Urbana, IL; [3]Psychology, [4]Nutritional Sci., [5]Kinesiology and Community Hlth., [2]The Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: The dietary carotenoid lutein is an important constituent of both macular and brain tissue across the lifespan. While the developmental and neuro-protective roles of lutein have been well characterized in the visual system, recent evidence suggests that lutein may also play an important role in cognitive function. In addition to the macula of the retina, lutein is also preferentially accumulated throughout the CNS and is the predominant carotenoid in early as well as late life (Johnson *et al.*, 2013; Vishwanathan *et al.*, 2014). Macular pigment optical density (MPOD), a measure of macular lutein abundance is correlated with lutein levels in brain tissue (Vishwanathan *et al.*, 2015; Vishwanathan *et al.*, 2013) and is positively associated with performance in a range of cognitive domains, including memory, executive function, and language in adults (Johnson *et al.*, 2013). Furthermore, there is evidence that lutein supplementation improves cognitive performance in older adults. However, no previous study has examined the relationship between macular lutein and cognition in a developing population. The current study investigated this relationship in preadolescent children (N=39; mean age=8.7 years) who underwent MPOD testing and completed a series of cognitive tasks, including a reconstructive memory task. We found that children with higher macular lutein performed better on the memory task, specifically by making fewer "swap errors", a type of relational error that has previously been linked to hippocampal function (Watson *et al.*, 2013). These results are the first evidence that macular lutein is positively associated with cognitive performance in preadolescent children and suggest that increasing dietary lutein has the potential to improve memory in this population.

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Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.01/X11

Topic: F.01. Human Cognition and Behavior

Title: Time course of attentional selection

Authors: A. DREW[1], *J. E. KOCH[2], Q. M. CHROBAK[1], A. T. KARST[1];
[1]Univ. of WI Oshkosh, Oshkosh, WI; [2]Univ. Wisconsin, Oshkosh, Oshkosh, WI

Abstract: The N2pc ERP component is associated with the deployment of visual attention to regions in visual space (Luck & Hillyard, 1994a, Luck & Hillyard, 1994b). Several hypotheses have been advanced regarding what specific aspect of spatially deployed attention the N2pc reflects (Luck & Hillyard, 1994b; Kiss, Velzen, & Eimer, 2008; Tan & Wyble, 2014). The Localization Hypothesis, the focus of the current study, posits that the N2pc reflects the localization of, or orientation to, relevant visual information in space in preparation for the enhancement of sensory processing. Recent research has supported this hypothesis by demonstrating that the N2pc elicited by target information is eliminated if this target follows a second target in the same location, as a second localization process is unnecessary (Tan & Wyble, 2015). Other research provides evidence that is incongruent with the localization hypothesis. Specifically, a cue that directs attention to a portion of the visual field prior to the occurrence of a target does not eliminate the N2pc for that target, as is predicted by the localization hypothesis (Kiss, Velzen, & Eimer, 2008). However, the stimulus onset asynchrony of the cue (700ms prior to target onset) established a temporal window that may have been too large to capture a single episode of attentional localization. The present study tested the localization hypothesis by using a cue presented within the time course of current theoretical models of attention (Wyble, Bowman, & Nieuwenstein, 2009; Olivers & Meeter, 2008; Nakayama & Mackeben, 1989). A target was presented laterally (right and left), embedded within streams of rapidly presented distractors. Half of the trials contained a target, the other half did not. Within each of these two conditions, on half of the trials, a cue was presented. If both a cue and target were presented, the cue preceded the target by 100ms. Preliminary analyses revealed that the N2pc elicited by a single target embedded in the stream of distractors is

eliminated when preceded by a cue occurring 100ms prior to the target. These results support the hypothesis that the N2pc reflects a visual localization process.

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Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Program#/Poster#: 172.02/X12

Topic: F.01. Human Cognition and Behavior

Title: Target detection, following central cues of different reliabilities, is associated with modulations of the P300 and N400 components

Authors: D. VALAKOS[1], D. MYLONAS[1], G. D' AVOSSA[2], *N. P. SMYRNIS[3,1];
[1]Lab. of Sensorimotor Control, Univ. Mental Hlth. Res. Inst., Athens, Greece; [2]Psychology, Univ. of Bangor, Bangor, United Kingdom; [3]Natl. Univ. of Athens Med. Sch., Athens, Greece

Abstract: An important issue in visual attention is how endogenous covert orienting and target detection are affected by an observer's spatial expectations. We examined the effects of partially valid cues, of high and low reliability, on simple detection reaction times and scalp recorded, electrophysiological potentials, evoked by the presentation of luminance targets. The paradigm began with a central arrow cue, which indicated one of four locations, where a target stimulus could appear. The cue could either be reliable or unreliable. Reliable cues indicated the correct target location in 75% of the trials, while unreliable cues indicated the correct target location in 25% of the trials, and were therefore uninformative. Reliable and unreliable cues had different colors, and participants were made aware of the mapping between color and reliability ahead of the testing procedure. Reaction times showed a larger validity effect following reliable than unreliable cues, while the ERPs revealed a P300 significantly bigger for invalid than valid trials, but not further modulated by target probability. Finally, a typical N400 response was found, suggesting the recruitment of domain general processes in target evaluation, at least under conditions of varying spatial uncertainty.

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Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.03/X13

Topic: F.01. Human Cognition and Behavior

Support: Medical Research Council

Title: The behavioural and cortical dynamics of reward-driven attentional capture

Authors: *L. TANKELEVITCH, M. F. RUSHWORTH, M. G. STOKES;
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Abstract: Selective attention is the prioritization and facilitation of processing via the enhancement of relevant information at the expense of that less relevant. Although it is often guided by task-relevant goals like searching for food, or captured by intrinsically salient stimuli like a car horn, recent work has shown that attention may also be shaped by reinforcement learning, in which visual stimuli with a history of reward capture attention even when they are task-irrelevant and unrewarded. Here we provide further evidence for reward-driven attentional capture, and investigate its behavioural timecourse and spatiotemporal dynamics in the cortex. Participants first completed a decision-making task in which they chose between different reward-associated visual tokens with the aim of maximizing their monetary payoff. Participants then completed a non-rewarded lateralized target discrimination task involving oriented gratings, in which they also encountered the previously valuable - but now irrelevant - tokens. Critically, a high or low value token could appear either around the target grating (congruent trials) or around the distracter grating (incongruent trials). The tokens appeared at ten different stimulus-onset asynchronies (SOA) relative to the gratings, ranging from 50 ms to 950 ms, allowing us to probe the timecourse of attentional capture via changes in reaction times and accuracy. In line with previous work, there was a significant difference in participants' reaction times between congruent and incongruent trials indicating that attentional capture was respectively facilitating or impairing task performance as a function of congruency. This effect occurred at both short and long SOAs, peaking at 50 ms and 450 ms prior to target onset. Importantly, attentional capture was significant only for high value tokens, implicating the role of reinforcement learning in shaping attentional selection. In a second experiment, we used magnetoencephalography (MEG), aided by structural magnetic resonance imaging (MRI), to probe the spatiotemporal dynamics of reward-driven attentional capture in the visual cortex and frontoparietal attention network. Using the same behavioural paradigm as above, we investigated the modulation of attentional allocation and target processing by the presentation of task-irrelevant tokens with different

reward histories. Our work furthers the understanding of the interaction between reinforcement learning and selective attention.

Disclosures: **L. Tankelevitch:** None. **M.F. Rushworth:** None. **M.G. Stokes:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: DFG SFB 779 TPA1

Title: An electrophysiological dissociation of craving and stimulus-dependent attentional capture in smokers

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Abstract: In addiction, the motivation to use a specific substance is driven in large part by strong feelings of craving. Additionally, there is behavioral evidence to suggest that attention may be captured by addiction-related images, rendering such stimuli highly salient in a given environment. The neural underpinnings of such attentional capture by substance-related images and how it interacts with craving, however, are largely unknown. Here, we examined the neural correlates of attentional capture by addiction-related stimuli in smokers as a function of craving. To assess these effects, simultaneous EEG and MEG data were recorded in two sessions: one in which participants had just smoked (noncraving), and one in which they had abstained from smoking for three hours (craving). Participants performed a visual search task in which two colored squares were presented on the left and right of fixation, with the task to shift attention to the square of a designated color (the target square) and discriminate the location of the missing corners. In a key non-task-related manipulation, smoking-related and smoking-unrelated images were embedded in the target and distractor squares, such that the shift of spatial attention to the target (reflected by the hallmark attention-related N2pc component) could be examined as a function of the addiction-related image being present in either the target or distractor, present in both, or absent from both. We observed that when the smoking-related image was present in

(versus absent from) the target, participants shifted attention less strongly to the target, as indicated by a decreased amplitude of the N2pc. Such an effect suggests that participants were actively suppressing the smoking-related images by directing attention away from them. Importantly, this effect was not modulated by craving. Craving did, however, influence the earlier P1 index of early extrastriate visual processing at 100 ms, with a higher P1 amplitude when participants were craving versus when they were not craving, likely reflecting a higher level of general arousal. Together, these results provide an electrophysiological dissociation between addiction-related stimulus processing and the more general arousal observed in a state of craving.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: DFG SFB 779 TPA1

Title: Smoking-related images capture the attention of heavy smokers outside of awareness: EEG and MEG evidence

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Abstract: Reward-associated visual stimuli capture attention, which can be either beneficial or detrimental depending on their relevance to the task at hand. It is well-established that such capture can occur at the level of reward-associated features, such as colors, but recent evidence suggests that this can also occur at the level of object categories. This phenomenon of attentional capture by reward-associated objects may play a central role in substance abuse, although the specific role and neural underpinnings of this effect in the context of addiction are not well understood. The present study investigated the automaticity of attentional capture by smoking-related visual targets in heavy smokers who were in a craving versus a sated state. Simultaneous

MEG and EEG measures of brain activity were taken during an object-substitution masking (OSM) task. In OSM, an array consisting of a number of distracter items, plus a single target appearing in an unpredictable location surrounded by a four-dot target-designating cue, is briefly presented. In half of the trials, the four-dot cue surrounding the target remains on the screen for a short period of time following the offset of the rest of the target/distracter array, which greatly reduces the visibility of the target. In the present study, these targets could be either smoking-related or office-supply-related images. The event-related N2pc MEG/EEG component, a hallmark index of spatial attentional shifts to a visual target, was extracted and tracked across masking conditions as a function of object category, as well as craving state. An enhanced-amplitude N2pc was observed in response to the smoking-related stimuli for all craving and masking conditions, with the exception of unmasked trials within the sated session. These results indicate that enhanced attentional capture by addiction-related images is largely automatic and can occur outside of conscious awareness. However, the results further suggest that this automatic capture of attention can be mitigated under conditions of clear target visibility and substance-specific satiety.

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Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Topic: F.01. Human Cognition and Behavior

Title: The control of attention is altered in the absence of subjective awareness

Authors: *T. WEBB, M. S. A. GRAZIANO;
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Abstract: We proposed that awareness is a crucial part of the control mechanism for attention. Many studies have shown that people can pay attention to a stimulus without being aware of it, suggesting that awareness is not needed for attention. However, awareness may still have effects on attention. We hypothesized that the presence or absence of awareness will change the control of attention. To test the hypothesis, we directly compared attention with and without awareness in human subjects performing a Posner task. Metacontrast masking of a visual stimulus was applied such that subjects reported being aware of the stimulus in one condition and unaware of the stimulus in another condition. The stimulus was used as the cue in the Posner task, allowing

us to measure the amount of attention drawn by the stimulus. Attention was measured at five time points during the first 500 ms after stimulus presentation. We found that attention was drawn to the stimulus regardless of whether or not people were subjectively aware of it. However, attention was significantly different in the aware and the unaware conditions. It was not simply the case that awareness was associated with more attention. At one time point, subjects showed significantly more attention to the stimulus when they were unaware of it, whereas at another time point subjects showed significantly less attention to the stimulus when they were unaware of it. The timecourse of attention was changed by the presence or absence of awareness. These differences show that awareness is not an epiphenomenon and that it plays some role in the control of attention.

Disclosures: T. Webb: None. **M.S.A. Graziano:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Topic: F.01. Human Cognition and Behavior

Support: NWO VIDI grant

NWO Research talent grant

Title: Transcranial direct current stimulation modulates the attentional blink

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Abstract: Our senses are continuously bombarded with more information than the brain can possibly process all the way up to the level of awareness. Hence, selection mechanisms that dynamically gate only goal-relevant information into working memory for further processing and conscious representation are critical for successful goal-directed behavior. In study 1, we examined whether this selection process can be facilitated by transcranial direct current stimulation (tDCS) over left dorsolateral prefrontal cortex (lDLPFC) - a region that plays a key role in working memory and conscious access. Specifically, we examined the effects of tDCS on the size of the attentional blink (AB): a deficit in identifying the second of two target stimuli presented in close temporal proximity in a rapid stream of distractor stimuli. tDCS works by creating a low, constant current between two surface electrodes, and can either increase or

decrease neuronal excitability depending on the kind of stimulation applied (anodal vs. cathodal). 34 subjects performed a standard AB task before (baseline), during and after 1 mA anodal and cathodal tDCS in two separate sessions. We hypothesized that anodal, but not cathodal tDCS over lDLPFC would reduce the magnitude of the AB, possibly as a function of individual baseline AB size. Indeed, while the effects of tDCS were not apparent at the group level, anodal tDCS decreased the AB in individuals with a large baseline AB, but increased the AB in individuals with a small baseline AB. This effect was only observed during (but not after) stimulation, was not found for cathodal tDCS, and could not be explained by regression to the mean. These findings support the idea that lDLPFC plays a critical role in the AB and in conscious access more generally. They are also in line with the notion that there is an optimal level of prefrontal activity for cognitive functioning, with both too little and too much activity hurting performance. In a follow-up combined tDCS-EEG study (study 2), we are currently examining the neural mechanisms underlying the observed anodal tDCS-related changes in the attentional blink. Results from this study will also be presented.

Disclosures: **L.C. Reteig:** None. **R.E. London:** None. **H.A. Slagter:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: DFG, Grant 779 (A03)

Title: Task-irrelevant novel sounds activate the orienting network of attention and improve performance in children and adolescents with and without ADHD

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Abstract: Introduction: Increased distractibility is one of the main symptoms in children with attention deficit hyperactivity disorder (ADHD) and could result from an increased orienting response towards irrelevant stimuli. However, there has been evidence that task-irrelevant novel sounds can have a beneficial effect on cognitive performance in ADHD (van Mourik, 2007). We

aimed to investigate the underlying neural mechanism of attentional modulation by task-irrelevant novel sounds in youth with and without ADHD. **Methods:** 46 children (50% ADHD, age: 11 to 16) executed a visual flanker task during functional magnetic resonance imaging (fMRI, 3T). Two thirds of the task displays were preceded by an auditory stimulus (environmental sound), half of them by a repeated standard sound and half by unique novel sounds. Analyses comprised the investigation of sound modulations on mean reaction times and error rates in the flanker task as well as activation differences between the sound conditions and groups. **Results:** We found a beneficial effect of novel sounds compared to the no sound baseline on reaction times and error rates in both groups. Moreover, novel sounds induced increased activation in the bilateral superior and middle temporal gyri as well as the right inferior frontal gyrus when compared to standard sounds and the no sound baseline. This pattern was comparable for typically developing children and children with ADHD. **Conclusion:** Novel sounds particularly activated the orienting/reorienting network of attention (Fan, 2005) which is engaged in attention orienting to sensory information and attention shifting. As this activation was accompanied by improved performance in an ongoing task, it shows the underlying correlate of the alerting benefits of novel sounds (SanMiguel, 2010). Furthermore, this mechanism seems to be intact in children and adolescents with ADHD indicating that they can benefit from external stimulation under certain conditions despite the disorder specific increased distractibility. **References:** Van Mourik, R. (2007). When distraction is not distracting: A behavioral and ERP study on distraction in ADHD, *Clinical Neurophysiology*, 118**(**8), 1855-1865. Fan, J. (2005). The activation of attentional networks, *NeuroImage*, 26(2), 471-479. SanMiguel, I. (2010). Attention capture by novel sounds: Distraction versus facilitation, *European Journal of Cognitive Psychology*, 22(4), 481-515.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NNRHA 5154/PEP1012-11

Title: ERP Indices of proactive and reactive attentional control in adult ADHD

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Abstract: Attention Deficit Hyperactivity Disorder (ADHD) is a prevalent early-onset, neurodevelopmental disorder characterized by inattention, hyperactivity, and impulsivity that is behaviorally maladaptive. The core symptoms persist into adulthood in a substantial number of individuals but may change over time in that difficulties with dynamic attentional control are commonly more pronounced than behavioral impulsivity in adulthood. The objective of this study was to examine whether electrophysiological markers of proactive and reactive attentional control are altered in adult ADHD. We studied event-related potential (ERP) indices of preparatory attention (Cue-evoked P3 and contingent negative variation [CNV]) and reactive response control (P3 Go, N2 NoGo and P3 NoGo) derived from a visual cued Go/NoGo task performed by treatment-naïve adults with newly diagnosed ADHD (n = 33) and a group of age-, sex- and education matched healthy controls (n = 31). The behavioral performance data showed that the ADHD group had significantly more omission errors to Go signals than controls (p = .037), but the groups did not differ in reaction time, reaction time variability, or in NoGo commission errors. The electrophysiological data revealed that ADHD patients did not differ from controls in the Cue P3 or CNV amplitudes. Furthermore, the ADHD patients were indistinguishable from controls in N2 NoGo amplitude. Adult ADHD patients had diminished P3 amplitudes to Go (p = .009) as well as NoGo (p = .035) signals at the central midline location. These ERP results indicate normal preparatory processes (Cue P3 and CNV), and conflict monitoring (N2 NoGo) in adult ADHD patients, but deficient allocation of attentional resources to targets and non-targets as revealed by the attenuated P3 Go and NoGo waves. The electrophysiological results highlight specific information processing deficits in the unmedicated adult ADHD population.

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grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NNRHA 5154/PEP1012-11.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.10/X20

Topic: F.01. Human Cognition and Behavior

Support: FT130101488

Title: Abnormalities in the direction of attention in space in ADHD are underpinned by weaker right-hemisphere orienting responses- an electrophysiological analysis

Authors: *M. A. BELLGROVE[1], D. P. NEWMAN[1], G. LOUGHNANE[2], S. P. KELLY[3], R. G. O'CONNELL[2];
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Abstract: Background: An abnormality in the direction of attention in space is an intriguing aspect of the cognitive profile associated with attention deficit hyperactivity disorder (ADHD). Neurologically healthy individuals tend to more efficiently process stimuli presented in the left, compared to right, hemi-field. In ADHD however this advantage is lost or even reversed such that attention is less effectively directed to the left hemi-field. This abnormality is reminiscent of hemi-spatial neglect and implies weaker right hemisphere function in ADHD. Methods: We used electroencephalography (EEG) to investigate the neural processes underpinning the direction of attention in space in ADHD. EEG was recorded from 22 children diagnosed with ADHD and from 29 typically developing control children while they performed a visuospatial attention task. Results: As expected, typically developing controls displayed a significant reaction time advantage for detecting left hemi-field targets, whereas this advantage was lost in ADHD. This difference in spatial bias was reflected in hemispheric asymmetry of the N2pc, an ERP marker of visuospatial attention orienting. Whereas the control children displayed right-hemisphere dominance of the N2pc, particularly for left hemi-field targets, this dominance was lost in the ADHD group. Further, a difference in the centro-parietal positivity (CPP), a marker of perceptual evidence accumulation, was observed between the groups, whereby controls displayed earlier CPP latency for left than right hemi-field targets, whereas the ADHD group did not. Discussion: These data confirm that ADHD children have an abnormality in the efficient direction of

attention to left space, the EEG correlate of which is a reduced right-hemisphere dominance for spatial orienting.

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Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Topic: F.01. Human Cognition and Behavior

Support: Klingenstein Third Generation Foundation

Title: Alpha desynchronization and fronto-parietal connectivity during spatial working memory deficits in ADHD: A simultaneous EEG-fMRI Study

Authors: ***A. LENARTOWICZ**, S. LU, E. LAU, J. T. MCCRACKEN, M. S. COHEN, S. K. LOO;
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Abstract: Spatial working memory (SWM) is reliably associated with deficits in attention-deficit hyperactivity disorder (ADHD), making it a potential gateway to understanding the neural sources of inattention symptoms. In prior research we have demonstrated that SWM performance, inattention symptoms and ADHD diagnosis are correlated with the degree of occipital, alpha (8-12Hz), event-related desynchronization (ERD) in electroencephalography (EEG) signals of neural activity during the encoding phase of SWM. In the current study we tested whether alpha ERD is correlated with the connectivity of right fronto-parietal network activity that is known to facilitate SWM. To do so, we tested 30 boys (Age=13.9, ADHD=15) on the Sternberg SWM task while concurrently measuring electrical scalp (EEG) and metabolic whole-brain (functional magnetic resonance imaging) measures of brain activity. We used single-trial alpha desynchronization during encoding and a psycho-physiological interaction between this measure and activity in fronto-parietal regions of interest to identify the neural-activity correlates of alpha and the neural-network correlates of alpha modulation, respectively. The results replicate our prior finding of weaker alpha ERD during encoding in children with ADHD. In addition, greater alpha ERD was associated with increased activity within lateral occipital cortices, and enhanced connectivity between right occipital cortices and a fronto-parietal network, which suggests that alpha ERD is a correlate of interactions within an occipito-

parieto-frontal network. The strongest predictor of inattention symptoms and performance was connectivity within right superior occipital cortex, suggesting a target for further study of ADHD-related impairment. Finally, while our study demonstrates for the first time the feasibility of performing EEG-fMRI concurrently for the study of SWM in ADHD, we also found considerable artifact in the EEG data, especially in children with ADHD, which highlights possible limitations and pressing challenges in applying simultaneous EEG-fMRI in this population.

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Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Topic: F.01. Human Cognition and Behavior

Title: Methylphenidate regulates electrophysiological signals that reduce lapses of attention

Authors: *P. M. DOCKREE[1], M. BELLGROVE[2], R. ABE[2], J. BARNES[3], N. MATTHEWS[3], R. O'CONNELL[4];
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Abstract: Abstract The mechanisms by which methylphenidate (MPH) reduce episodes of inattention are not fully understood. However, electrophysiological markers that characterise error-prone states provide a means to examine the influence of MPH on dissociable neural signals that foreshadow lapses of attention. In a placebo-controlled, crossover design, we examined the performance of healthy adults (n=36) on a simple target detection paradigm. MPH improved task performance and suppressed α-band (7-11hz) activity across the task. Change in α-band power was also correlated with change in accuracy between MPH and placebo conditions. In the 4s period preceding targets, there was marked changes to attention-relevant signals in the MPH condition (α-band and θ-band suppression and P3 amplitude increases),

which predicted target detection. By contrast, there were no changes to early visual processes (visual P1 and 25Hz steady-state response) indicating that MPH exerts its influence primarily on higher-order endogenous mechanisms instead of facilitating bottom-up sensory processing. These data show the physiological basis by which MPH improves attention offering candidate markers for remediation in ADHD.

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Title: Effect of ADHD candidate risk SNPs on cortical thickness in typical children and young adults

Authors: *A. J. KRAFNICK[1], D. A. RINKER[2], T. T. BROWN[3], A. W. TOGA[1], P. M. THOMPSON[2], K. A. CLARK[1];
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Abstract: Attention deficit hyperactivity disorder (ADHD) is a complex behavioral neuropsychiatric disorder highlighted by inattentiveness, impulsiveness, and hyperactivity (APA, 2000). There is a strong genetic component with estimates of the additive genetic effects approaching 80% (Faraone et al., 2005). Many genes have been implicated in ADHD, with the most consistently identified genes being related to the catecholamine lifecycle (Akutagava-

Martins et al., 2013; Gizer et al., 2009). Here, using data from the Pediatric Imaging, Neurocognition, and Genetics (PING) study, we investigated how SNPs from ADHD candidate risk genes effect cortical thickness in a group of typically developing children and young adults. 409 subjects (average age 11 years, range 4-21 years; 200 female) from the PING database were processed with Freesurfer (Fischl, 2012) in order to estimate cortical thicknesses for each subject across the whole brain. We identified three SNPs consistently related to ADHD from genes related to the catecholamine lifecycle: rs27072 (dopamine active transporter 1, DAT1; risk allele G), rs4680 (catecholamine-o-methyltransferase, COMT; risk allele G), and rs6314 (serotonin receptor 2A, HTR2A; risk allele A). Based on subject genotypes we were able to compare two genotypes for DAT1 (n = 120 AG, 276 GG) and HTR2A (n = 60 AG, 346 GG), and three genotypes for COMT (n = 83 AA, 226 AG, 100 GG). Of the three SNPs, DAT1 and COMT survived corrected thresholds of $p<0.01$ (vertex) and $p<0.05$ (cluster). For DAT1 (rs27072), subjects with two copies of the risk allele (GG) showed reduced cortical thickness in left orbitofrontal cortex (OFC) compared to subjects with one copy of the risk allele (AG). For COMT (rs4680) there was a main effect of genotype in left lateral occipital cortex, with post-hoc t-tests showing this was due to less cortical thickness in subjects with two risk alleles (GG) compared to subjects with no risk alleles (AA). These results suggest unique contributions of these risk SNPs to variation in cortical thickness in a typically developing population. The left orbitofrontal cortex result for rs27072 (DAT1) is in line with a recent study of another DAT1 polymorphism that found variation in white matter volume here was related to both the polymorphism and executive function (Chung et al., 2015). This suggests that OFC may be of particular interest for investigating the interaction of genetics, executive function, and ADHD. The COMT SNP is more commonly investigated as it has been implicated in several disorders, and occipital cortex has been previously implicated. Future work will need to continue investigating these polymorphisms in multiple imaging modalities.

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Poster

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Support: NIH Grant MH086530

Title: The “good” choline transporter gene variant? Resilience against distractibility and depression

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Abstract: Forebrain cholinergic systems play a critical role in perception and cognition. While the contributions of cholinergic dysregulation to the severity of cognitive symptoms have been extensively demonstrated, long-standing hypotheses linking such dysregulation with depression remain less well supported. The high-affinity choline transporter is an essential regulatory step for the synthesis of acetylcholine (ACh) and the ability to sustain elevated levels of cholinergic activity. We have previously reported that a single nucleotide polymorphism (SNP) of the CHT gene (SLC5A7; rs1013940) is associated with increased behavioral distractibility on both laboratory and self-report measures, and with reduced right prefrontal brain activation in the face of distraction (Berry et al., 2014, 2015). Another CHT SNP (rs333229; G vs. T base pair substitution at the 3’ untranslated region; 3’UTR) was associated with differences in heart rate variability and corticolimbic reactivity (Neumann et al., 2005, 2006). On this basis GG homozygosity has been suggested as a risk factor for depression but to our knowledge no behavioral correlates have been reported thus far. We found that in individuals homozygous for the major allele at SLC5A7, those with a T allele at the 3’UTR site self-report less boredom in everyday life (Short Imaginal Processes Inventory, Huba et al., 1982) and better sleep (Pittsburgh Sleep Quality Index, 1989), as well as showing dramatically reduced distractibility on a laboratory task. These participants also show a moderate trend (d = 0.68) for lower scores on a self-report measure of depressive symptoms (Patient Health Questionnaire depression subscale, Spitzer et al., 1989). Although these results require replication, they provide the first evidence for behavioral differences associated with variation at the 3’UTR and further reinforce our previous findings that genetic variations of the CHT impact cognitive function and psychiatric risk. Furthermore, these findings suggest relationships between resilience against distraction and against depression.

Disclosures: Y. Isaacs: None. **Z. Lin:** None. **P.J. Deldin:** None. **R.D. Blakely:** None. **M. Sarter:** None. **C. Lustig:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.15/X25

Topic: F.01. Human Cognition and Behavior

Title: The effect of alertness on the detection of semantic incongruities and generation of the N400 event related potential

Authors: *E. A. SILVA, JR[1], S. MENESES ORTEGA[2];
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Abstract: Introduction: It is known that alertness and selective attention are strongly related. Therefore, variations in alertness influence the capacity of detection of relevant stimuli. It has been found that different cognitive processes are affected when alertness levels and attention decrease. Nevertheless, it is not yet clear with accuracy if changes in the alertness level, during waking, affect the recognition of semantic incongruities, despite being a complex cognitive process. The aim of this study was to determine whether the detection of incongruent stimuli, evaluated by recording the N400 component, is influenced by the alertness level during waking. Method: ERPs were recorded in three derivations (Fz, Cz, Pz) in 11 male subjects. Seven-word sentences visually presented were used. Each word of the sentence was presented individually. Each sentence can be classified in two ways: congruent, and strongly incongruent. Trials began with a black screen (2 seconds) followed by each word of the sentence. The words were presented for 100 milliseconds with an interstimulus interval of 2 seconds. At the end of each sentence participants had to press the left button to indicate that the sentence was congruent or the right button for the incongruent sentences. In order to determine the level of arousal we analyzed the power spectrum of the brain electrical activity one second prior to the presentation of each congruent or incongruent stimuli. The segments were classified according the type of stimuli (congruent - incongruent) and the alertness (high - low alertness). The averages for each condition were obtained. Difference waves were created by subtract the ERP waveform elicited by the incongruent stimuli from the ERP waveform elicited by the congruent stimuli. A two-way repeated-measures ANOVA (alertness level x derivations) were used to analyze the amplitude and distribution of the N400 component. Results: A significant main effect for alertness was found ($F(1,50)=4.19$; $p<0.05$), with a smaller amplitude when the alertness was low. No significant differences in amplitude were found for Derivations ($F(2,50)=0.71$; $p>0.05$), nor for interaction ($F(2,50)=0.12$; $p>0.05$). Conclusions: The results show that the N400 amplitude is reduced when alertness decreases, which suggest that efficiency in the processing of semantic inconsistencies is reduced with decreasing alertness. Furthermore, no differences on the N400 amplitude were found between derivations in each alert condition, there is no interaction; this could indicate that the circuit underlying the processing related to the generation of N400 are affected equally under reduced alertness.

Disclosures: **E.A. Silva:** None. **S. Meneses Ortega:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

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Program#/Poster#: 172.16/X26

Topic: F.01. Human Cognition and Behavior

Title: Influence of gender role on brain lateralization and error detection during a semantic reasoning task

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Abstract: 42 undergraduates took part in a study investigating the relationship between brain lateralization, gender role, and reasoning. Brain lateralization was measured using a newly developed semantic retrieval task where the names of objects were simultaneously projected to the left, center, and right visual fields (2 words per field). Prior to the task, participants were invited to name each of the 90 objects used in the study. The task was composed of 15 blocks of 6 objects to retrieve. To control for eye gaze, we first verified that the two objects named in the middle of the visual field were remembered, then the number of retrieved objects from the right (left hemisphere projection) or left (right hemisphere projection) visual fields were scored. Gender role was determined using the Bem sex-role inventory, and participants were classified into one of four groups: feminine, masculine, androgynous, or undifferentiated. Participants performed the Remote Associate Task (RAT): finding a target word related to three stimulus word (i.e. Cottage, Swiss, Cake: Cheese). The RAT was coded for correct answers and errors (failure to recognize that the selected word does not work for every stimulus word). Right hemisphere recruitment (Number of objects retrieved from the left visual field/total number of correct answers) was computed and an analysis of variance performed: A significant difference was found among the four groups ($F=3.23$, $p<.05$). Post hoc analyses (LSD) revealed that the androgynous group recruited significantly less information from the right hemisphere than the male and the undifferentiated group. The androgynous group also demonstrated a fewer errors during the RAT. T-tests revealed that the androgynous group had significantly fewer errors than the feminine group ($t=-2.77$, $p<.05$), and nearly significantly fewer errors than the masculine group ($t=-1.85$, $p=.08$. The difference from the undifferentiated group ($t=-1.56$, $p=.13$) was close to one-tail significance. These results suggest that the androgynous gender role is associated with less right hemisphere recruitment. In addition, despite the small sample size, the reasoning data gave us some promising venue to explore. The androgynous group produced significantly fewer

errors during the RAT than the female group; and almost significantly fewer errors than the two other groups. The right prefrontal cortex is often associated with error detection; yet our results suggest that less lateralization toward the right hemisphere is associated with fewer errors in semantic reasoning. Future studies, with larger samples, which replicate our findings is necessary to confirm our findings/interpretation.

Disclosures: J. Quillien: None. **R.L. Lloyd:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.17/X27

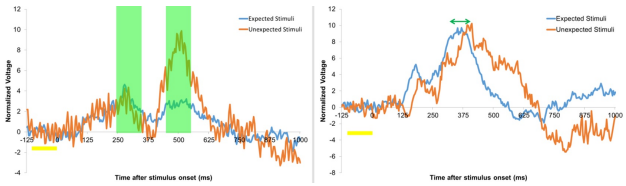
Topic: F.01. Human Cognition and Behavior

Title: Event-related potentials analysis of mental arithmetic processing caused by numerical versus symbolic stimulation

Authors: M. J. FRONDORF, *A. W. CHIU, M. THAKKER, W.-W. JEONG;
Biololgy and Biomed. Engin., Rose-Hulman Inst. of Technol., Terre Haute, IN

Abstract: Early, and late waves exhibited in event-related potentials (ERPs) at around 0-300ms, and 300-500ms after the presentation of questions are associated with mental arithmetic processing (MAP). Unlike traditional P300 oddball experiments where subjects observe visual stimuli passively; the purpose of this research was to determine the difference in the VEP when the subject was presented with an unexpected stimulus while actively visualizing and imaging a particular visual cue. Here we focused our efforts in the evaluation of human subject responses to the presentation of unexpected solutions to mental arithmetic problems in the form of incorrect numerical values or inconsistent form of representation to aid in the study of attention and cognitive functions. Right handed, male subjects with healthy (or corrected) eye sight, between the ages of 18-55 were recruited for this study. In each trial, the subjects were exposed to an arithmetic equation with the answer absent. Each subject underwent 360 trials (80% correct, and 20% incorrect solution). There was a small increase in the amplitude of the averaged VEP 300ms after the onset of unexpected stimulus when the subjects were presented with simple X's and O's, with a much stronger increase in the averaged VEP amplitude (over four times as large as that of the expected stimulus) 500ms after the stimulus onset ($p<<0.01$). When presented with errors in mathematical solutions, the averaged VEP showed a longer latency without any difference in amplitude. For the Mental Arithmetic study, there was no significant increase in the amplitude of the averaged VEP between 250-400ms after the onset of unexpected stimulus. However, there

was a statistically significant delay (~80ms) in the positive peak in the averaged VEP amplitude near 375ms after stimulus onset. The longer latency suggests the possibility of a longer processing time when subjects realized that the answers were not what they had in mind. This research may offer alternative ways to evaluate cognitive function and attention in a quantitative manner.



Disclosures: **M.J. Frondorf:** None. **A.W. Chiu:** None. **M. Thakker:** None. **W. Jeong:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

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Program#/Poster#: 172.18/X28

Topic: F.01. Human Cognition and Behavior

Title: The role of visuospatial attentional mechanisms during retention of information in visual short-term memory

Authors: ***M. E. VISSERS**[1], R. GULBINAITE[2], B. P. BRAMSON[1], T. VAN DEN BOS[1], H. A. SLAGTER[1];
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Abstract: There is ample evidence showing that attentional control plays an important role during encoding of information in visual short-term memory (VSTM). Yet, at present it remains unclear to what extent the allocation of spatial attention plays a functional role in preserving the content of visual short-term memory after initial encoding. In the current study, we investigated the allocation of visuospatial attention during VSTM retention. Using EEG and frequency tagging of individual stimulus positions, we measured attentional allocation to each stimulus position during VSTM retention, as reflected by amplitude modulations of the SSVEP. In the first experiment we manipulated memory load to differentiate between parallel vs. sequential attention to stimulus positions, and the degree to which this is dependent of memory load. In the second experiment we manipulated the presence of irrelevant stimuli, in order to study attention to locations previously containing relevant vs. irrelevant information. First findings suggest that

attention to relevant stimulus positions occurs in a sequential fashion, suggesting attentional looping over stimulus positions during VSTM retention. Furthermore, positions previously containing relevant stimuli are attended more than positions containing irrelevant stimuli. Using time-frequency decomposition of the EEG-data, we relate the extent of attentional modulation during VSTM retention to activity in the brain networks involved (i.e., inter-electrode α-phase synchronization). Together, our findings provide novel evidence for the importance of visuospatial attention for VSTM, and show that visuospatial attention may serve to preserve memories during VSTM retention.

Disclosures: **M.E. Vissers:** None. **R. Gulbinaite:** None. **B.P. Bramson:** None. **T. van den Bos:** None. **H.A. Slagter:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Program#/Poster#: 172.19/X29

Topic: F.01. Human Cognition and Behavior

Title: Working memory allocation reflects task demands during prospective remembering

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Abstract: Prospective memory can be achieved with proactive control by actively maintaining the goal in working memory or with reactive control by relying on external cues to retrieve the goal when necessary. The flexible choice of strategy involves effort and performance tradeoffs. Prior work has used multivariate pattern analysis of fMRI data in a dual-task prospective memory experiment to profile the allocation of working memory resources underlying task performance. Behavioral metrics indicated that both proactive and reactive control strategies were used for prospective remembering. However, neural readouts of working memory usage indicated a bias towards the more effortful proactive control strategy: goal-related processing during the delay period was predictive of prospective memory performance at the end of the trial. The strength and predictiveness of these readouts were sensitive to changes in task demands, such that goal-related working memory was strongest and most predictive of performance on trials with both (a) lower ongoing task demands and (b) higher prospective memory demands. Yet, the unique influence of ongoing task demands remains unclear. We developed a modified version of this experiment to assess how the dynamic selection of control strategy varies with the attentional demands of the ongoing task. We recorded whole-brain fMRI data every second and

used multivariate pattern analysis to decode the contents of working memory throughout the trials. Each trial began with a picture target (a face or scene), followed by a variable-length sequence of 2-sec memory probes containing four pictures and a field of moving dots. Participants balanced two objectives: 1) to make repeated direction-of-motion judgments about the dots (ongoing task); and 2) to identify when the picture target reappeared (prospective memory task).The motion coherence of the dots in the ongoing task was parametrically varied across trials. As the coherence of the dots increased, the attentional demands of the ongoing task decreased. We hypothesized that a reduction in cognitive load would lead to an increase in proactive control, thus stronger and more predictive goal-related working memory readouts. Pilot results indicate that prospective memory performance remained stable, yet the use of proactive control increased as the ongoing task became easier. This was reflected both behaviorally (slower responses in the ongoing task) and neurally (stronger and more behaviorally predictive readouts of goal-relevant working memory processing). These results highlight that prospective memory control is quite flexible and sensitive to changes in concurrent cognitive demands.

Disclosures: **A. Mukerji:** None. **J.A. Lewis-Peacock:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Topic: F.01. Human Cognition and Behavior

Support: NMRC StaR 0004/2008

Title: EEG spectral changes during involuntary eyelid closures in resting-state fMRI studies

Authors: *J. L. ONG, C. WANG, K.-K. NG, J. ZHOU, M. W. L. CHEE;
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Abstract: Inadvertent sleep has been shown to occur in a third of subjects studied in task-free fMRI studies, affecting downstream data interpretation (Tagliazucchi and Laufs 2014). EEG remains the gold standard for measuring sleep but simultaneous EEG-fMRI is resource intensive. Spontaneous eyelid closures in fatigued persons correspond to periods of diminished responsiveness to auditory stimuli and reduced functional brain connectivity. Here we investigated EEG features associated with spontaneous eyelid closures in drowsy persons, to consolidate the proposition that monitoring the latter is an excellent alternative to simultaneous EEG-fMRI for task-free fMRI studies. 17 undergraduates underwent two 6-minute task-free

fMRI scans in darkness after a 5h night of sleep. Simultaneous eye video and 64-channel EEG was collected. Eye videos were scored in 4s video epochs from 1-9 using a semi-automated algorithm and collapsed into 3 Eyescore (ES) conditions (ES 1-3: fully closed; ES 4-6: semi opened; ES 7-9: fully opened). Gradient, pulse, eye and muscle artifact removal were performed before re-referencing to the average of both mastoids. The EEG data was then segmented into 4s epochs. Epochs contaminated by heavy movement were removed from further analysis. Subjects were instructed to stay awake and to keep their eyes open. However subjects frequently dipped into microsleeps. EEG power collected during varying degrees of eyelid closure at Cz and O1 is shown in Fig 1. Epochs with lower ESs (greater eyelid closures) were associated with higher delta and theta power but lower beta power. Alpha power increased from ES 1-3 to ES 4-6, but decreased in ES 7-9. These results are in line with prior behavioral-EEG correlations. Even during resting-state studies where no measure of performance is directly observable, periods of decreased vigilance can be obtained by observing eyelid closures. These findings underscore the value of monitoring eyelid closures in order to exclude confounding periods that may contaminate true task-free or baseline data.

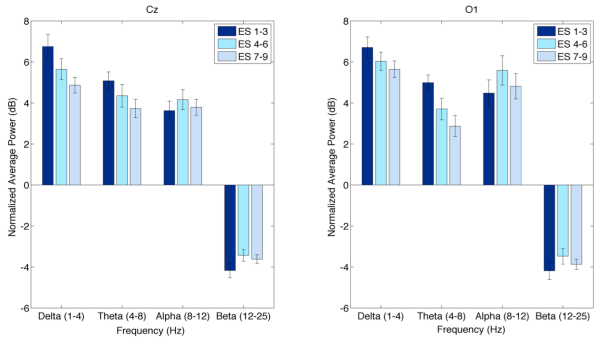

Figure 1 Average normalized power at electrode Cz and O1 by frequency band in 4s-epochs grouped into three eyescore (ES) conditions (ES 1-3: fully closed; ES 4-6: semi opened; ES 7-9: fully opened). Power was normalized by subtracting the mean log spectrum (1-25 Hz) in the ES 7-9 condition. Significant differences (p's < 0.05) were present in the delta (Cz only) and theta bands (Cz and O1), where lower eye scores were associated with greater delta and theta power. The converse was found for the beta band (Cz and O1) where higher eye scores were associated with greater beta power. In the alpha band however, alpha power significantly increased from ES 1-3 to ES 4-6 and decreased again in ES 7-9 (O1 only).

Disclosures: **J.L. Ong:** None. **C. Wang:** None. **K. Ng:** None. **J. Zhou:** None. **M.W.L. Chee:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.21/X31

Topic: F.01. Human Cognition and Behavior

Title: Examination of the effect of light environment on brain activity during visual search task by fNIRS

Authors: *H. TANAKA, T. HIROYASU;
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Abstract: [Purpose] In this study, examination of light environment to improve intelligent productivity and comfort is conducted. It is reported that light environment effects on working efficiency and psychological states of office workers. However, working efficiency and psychological states are processed in the brain and we investigated the effect of light environment on brain activity. Brain activities were measured by functional near-infrared spectroscopy (ETG-7100; Hitachi Medical Corporation). We have constructed the special experimental environment whose light color can be changed. Because of this environment, more channels of fNIRS can be used compared to the former study. [Methods] Under each type of light, cerebral blood flow (CBF) changes in the frontal lobe (22ch) were measured. Task performance was measured on reaction time (RT) without errors. The reaction was assumed as error when subjects made mistakes and could not react while image stimuli were to be submitted. Subjects were divided into two groups by an average of RT in all of the light environment: high performance group (high-group; 6 subjects) and low performance group (low-group; 6 subjects). [Results] In task performance, t-test was conducted and there was significant difference between two groups in all of the light environment (Red: $p<.05$, White: $p<.01$, Blue: $p<.01$). Brain activity by CBF changes during the task was visually confirmed. In White, CBF changes in high-group became constantly more extensively after CBF increased. In Red, CBF changes in high-group increased slowly. On the other hand, the tendency of CBF changes in low-group to increase was observed from the middle of the task team. In Blue, CBF changes in both groups did not increase extensively. [Discussions and Conclusions] In task performance, RT in White was the fastest in both groups. By examination of CBF changes, CBF in many channels became constant after they increased in white. We consider that part where CBF change increase is active. Therefore, it was found that the continual activation of frontal lobe effects on task performance in white. Under different light environment, CBF changes were examined. These results indicated that different light environment effected on brain activity.

Disclosures: **H. Tanaka:** None. **T. Hiroyasu:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Program#/Poster#: 172.22/X32

Topic: F.01. Human Cognition and Behavior

Support: Air Force Office of Scientific Research Grant FA9550-10-1-0385

Title: Transcranial direct current stimulation (tDCS) affects cognitive performance in only a subset of individuals - inter-individual differences in tDCS responsiveness during complex cognitive training

Authors: *M. R. SCHELDRUP[1], P. DWIVEDY[1], R. MCKINLEY[2], P. GREENWOOD[1]; [1]George Mason Univ., Fairfax, VA; [2]Air Force Res. Lab., 711 Human Performance Wing, Dayton, OH

Abstract: Complex multi-tasks that make simultaneous demands on several cognitive components are required during many real-world operations. It was previously argued that efficient performance of multi-tasks requires coordination of attention between subtasks (Kramer et al., 2005, Strobach et al., 2014). The most effective way to facilitate that coordination has not yet been established. Transcranial direct current stimulation (tDCS) has been used to facilitate single task performance (reviewed in Coffman et al. 2014), but only recently used to facilitate multi-task acquisition (Scheldrup et al., 2014). We hypothesized that in a multitask with verbal and spatial working memory (WM) subtasks, tDCS over left dorsolateral prefrontal cortex (DLPFC) would facilitate verbal WM while tDCS over right DLPFC would facilitate spatial WM. We tested this in a multi-task that simulates aircraft carrier operations - Warship Commander (WSC). Participants were randomly assigned to receive sham, anodal, or cathodal stimulation to either F4 or F3 of the 10-20 EEG system (corresponding with right and left DLPFC). Results indicate that both anodal and cathodal stimulation affected performance but only for a subset of participants. Our results are in concert with recent findings indicating the large role that inter-individual differences play in not only cognitive performance but in responsiveness to tDCS (Jaeggi et al., 2014, Krause and Cohen Kadosh; Lopez-Alonzo et al., 2014). Our results demonstrate the need for adequate behavioral as well as neurophysiological assessments of tDCS responsiveness within a given paradigm.

Disclosures: **M.R. Scheldrup:** None. **P. Dwivedy:** None. **R. McKinley:** None. **P. Greenwood:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.23/X33

Topic: F.01. Human Cognition and Behavior

Title: Only random alarm?! Situation and perspectives on civil aviation security

Authors: *J. K. KRÜGER, B. SUCHAN;
Ruhr-Universität Bochum, Bochum, Germany

Abstract: Examinations of performance of security screeners by the European Commission demonstrated striking results. Nearly every second bag containing a forbidden item (e.g. Improvised Explosive Device) was transferred through security check without any difficulties. Aviation security screeners are responsible for controlling carry-on luggage and customers, here screeners perform amongst others analyses of X-ray images to detect prohibited objects (6). X-ray screening includes the search for very rare and infrequent items (7). This work is performed under highly noisy circumstances with time pressure and high distractibility (2). Screeners have to be very concentrated and focused while visually scanning the image, recognize and categorize the shown items and have to decide quickly whether there are any suspicious objects (3). Attentional functions were assessed by a computerized neuropsychological test battery (TAP 8) in combination with parts of the WMS - III (1) and WMS-IV (4) and a computerized full version of the Mental Rotation Test (5). 50 security assistants working at various German airports were assessed. To assess circadian variability and the influence of shift-work, participants were tested before and/or after working shifts early in the morning as well as in the late evening. Performance of each subtest provided average or marginal average results of the participants for the above mentioned cognitive aspects. Results suggest no significant influence of shift duration, time in day, and time of testing (before versus after work). Contrary to this, were the results of subjective evaluation via visual analogue scales of concentration, motivation, and attention as well as the Stanford Sleepiness Scale. As expected, screeners declared an increase of sleepiness and a depression of the named mental factors with the passing of time. One could argue that the overall fatigue was so severely pronounced that small variations by the time of the day were not enough influential to additionally impact the cognitive ability in a significant manner. Despite modern and cutting-edge technology, it is always the human who makes the last decision whether a handbag contains a forbidden threating item.

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Disclosures: J.K. Krüger: None. **B. Suchan:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.24/X34

Topic: F.01. Human Cognition and Behavior

Support: DoD Defense Health Program Grant NF90UG

Title: A real-time objective assessment of cognitive workload during ambulation

Authors: *E. P. SHAW[1,2], J. C. RIETSCHEL[3], C. G. MCDONALD[4], M. W. MILLER[5], R. J. GENTILI[6,7,8], B. D. HATFIELD[6,7];
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Abstract: Safe ambulation within a real-world environment often requires the concurrent performance of secondary tasks (e.g. attending to oncoming traffic while crossing a street). Due to limited attentional resources available for cognitive processes, the manner in which such resources are allocated during ambulation is of much interest. In particular, individuals with lower limb amputations may require additional attentional resources to ambulate with their prosthetic limb. Here, we present a novel and objective approach for the assessment of cognitive workload during ambulation in healthy individuals, which will later serve as a baseline for comparison to individuals with unilateral lower limb amputation. In the ongoing study, EEG was recorded from 12 uninjured healthy males performing cognitive tasks of varying difficulty (easy and hard), while seated and while walking on a treadmill in a Computer Assisted Rehabilitation Environment at Walter Reed National Military Medical Center. Attentional reserve was assessed by evaluating the P3a component of the event-related potential, an index of the involuntary orienting of attention, elicited by intermittently presented task-irrelevant novel complex auditory stimuli. To quantify the P3a component, a temporal-spatial principal components analysis was performed. Factor scores were subjected to a 2 x 2 (Task [seated vs walking] x Difficulty [easy vs hard]) repeated-measures ANOVA, which revealed no significant main effects or interaction. These findings suggest ambulation does not impose additional cognitive workload in healthy individuals. However, individuals with lower limb amputation may require additional attentional resources during ambulation. Accordingly, we are initiating an investigation of cognitive workload among these individuals with the same pragmatic approach utilized for evaluating healthy controls, with a plan to collect data for 15 males with unilateral lower limb amputation. We predict ambulation will impose additional cognitive workload in these individuals. This increase in cognitive load is expected to be reflected by diminished attentional orienting, as indexed by the P3a component. The present study provides support for the utility of EEG as an objective real-time assessment of cognitive workload during ambulation within an ecologically valid environment. This work was supported by the DoD Defense Health Program (NF90UG) and the DoD-VA Extremity Trauma & Amputation Center of Excellence. Views expressed are those of the authors and do not reflect the official policy or position of the Departments of the Army, Navy, or Defense, or the U.S. Government.

Disclosures: **E.P. Shaw:** None. **J.C. Rietschel:** None. **C.G. McDonald:** None. **M.W. Miller:** None. **R.J. Gentili:** None. **B.D. Hatfield:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Topic: F.01. Human Cognition and Behavior

Support: ARL W911NF-10-D-02-0022

MOST 103-2221-E-009-149

MOST 103-2911-I-009-101

Title: Time pressure on brain inhibition for emergency driving

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Abstract: How to deal with the upcoming emergency situations is a key to avoid car accidents. Previous study used brain imaging to reveal that the efficiency of inhibition function is responsible for copying such situations. However, other factors, such as stress, on driving inhibition are still unknown. Hence, in this study, we aim to get an insight into brain activities of emergency management in stress conditions. To investigate driver's brain responses of inhibition function, a modified stop-signal driving task was implemented in a virtual-reality driving environment. The electroencephalography (EEG) was recorded from 16 subjects as they performed the experimental tasks under normal (without time pressure) and stress (with time pressure) conditions. Given a fixed road distance, each subject was instructed to arrive at the finishing line within a limited time under the stress condition. In signal processing, independent component analysis (ICA) and event-related spectral perturbation (ERSP) analysis were applied to investigate the spectral dynamics of independent brain processes. The behavioral results showed that the stop-signal reaction time (SSRT) was shorter under the stress condition than that under the normal condition. This result indicated that the stress could help to improve the efficiency of inhibition ability. The ERSP results showed that the augmentation of delta (1-3 Hz) and theta (4-7 Hz) powers in frontal and central areas are related to the inhibition mechanism. There is no statistically significant difference between two conditions. However, beta (13-30 Hz) and gamma (30-50 Hz) powers in frontal and central areas increased only in the stress condition. The beta and gamma powers of the central area under the stress condition were significantly higher than those under the normal condition. Because the gamma band is thought to reflect the top down modulation, the time pressure could possibly improve the driving inhibition efficiency by the proactive control which prepares to stop before the signal onset.

Disclosures: J. King: None. **C. Chuang:** None. **C. Lin:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.26/X36

Topic: F.01. Human Cognition and Behavior

Support: MRC Grant WMCR P36349

MRC Grant WMCR P39389

Title: Impaired memory and the cocktail party

Authors: *S. KAMOURIEH, R. LEECH, R. WISE;
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Abstract: Background 'Top-down' attention is required when listening to a speaker partially masked by background speech. The cortical systems for attention and cognitive control are affected early in the course of cortical neurodegenerative disease. This affects a patient's ability to register what a speaker has said when masked by background speech or noise in social situations. Objectives To investigate impairment in the functional anatomy of the fronto-parietal attentional systems in patients with memory impairment during auditory speech stream segregation (the "cocktail party" phenomenon), using functional magnetic resonance imaging (fMRI). To explore the modulation of top-down cognitive control regions by a central cholinesterase inhibitor. Design/Method 22 healthy volunteers and 31 patients with self reported and objective memory impairment were included in this fMRI study. T2*-weighted gradient echo planar images were collected on a 3T scanner using interleaved silent steady state (ISSS) imaging. The task involved attending to a female speaker, either in the absence or the presence of a masking male speaker, with or without spatial cues. The patient group were scanned twice, 6 weeks apart, with 18 patients started on Galantamine after the first scan. Results A repeated measures ANOVA on behavioural results between the healthy volunteers and patients identified a significant effect of group and condition but no group by condition effect. Univariate analysis identified activity in similar systems between the two groups, but the poorer performance in the patients was reflected by reduced activity in dorsolateral prefrontal cortex and dorsal anterior cingulate. Multivariate analysis identified reduced connectivity between the left superior temporal gyrus with the left fronto-parieto-temporal cortex and the cingulo-opercular network in the patients. The effect of Galantamine was to increase dorsal anterior cingulate cortex response. Conclusions A complaint of poor recent verbal memory is often due to impaired encoding but it also has its origins in poor registration. This is a consequence of impaired high-order systems affected by neurodegenerative diseases. An inability to register verbal information when listening in the presence of distracting background speech will contribute to complaints of 'impaired memory' and social isolation.

Disclosures: S. Kamourieh: None. **R. Leech:** None. **R. Wise:** None.

Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Topic: F.01. Human Cognition and Behavior

Support: Foundation grants from Hitachi Med Co (A. K.)

Brain Science Foundation (A.K.)

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Title: Near-Infrared Spectroscopy (NIRS) measurement for evaluating mild delirium in hepatic disease

Authors: *A. YOSHIMURA[1,4], M. M. TOWE[1], L. K. MAX[2], A. LAFLAM[2], J. JOHNS[5], D. H. EDWIN[1], M. LINDQUIST[5], C. W. HOGUE[2], A. GURAKAR[3], K. J. NEUFELD[1], A. KAMIYA[1];
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Abstract: Delirium, also known by some as “encephalopathy”, is a common and serious clinical syndrome that is associated with significant mortality and increases in healthcare resource utilization. Although early detection of delirium is crucial for improving patient outcomes, it is made difficult due to the lack of quick, easy screening tools. As a result, such syndromes often go undetected in hospital and clinical settings. In this study, we aim to reveal brain function in mildly delirious patients with liver disease by using near-infrared spectroscopy (NIRS), an optical topography system designed to measure the changes in concentration of oxy- and deoxy-hemoglobin in the cerebral cortex. Specifically, we examined these changes in the frontal and temporal areas of the brain during cortical stimulation using tasks for attention and executive function. We found a significant correlation between Hb integral values on NIRS during these tasks and participants’ values on the delirium rating score revised 1998 (DRS-R-98), a commonly used rating scale for measuring delirium. These findings suggest that the NIRS optical topography system might be a useful tool for evaluating and detecting mild delirium.

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Poster

172. Functional Mechanisms of Attention and Disorders of Attention

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 172.28/X38

Topic: F.01. Human Cognition and Behavior

Title: Disturbance of fast frontoparietal oscillatory activity and hemispheric dysbalance in delirium: evidence for a common pathophysiological framework from a large retrospective quantitative EEG study

Authors: *R. FLEISCHMANN, S. TRÄNKNER, M. RÖNNEFARTH, S. SCHMIDT, S. J. SCHREIBER, S. A. BRANDT;
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Abstract: Objective: Delirium is a common disorder that is associated with increased morbidity. The clinically heterogenous phenotype and lack of objective disease markers are major causes of misdiagnosis leading to considerably variable and potentially detrimental treatment decisions. A better understanding of pathophysiological mechanisms underlying this disorder are necessary to facilitate diagnosis and guide therapy. Methods: We searched the EEG database of a German university hospital between years 2004 and 2014 for recordings of patients diagnosed with

delirium (DSM-V criteria) and age-matched controls (n = 129 and 414, respectively). Normalized total and frequency band specific power spectra of continuous EEG data were compared between conditions (i.e. control vs. delirium) and topographically by ANOVA. Results: There was a significant interaction of CONDITION x FREQUENCY x SENSOR ($F(120,540) = 9.1$, $p < 0.001$) and CONDITION x HEMIPSHERE ($F(2,541) = 26$, $p < 0.001$). Post-hoc analyses revealed that total power was significantly increased centrally and decreased in the right hemisphere in the delirium group ($p < 0.01$). Furthermore, right frontal and parietal fast oscillations were significantly changed (increased gamma and decrease beta power, respectively; $p < 0.01$). Conclusions: This retrospective study yielded specific EEG patterns in patients with delirium that provide correlates of typical behavioral changes and concepts for underlying pathophysiological mechanisms. Spontaneous frontal gamma increase is well described to interfere with working memory and linked to a breakdown of local inhibitory interneuron circuitry. Similar mechanisms possibly account for changes in parietal beta activity since it is inversely correlated with input at gamma frequencies with consequences for sensory perception and integration. Although loss of inhibition provides an appealing pathophysiological model to elaborate on diagnosis and therapy of delirium, current data require confirmation by prospective studies including standardized diagnostic criteria and behavioral tests. Additionally, source reconstruction and neurophysiological characterization of affected networks are required.

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Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Title: Transient left spatial neglect as unique clinical expression of status epilepticus

Authors: *L. VERONELLI, S. BOVO, P. DE GIAMPAULIS, I. PASSARO, M. CORBO; Dept. of Neurorehabilitation Sci., Casa Di Cura Del Policlinico, Milano, Italy

Abstract: Unilateral spatial neglect (USN) is defined as a failure to report, respond, or orient to stimuli presented on the side of space contralateral to a cerebral lesion. It has been widely documented after brain damage to right fronto-temporo-parietal networks, and to lesser degree, to left ones. Seizures and status epilepticus causing USN syndromes are rarely diagnosed. We report the case of BG, a right-handed 76-year-old man, affected by hypertension and diabetes

without previous history of epilepsy, who was hospitalized for post-traumatic intracranial hemorrhagic lesions as a consequence of cardiogenic syncope, due to complete atrio-ventricular block. Anticonvulsant treatment was started as prophylaxis. To treat the atrio-ventricular conduction disease, the patient received definitive pacemaker implantation. At admission to our clinic three weeks after the traumatic event, the neurological examination was normal, excluding slight right hemiparesis. A brain computed tomography (CT) showed the presence of bilateral intraparenchymal hemorrhagic lesions, including right fronto-temporal areas, and a right parieto-occipital subdural hematoma. A comprehensive neuropsychological evaluation detected slight cognitive deficits affecting executive, memory and non-lateralized attentive functions, in absence of signs of USN. Three weeks later, BG presented with acute severe left USN, both clinically and formally assessed through a deep neuropsychological battery. In particular, he showed rightward line bisection error, a large number of left omissions on cancellation and drawing tasks, USN dyslexia, and personal USN. A control CT ruled out the possibility of a new brain lesion. The EEG showed abundant and subcontinous spikes and sharp-waves prevailing in the right temporo-parietal derivations, while the USN was the only clinical correlate (non-convulsive status epilepticus). After adaptation of the anticonvulsant treatment, EEG exams depicted a gradual resolution of the electrical abnormalities and the neuropsychological assessment confirmed the reduction of USN. In conclusion, transient USN was shown to be present as unique clinical expression of non-convulsive epilepsy and resolved after adequate anticonvulsant treatment. This observation provides further evidence that epileptic discharges, due to specific cerebral lesions, could selectively affect cognitive functions, even in terms of attention disorder as USN.

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Poster

172. Functional Mechanisms of Attention and Disorders of Attention

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Topic: F.01. Human Cognition and Behavior

Support: Mission Connect, a program of TIRR Foundation

NIH P30-EY010608

Title: Detection of subtle cognitive changes in mTBI using a novel tablet-based task

Authors: *T. D. FISCHER[1], S. D. RED[1], A. Z. CHUANG[2], E. B. JONES[3], J. J. MCCARTHY[3], A. B. SERENO[1];
[1]Neurobio. and Anat., [2]Ophthalmology and Visual Sci., [3]Emergency Med., Univ. of Texas Med. Sch. Houston, Houston, TX

Abstract: This study examined the potential for novel tablet-based tasks, modeled after eye tracking tasks, to detect subtle sensorimotor and cognitive deficits after mild traumatic brain injury (mTBI). Specifically, we examined whether performance on these tablet-based tasks (Pro-point and Anti-point) was able to correctly categorize concussed versus non-concussed participants compared to performance on other standardized tests for concussion. Patients admitted to the emergency department with mTBI were tested on the Pro-point and Anti-point tasks, a current standard cognitive screening test, the Standard Assessment of Concussion (SAC), and another eye movement-based tablet test, the King-Devick® (KD). Within hours after injury, mTBI patients showed significant slowing in response times compared to both orthopedic and age-matched control groups in the Pro-point task, demonstrating deficits in sensorimotor function. mTBI patients also showed significant slowing compared to both control groups on the Anti-point task, even when controlling for sensorimotor slowing, indicating deficits in cognitive function. Performance on the SAC test revealed similar deficits of cognitive function in the mTBI group compared to the age-matched control group; however, the KD test showed no evidence of cognitive slowing in mTBI patients compared to either control group. Furthermore, measuring the sensitivity and specificity of these tasks to accurately predict mTBI with ROC analysis indicated that the novel pointing measures reached good to excellent levels of accuracy, and fared better than current standardized tools for assessment of concussion. Our findings suggest that these rapid tablet-based tasks are able to reliably detect and measure functional impairment in cognitive and sensorimotor control within hours after mTBI. These tasks may provide a more sensitive diagnostic measure for functional deficits that could prove key to earlier detection of concussion, evaluation of interventions, or even prediction of persistent symptoms.

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Poster

173. Language I

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 2R25GM078441

NIH Grant R03-DC011304

Title: Effects of glottal source modulation on speech perception and production

Authors: *T. SALAZAR, J. BRUMBERG;
Univ. of Kansas, Lawrence, KS

Abstract: The ability to differentiate between self-generated sensory inputs and exogenous sensory stimuli is essential for accurately perceiving the world around us. One of the acoustic characteristics of speech, pitch, is the result of rapid vibration (fundamental frequency) of the vocal folds in the presence of airflow through the larynx during phonation. These vibrations also give rise to harmonic frequencies that provide the spectral basis for acoustic resonance and harmonic structure. Pitch, intensity and timbre (which includes harmonics) are important for separating self-generated speech from other external acoustic information. In auditory processing, the P300 auditory event related potential (ERP) can be evoked through an unexpected deviant associated with a shift/orientation in attention. The neural mechanisms of pitch and intensity perception have been extensively researched using ERPs, but the significance of higher frequency sound qualities such as timbre on speech perception have not been explored. In the present study, the harmonic structure of speech is systematically varied while maintaining equal fundamental frequency. Native English speakers with normal hearing are recorded using electroencephalography (EEG) while they engage in a target recognition task with five modulated glottal source conditions: natural voice, synthesized glottal source, sawtooth, square, whisper. We expect that the P300 ERP amplitudes will be more pronounced when the target stimulus is the natural voice or whisper due to their salience and predict that the synthesized, square and sawtooth conditions will elicit reduced P300 ERPs due to their similarities in sound quality. These results, combined with prior evidence in pitch perception, will provide a comprehensive characterization of the importance of source signal qualities on speech perception and production.

Disclosures: **T. Salazar:** None. **J. Brumberg:** None.

Poster

173. Language I

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Support: Lundbeck Foundation, DK

Erasmus, EU

Title: First-pass neocortical processing of language takes only 30 msec: optimising eletrophysiological techniques for capturing transient early brain responses to words

Authors: *Y. Y. SHTYROV, M. LENZEN;
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Abstract: The nervous system has developed through evolution to ensure the timely processing of incoming information and appropriate reaction to external stimulation; the speed of this processing is vital for biological survival in the constantly changing and volatile environment. From low-level studies of structural and functional connectivity, it can be inferred that information arriving at the auditory sensory input can in theory be transferred, through a series of relay stations, to high-level associative cortices (including Wernicke and Broca's language areas) within 30-60 ms. Yet, it is commonly accepted in cognitive neuroscience that language, the vital messaging tool used by humans for information transfer, is not processed by the brain until hundreds of milliseconds after the signal arrives at the sensory input. Here, we show that this is a misconception caused, among other things, by the use of analysis techniques inappropriate for registering early transient responses generated by the human neocortex to spoken language. Whereas cognitive EEG and MEG evoked responses are usually analysed with a low-pass filter of 20-40 Hz, earlier brain responses tend to be in a much higher frequency range; for example, P1, the first marked cortical neurophysiological deflection caused by auditory stimuli, requires a high-pass filter of >10 and low-pass of >70 Hz to be reliably registered. To test the putative ultra-early linguistic activity, we recorded EEG responses to a set of meaningful words and acoustically and psycholinguistically matched meaningless word-like stimuli ('pseudowords'). We used the oddball lexical stimulation paradigm, an established experimental design known to elicit (in a later time range) robust EEG indices of neural access to linguistic memory traces (e.g. Shtyrov et al, 2010 J Neurosci). The data were then subjected to a P1-optimised analysis: bandpass-filtered at 10-100 Hz, with artifacts corrected using ICA. We found that all stimuli elicited P1 responses, which peaked on average just 30 ms after the stimulus recognition points and showed a significant amplitude increase for real words as opposed to matched pseudowords, a response enhancement which likely reflects activation of long-term memory circuits for meaningful words. The results suggest that the previously reported neurolexical effects at 100-500 ms reflect secondary and later processes, which are preceded by a first-pass lexical access that takes place in the neocortex already well within 50 ms and can only be captured with appropriate data analysis techniques.

Disclosures: Y.Y. Shtyrov: None. **M. Lenzen:** None.

Poster

173. Language I

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Topic: F.01. Human Cognition and Behavior

Support: MRC Grant MR/J004146/1

Rosters Trust A445

Title: Using principle component analysis to investigate lesion correlates of naming errors post-stroke

Authors: *A. HALAI[1,2], A. WOOLLAMS[2], M. LAMBON RALPH[2];
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Abstract: Aphasia classification generally includes deficits in speech fluency (effortful or effortless speech), comprehension (verbal/non-verbal), repetition (word/pseudoword) and the types of errors made during these tasks. Two major classes of errors are routinely investigated; semantic errors relating to the meaning and phonological errors relating to sounds. Lesion mapping studies post-stroke have identified damage to left anterior temporal lobe (ATL) and precentral gyrus (preCG) results in increased semantic and phonological errors, respectively. However, such studies rarely investigate the whole range of naming errors and do not account for the multi-dimensional deficits observed in post-stroke aphasia, such as phonological, semantic and executive-demand impairments. We used a novel combination of principle component analysis (PCA) and voxel based correlation methodology (VBCM) to identify unique lesion sites for naming errors and general language impairments. We coded naming errors during two picture naming tasks (Boston Naming Test and Cambridge Naming Test), performed by 40 chronic left hemisphere stroke cases with aphasia. Errors were categorised into twelve types; semantic, phonemic, neologism, formal, mixed, initial phoneme, dysfluency, circumlocution, not a correct or incorrect, omission and other. Furthermore, we obtained detailed neuropsychological data which included receptive and expressive tests of phonology and semantics and measures of executive-demand. We confirmed previous results by identifying damage to ATL and preCG correlated with semantic and phonological errors. The PCA on a wide range of naming errors produced semantic and phonological error factors, but included three additional factors: 1) circumlocutions/other, 2) dysfluency, and 3) neologisms. Circumlocutions/other errors were correlated to damage to ATL, while dysfluency correlated with the insula. An omnibus PCA including all assessments produced 6 unique factors. Preserved phonological skill results in less phonological and neologism errors, however semantic skill was independent to semantic errors,

which loaded with speech variety. Semantic skill was related to ATL damage, while semantic errors were related to posterior temporal and inferior parietal damage. In addition, factors were identified for working phonological memory, speech quanta and executive-demand. The working phonological memory was related to middle and superior temporal gyrus damage, while speech quanta related with preCG and insula damage. In conclusion, PCA and VBCM can be used in conjunction to investigate the neural substrates of large-scale behavioural data.

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Poster

173. Language I

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Topic: F.01. Human Cognition and Behavior

Support: IARPA

Title: Behavioral and neural indices predict different aspects of language learning ability

Authors: ***A. S. FINN**, J. MINAS, S. S. GHOSH, M. LUESSI, C. GOETZ, J. D. E. GABRIELI; MIT, Cambridge, MA

Abstract: We asked whether brain measures improve, above and beyond behavioral measures, the identification of adults who excel in language learning. An additional goal was to understand more precisely which behavioral and neural characteristics were most predictive of learning success, either independently or in combination. 42 native English speakers were taught a novel miniature artificial language in the lab. Prior to learning, participants completed a large battery of cognitive and aptitude measures and completed an fMRI scan while performing three separate tasks and during rest. The artificial language (MAL) was learned somewhat naturalistically over the course of 4 days; participants observed 360 narrated scenes on a computer and repeated the narrations while trying to learn the language, which was comprised of 30 nouns, 4 verbs, 2 determiners, followed subject-object-verb word order, and had regular determiner-word pairings and subject-verb agreement. After each learning session, participants were tested for knowledge of all aspects of the language. Tasks during scanning measured neural recruitment during working memory, skill learning and language processing. Data show that behavioral measures are quite successful at predicting which learners will fall above or below median performance on the measures of grammatical learning, but not successful in predicting which learners will fall above or below median performance on a measure of semantic processing (the meaning and

content of the language). The opposite was true of the brain data, which predicted semantic processing but not grammatical learning. Thus, brain measures can enhance the identification of adult language learners who are more likely to succeed in processing the semantics of a newly learned language. This experiment also provides insight on which behavioral and neural characteristics are most predictive. For behavior, extant aptitude tests, IQ tests, working memory and open-loop (feedback based) forms of skill learning were predictive. Neural regions that were especially sensitive to working memory and sequence learning during scanning were also most strongly related to learning outcomes. In all, this experiment provides the first demonstration that brain measure can help identify successful learning and contributes to our understanding about which aspects of cognitive and neural architecture contribute to learning success.

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Poster

173. Language I

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Topic: F.01. Human Cognition and Behavior

Support: Arizona State University

Title: Interaction of working memory, modality & sentence comprehension in Broca's area: an fMRI study

Authors: ***Y. YI**[1], A. DIAZ[1], C. HOULIHAN[1], L. C. BAXTER[2], G. HICKOK[3], C. ROGALSKY[1];
[1]Speech and Hearing Sci., Arizona State Univ., Tempe, AZ; [2]Barrow Neurolog. Inst. & St. Joseph's Hosp. and Med. Ctr., Phoenix, AZ; [3]Cognitive Sci., Univ. of California, Irvine, Irvine, CA

Abstract: Broca's area is a functionally and anatomically diverse region. The contributions of Broca's area to language comprehension, and sentence processing in particular, remain highly debated. There is however overwhelming evidence that inferior frontal regions are activated during the maintenance and manipulation of verbal information. The present study addresses the possible role of inferior frontal working memory resources in sentence comprehension and how reading versus listening to sentences may engage these regions differently. In a sparse sampling fMRI paradigm, 17 native English speaking, right-handed participants were presented sentences

with either canonical or noncanonical structure in two ways: auditorily (listening via headphones) and visually (reading the sentences with one word presented at a time). The canonical sentences were subject-relative (SR) sentences and the noncanonical sentences were object-relative (OR) sentences. In addition, participants performed a working memory task, with each block consisting of listening to three non-words and then rehearsing the non-words covertly for 10 seconds. Multiple regression analyses were used to identify brain regions modulated by sentence modality and sentence type. We then examined the response properties of these regions during the working memory task. Significant main effects and interactions for modality and sentence type were identified in Broca's area. For both reading and listening, both sentence types elicited activation in a large fronto-temporo-parietal network, including Broca's area. As expected, significantly greater activation for listening than reading was found in bilateral superior and middle temporal gyri, while reading elicited more occipital lobe activation. Notably, reading sentences also elicited more activation than listening to sentences in left inferior frontal (~BA 44) and middle frontal regions. The comparison of OR > SR in the listening condition did not identify any inferior frontal regions at the group level, but in the reading condition the OR > SR contrast identified a left inferior frontal cluster (~BA 45). The left IFG region identified by the visual OR> SR contrast exhibited sustained activation during the working memory task's rehearsal period. The inferior frontal regions more activated by reading than listening also exhibited sustained activation during rehearsal. These preliminary findings suggest that Broca's area is more sensitive to sentence structure in visual than auditory presentation, and the regions of Broca's area that are sensitive to sentence structure are also recruited during articulatory rehearsal.

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Poster

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Support: US Dept Veterans Affairs N1549-M

Title: Frontal lobe language pathways

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Abstract: The present study investigated structural connectivity of Broca's region, a ventral lateral prefrontal cortical area consisting of pars orbitalis (pOrb), pars triangularis (pT), pars opercularis (pO), and inferior precentral gyrus (infPCG) (Hagoort, 2005). To investigate this connectivity we collected structural MRI data (T1 and diffusion weighted images) from 18 right-handed healthy volunteers (8M, mean age=26.5 y.o). T1-weighted scans were used to delineate cortical regions of interest using an automated cortical parcellation procedure in FreeSurfer. This procedure was used to create six regions of interest: pOrb, pT, pO, infPCG, middle frontal gyrus (MFG), and superior frontal gyrus (SFG). White matter pathways connecting these regions were inferred using the Mixture of Wischarts method distributions (Jian & Vemuri, 2007a, 2007b) implemented in an in-house software package written in IDL. We inferred a total of twelve pathways connecting ventral prefrontal cortical areas with MFG and SFG: pO/pT/pOrb/infPCG_SFG pathways (4 pathways), pO/pT/pOrb/infPCG_MFG pathways (4 pathways), pO/pT/pOrb/infPCG -SFG+MFG pathways (4 pathways). After delineating these pathways in each participant individually, we created population maps for each of the pathways by registering them to the common template, MNI152. These population maps were used to examine the amount of spatial overlap across participants. Our results (Figures 1 and 2) showed an anterior to posterior connectivity gradient, where the most anterior aspects of Broca's region (pOrb and pT) project to anterior and middle portions of MFG and SFG, while more posterior Broca's region (pO and infPCG) connected with middle and posterior MFG and SFG. The population maps of these pathways showed a considerable amount of spatial agreement across participants (brighter colors, Figure 2), especially for pO and infPCG pathways. Our results also showed that pO shares the most extensive and robust connectivity with MFG and SFG compared to other areas within Broca's region (p-value<0.001). Taken together, these findings suggest that the cortical areas comprising the anterior to posterior extent of Broca's region are linked to the entirety of frontal convexity cortex by corresponding anterior to posterior arrays of overlapping white matter connections. Moreover, pO appears to be the most extensively connected portion of Broca's region, which may allow it to play a particularly important role in language processing. Our results provide further support for the concept that neural representations are population encoded and they suggest that most of frontal convexity cortex contributes to language function.

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Poster

173. Language I

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

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Title: The influence of age of acquisition in bilingual reading

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Abstract: Neuroimaging evidence has suggested the involvement of left lateralized ventral and dorsal networks in reading. Nevertheless, it is still under debate to what extent different factors can modulate the engagement of key regions within these routes, such as the ventral occipito-temporal cortex (vOT), inferior frontal gyrus (IFG) and superior temporal gyrus (STG). Theoretical accounts suggest that the left vOT is mostly involved in pre-lexical computation of visual word forms, while others consider that it is also involved in integrating visuospatial features with higher-level associations. The present fMRI study sought to investigate the functional dynamics of these networks, as a function of the age of acquisition of their second language or L2 (early, late), language used to read (L1, L2), task demands (perceptual, semantic) and stimuli (word, pseudoword, consonant strings). Thirty-six bilinguals with Spanish as their L1, who learned Basque as their L2, before age 3 (early bilinguals) or after age 6 (late bilinguals), participated. In the scanner, they performed two separate tasks during which they were asked to press a button when they saw a colored letter within a given string (perceptual-task) or when they saw an animal word (semantic-task). Region of interest analyses revealed that the vOT showed stronger activation for words in the L2 versus L1 and in the semantic versus perceptual task. Within the IFG, late bilinguals exhibited stronger pars triangularis activation during word reading in the semantic, versus the perceptual task, across languages relative to early bilinguals, who show this same effect in their L2 but not in their L1. In contrast, pars opercularis showed higher activation for pseudowords than words in the L1, but a stronger activation in the L2 similar across words and pseudowords. The same profile was present in the STG. Functional connectivity analysis revealed that, compared to late bilinguals, early bilinguals exhibited tighter coactivation among ventral regions during word reading in the semantic task, suggesting a more specialized recruitment of this network for semantic processing in this group across languages. Our results suggest that, whereas ventral pathway regions (vOT, pars triangularis) were more strongly recruited for semantic-related processes, regions within the

dorsal pathway (STG, pars opercularis) showed a more phonological pattern of activation. Importantly, functional connectivity analysis indicated that coupling between regions within the ventral network are modulated by task demands and age-of-acquisition of the L2.

Disclosures: **M. Oliver:** None. **M. Carreiras:** None. **P. Paz-Alonso:** None.

Poster

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Support: NICHD R01 HD059858-01A

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T32 NS 47987-8

Title: Bilingual cortical control of between- and within- language competition

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Abstract: Spoken language unfolds gradually over time. As a result, words with similar onsets receive partial activation (e.g., clock activates the phonologically-similar word cloud) and compete for selection during comprehension. In bilingual speakers, this competition can also emerge across languages (e.g., the English word clock activates the Spanish word clavo, meaning nail). Controlling cross-linguistic competition may be more effortful than resolving competition from within a single language, because the cross-linguistic competitor can raise the global activation level of the non-target language, providing an additional level of interference. The current study examined cortical responses to within- and between-language competition in bilinguals to determine how the two types of linguistic interference are controlled. Sixteen highly-proficient Spanish-English bilinguals completed a word recognition task in an fMRI scanner. In each trial, participants heard a spoken English or Spanish word and identified its matching picture in a four-object display using a four-choice handheld button box. In competitor trials, the name of another picture phonologically overlapped with the target, either in the same language (within-language competition) or in the non-target language (between-language competition). In unrelated trials, competitor pictures were replaced by pictures with non-overlapping names in either language. The effect of competition in each condition was assessed

by creating contrast images between Competitor and Unrelated trials. We then analyzed the effect of competition Type (Between-, Within- language) on the Competitor vs Unrelated contrasts (collapsed across languages). In bilateral middle frontal gyrus (MFG), superior frontal gyrus (SFG), and inferior frontal gyrus (IFG), we found greater activation when competition occurred across the bilinguals' two languages compared to when it occurred within the same language. This finding suggests that between-language competition is processed differently than competition within a language and requires more frontal resources to resolve. Greater activation for between- vs within-language competition in right MFG and SFG was also correlated with participants' performance in an offline test of non-linguistic inhibitory control ability (Simon task), suggesting an overlapping mechanism for bilinguals' control of linguistic and non-linguistic interference. These results suggest that bilinguals recruit cognitive resources differently depending on the source of interference, providing insight into how multiple languages interact during spoken language processing.

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Poster

173. Language I

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Topic: F.01. Human Cognition and Behavior

Support: NLM Grant T15LM007093

Title: Dynamics of brain networks during word reading

Authors: ***M. WHALEY**[1], C. KADIPASAOGLU[2], S. COX[1], N. TANDON[2];
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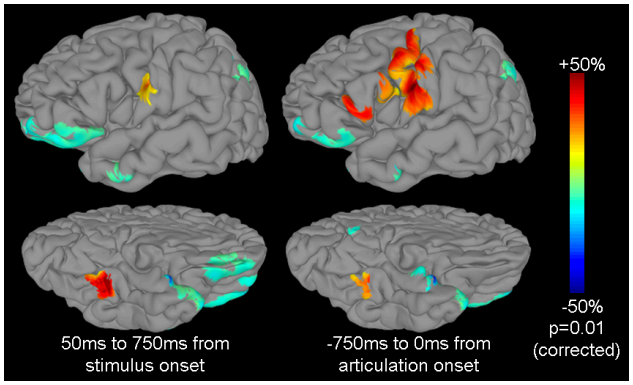
Abstract: We recorded electrocorticographic (ECoG) data from 15 patients with intractable epilepsy during a word completion task to precisely describe the spatiotemporal brain dynamics underlying word reading. Using a novel technique of analyzing grouped ECoG, cortical regions distributed throughout the left hemisphere were identified as significantly active versus baseline during our word stem completion task. Activity spread from fusiform to frontal regions, including pars opercularis, pars triangularis, and pre, post, and subcentral gyri during the time period approaching articulation onset (see figure). The ECoG data recorded from electrodes within these regions were fit into linear multivariate autoregressive models (MVAR), which precisely reveal the time, frequency, and magnitude of information flow between localized brain

regions. Grouped network dynamics were quantified by evaluating statistical significance of poststimulus interactions compared to baseline. Results reveal bidirectional exchanges between frontal regions with fusiform, supporting theories that incorporate top-down and bottom-up processing during single word reading. To our knowledge, this is the first application of MVAR for studying grouped connectivity of an ECoG dataset.



Disclosures: **M. Whaley:** None. **C. Kadipasaoglu:** None. **S. Cox:** None. **N. Tandon:** None.

Poster

173. Language I

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 173.10/Y2

Topic: F.01. Human Cognition and Behavior

Support: FCT Grant SFRH/BD/77908/2011

Title: How language shapes the brain: cross-linguistic differences in structural connectivity

Authors: ***T. GOUCHA**[1,2], A. ANWANDER[1], J. D. GRIFFITHS[3], L. K. TYLER[4], A. D. FRIEDERICI[1,2];
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Abstract: Language contributes to the architecture of the human brain. Whereas some of the perisylvian white matter pathways seem to be hard wired, other develop in parallel with language acquisition. However, studies on language-related neuroplasticity have focused on developmental aspects that are transversal to all languages, ignoring possible language

differences. For example, languages like German require online processing of abstract structural information (morphosyntax), anatomically supported by the dorsal arcuate fascicle, whereas languages like English more strongly engage lexical-semantic processing, which involves predominantly ventral fibre tracts. We therefore investigated how languages with different processing demands shape the language network. We compared diffusion MRI scans for three age, sex and education-matched groups with three different native languages: German, English and Mandarin Chinese. Anatomical regions of interest (ROIs) were defined in a template generated for this subject group, both in the inferior frontal gyrus and in the anterior and posterior superior and middle temporal gyri. Using probabilistic fibre tracking, we computed anterior-posterior, fronto-temporal and whole-brain connectivity of these ROIs and compared the respective connectivity strengths and maps of connection probability. We found higher dorsal fronto-temporal connectivity in the German group than in both the English and Chinese groups. Conversely, the English group showed higher ventral connectivity between the posterior temporal cortex and anterior frontal regions. In turn, the medium and short-range connectivity to the neighbouring temporal cortex and to the inferior parietal lobe was higher in Chinese speakers. These differences in connectivity indeed reflect the particular demands of the native language of the individuals as hypothesised. This study is a first indication that the wiring of language-relevant areas depends on the specific demands of each language. Further studies taking into account the genetic background and functional connectivity are needed to understand the implications of the study.

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Poster

173. Language I

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The Wyncote Foundation

Title: Longitudinal decline in speech in primary progressive aphasia

Authors: *S. ASH, C. YORK, T. BISBING, K. COHEN, F. MIN, E. MORAN, R. WILLIAMS, M. GROSSMAN;
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Abstract: Objective: To identify features of spontaneous speech for which progressive impairment is specific to distinct aphasic conditions Methods: We studied 31 patients, including 7 with nonfluent agrammatic primary progressive aphasia (naPPA), 12 with semantic variant PPA (svPPA), and 12 with non-aphasic behavioral variant frontotemporal dementia (bvFTD), and 20 age- and education-matched controls. All patients completed the task of describing the Cookie Theft picture from the BDAE (Goodglass & Kaplan, 1972) at least twice with an interval of at least 9 months. The Cookie Theft descriptions were recorded, transcribed, and analyzed for fluency, grammaticality, syntax, and adequacy of the report of content. Average disease duration at the time of the first recording was approximately 3 years. Results: naPPA patients were impaired relative to controls on all measures at the time of the first Cookie Theft recording. svPPA patients were impaired on measures of fluency, but not on grammaticality or the report of content. bvFTD patients exhibited reduced speech rate and impaired report of content but were not impaired on grammaticality. Patients in all groups were more impaired relative to controls at the time of retest (T2) than they were the first time they performed the task (T1). However, they differed on the performance measures for which they declined relative to their own performance at T1. naPPA patients declined significantly on measures of fluency (speech sound errors and speech rate), but not grammatical complexity. svPPA patients declined in the proportion of grammatically correct sentences they produced. Non-aphasic bvFTD patients declined on speech rate and measures of syntactic complexity. All three groups declined in the adequacy of their reports of the content of the Cookie Theft scene. Conclusions: Significant declines occur in spontaneous speech in both aphasic and nonaphasic patients with neurodegenerative disease. The areas in which the decline is most prominent are distinct in different patient groups. However, in all groups, the progression of disease reduces the patient's ability to perceive and describe the content of the Cookie Theft scene, thus progressively impeding communication at the global level.

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Poster

173. Language I

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant HD069162

Title: Microstructural white matter differences between 6-year old readers and pre-readers

Authors: J. N. ADAMS[1], V. N. KOVACHY[1], K. E. TRAVIS[1], M. BEN-SHACHAR[3], *H. M. FELDMAN[2];
[1]Pediatrics-Neonatology, Stanford Univ., Stanford, CA; [2]Stanford Univ., Palo Alto, CA; [3]English Literature and Linguistics, Bar-Ilan Univ., Ramat Gan, Israel

Abstract: Background: Recent studies reported white matter microstructural differences between individuals who learned to read as adults and those who remained illiterate. Reading is typically learned during early childhood when brain plasticity is pronounced. We set out to examine whether the ability to read in children is associated with differences in white matter microstructure. We hypothesized that compared to peers who had not yet learned to read, 6-year old readers would demonstrate differences in white matter properties within tracts implicated in decoding. **Methods:** We conducted diffusion MRI (dMRI) scans and behavioral tests of intelligence, expressive language, phonological awareness (PA) and reading skills in 33 children (mean age=6.2 years, 10 males). Readers (n=22) were defined as those with a standard score greater than the group mean (>110) on pseudoword reading. Children who read ≤1 word on the same task were defined as pre-readers (n=11). 30-direction dMRI and high resolution T1-weighted data were obtained on a 3T scanner. Six bilateral white matter tracts were identified using deterministic tractography: anterior Superior Longitudinal Fasciculus (aSLF), Inferior Longitudinal Fasciculus (ILF), Arcuate Fasciculus (Arc), Forceps Major (FMajor), Corticospinal tract (CST) and Uncinate Fasciculus (UF). We quantified fractional anisotropy (FA) values along the trajectory of each tract. Group differences in behavioral tests and tract FA were examined with independent samples t-tests. **Results:** Readers and pre-readers did not differ significantly on the basis of socioeconomic status, sex, second language exposure, stage in school, or non-verbal IQ. Readers performed significantly higher on tests of expressive language, PA, and verbal IQ ($p<0.05$). Readers demonstrated significantly higher FA than pre-readers within segments of the left ILF, left aSLF, FMajor, and right UF ($p<0.05$) and significantly lower FA than pre-readers within segments of the right Arc and left and right CST ($p<0.05$). Group differences observed within the left aSLF and right UF remained significant after correcting for multiple comparisons ($p<0.05$, corrected). **Conclusions:** 6-year old readers had both significantly higher and lower FA than pre-readers in several white matter tracts implicated in reading processes. Such group differences may reflect inherent differences in white matter microstructure or variability in

experiences with learning to read. Future longitudinal analyses in this sample will examine whether individual variations in FA observed during the early stages of learning to read are predictive of later reading abilities.

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Poster

173. Language I

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Program#/Poster#: 173.13/Y5

Topic: F.01. Human Cognition and Behavior

Title: Atypical visuospatial attentional orienting in dysfluent readers during classifying nonword stimuli

Authors: *O. H. LOBERG[1], J. HAUTALA[2], J. HAMALAINEN[1], P. H. T. LEPPANEN[1]; [1]Dept. of Psychology, [2]Agora Ctr., Univ. of Jyvaskyla, University of Jyvaskyla, Finland

Abstract: Recent research on eye-movements has implicated that Dysfluent readers have impaired attentional shift during lexical decision task (Hautala & Parviainen 2014). In modified version of the experiment, we presented to Fluent (N=15) and Dysfluent (N=9) adult readers words, nonwords, lines of symbols and gray rectangle while we registered eye-movements with eye-tracker and brain activation with brain event-related potentials (ERP). Participants were instructed to decide whether the stimulus on the screen was a word or not with a button press. Nonwords were generated by replacing the second or fifth letter within a six lettered word with a rare letter to cause violation to Finnish orthography. In this study we focus on the effects resulting from difference in spatial placement of this orthographical violation within a normal word and how Dysfluent readers differ from Fluent readers. Reaction times to nonwords with orthography violations in the end of the words were later in both groups and that the reaction times of Dysfluent readers were delayed for both stimulus types. Analysis of the first microsaccades(MS) during these stimuli revealed that the first MS tended to be oriented towards the violation, but that Dysfluent readers launched the first MS away from the violation in the word end to the left more often. Analysis of the ERP-components prior to the first MS onset revealed differences between the groups in anterior positive response at 180-240 ms (P2 at electrodes F3-F4) and posterior negative response at 190-240 ms (N2 at electrodes P7-P8). Fluent readers had larger anterior P2 amplitude for the violation in the beginning than for the one in the end of words but for Dysfluent readers this pattern was the opposite. The pattern for the

posterior N2 was very similar in Fluent readers showing stronger response for violation in the beginning, while Dysfluent readers showed again an opposite pattern. These results suggest that Dysfluent readers orient their attention differently to the beginnings and ends of words compared to Fluent readers as both anterior P2 and posterior N2 are linked to feature based attention in the earlier research. The results implicate that attention during word recognition is distributed differentially in Dysfluent readers which leads to atypical critical feature detection during word recognition, which in turn could lead to faulty orienting of microsaccades.

Disclosures: O.H. Loberg: None. **J. Hautala:** None. **J. Hamalainen:** None. **P.H.T. Leppanen:** None.

Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Support: Alexander von Humboldt Foundation

Takeda Science Foundation

Kanae Foundation for the Promotion of Medical Science

Title: Auditory-motor mapping and phonological processing in the left dorsal speech stream

Authors: *T. MURAKAMI[1,2], C. KELL[2], J. RESTLE[2], Y. UGAWA[1], U. ZIEMANN[3,2];
[1]Fukushima Med. Univ., Fukushima, Japan; [2]Goethe-University, Frankfurt am Main, Germany; [3]Eberhard-Karls-University, Tuebingen, Germany

Abstract: Introduction: Speech models propose an auditory-motor mapping via a left-hemispheric speech-processing stream, yet its detailed contributions to speech perception and production are unclear. Using fMRI-navigated continuous transcranial magnetic theta burst stimulation (cTBS), we virtually lesioned left speech stream components to investigate the issue of their functional contribution of speech processing. **Methods**: In total, 24 healthy right-handed native German subjects participated in this study. Inhibitory cTBS was applied over the posterior superior temporal sulcus (pSTS), the sylvian parieto-temporal region (SPT), pars opercularis of the inferior frontal gyrus (pIFG) and the dorsal premotor cortex (dPMC) consisting of the dorsal stream, and over the anterior superior temporal gyrus (aSTG) of the ventral stream, and an occipital control site. We measured speech-related facilitation of articulatory motor cortex (M1)

excitability, as indexed by increases in motor evoked potential (MEP) amplitude of a lip muscle, and performed speech processing performance tests. **Results**: Speech-related MEP facilitation was disrupted by cTBS of pSTS, SPT, and by double-knockout but not individual lesioning of pIFG and dPMC, and not by cTBS of aSTG or an occipital control site. Virtual lesions of the dorsal stream but not of the ventral stream or the occipital control site increased phonological errors. Disruption of aSTG led to enhancement of semantic errors but not of the dorsal stream or an occipital control site. Performance of syllable and pseudoword repetition correlated with speech-related MEP facilitation, and this relation was abolished with cTBS of pSTS, SPT and pIFG. **Conclusion**: Findings provide direct evidence that auditory-motor mapping in the left dorsal stream causes reliable and specific speech-related MEP facilitation in left articulatory M1. The left dorsal stream targets the articulatory M1 through pSTS and SPT constituting essential posterior input regions and parallel via frontal pathways through pIFG and dPMC, while the ventral stream mainly engages in semantic processing.

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Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Title: Expectation effects in syntactic processing - evidence from ambiguous sentence structures

Authors: *L. KROCZEK, T. C. GUNTER;
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Abstract: Sentence comprehension is a rapid process that takes place within milliseconds after a linguistic input is presented. Generally, it has been hypothesized that the brain enables such efficiency by means of predictive processing. In language comprehension, expectation effects have been demonstrated mainly for the semantic domain. However, processing the syntactic structure of a sentence ("who is doing what to whom") is a crucial part in sentence comprehension. Accordingly, top-down expectations could also play an important role with regards to syntactic structure processing. In the current EEG study a speaker's voice (male, female) was coupled to the expectancy for a particular syntactic structure. Thus, one speaker produced complex Object-Subject-Verb (OSV) sentences with a higher probability than easy Subject-Object-Verb (SOV) sentences (the O-speaker) and vice versa for the other speaker (the

S-speaker). Importantly, experimental sentences were ambiguous towards their syntactic structure up to the sentence final word. We hypothesized that speaker information would make the disambiguation easier. Preliminary analysis showed that participants were sensitive to a particular speaker identity as demonstrated by an increased positivity for the O-speaker compared to the S-speaker that was elicited at a time-point before the actual syntactic structure was disambiguated. ERPs time-locked to the disambiguating final word showed a main effect of structure, with complex OSV structures having a more positive waveform than easy SOV structures. Additionally, the probability of a structure also had an effect approximately 200 ms later in time. Sentences with a congruent speaker-structure pairing (S-speaker/SOV and O-speaker/OSV) showed a greater positivity than sentences with an incongruent speaker-structure pairing (S-speaker/OSV and O-speaker/OSV). These findings suggest that although the participants coupled the probability for a particular sentence structure to a particular speaker, this had no beneficial effect for syntax processing per se. Probably the ambiguity of the sentences led to these results.

Disclosures: **L. Kroczek:** None. **T.C. Gunter:** None.

Poster

174. Language II

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 174.03/Y8

Topic: F.01. Human Cognition and Behavior

Title: Processing meaningful and meaningless verbal material in different languages

Authors: *N. PLAKHOTNYK, S. TUKAIEV, I. ZYMA;
Educational and Scientific Ctr. “Institute of Biology”, Natl. Taras Shevchenko Univ. of Kyiv, Kiev, Ukraine

Abstract: The study of the emotional component of the perception of verbal information in human remains a considerable question as well as identification of possible universal and language-specific EEG characteristics. The purpose of this study was to evaluate phychophysiological changes during the exposure to information in different languages. We recorded EEG activity in 24 subjects, exposed to series of poetic passages in English and Ukrainian languages. The experimental material consisted of W.Shakespeare’s sonnets and L.Carrol’s Jabberwocky poem, which allowed to study phonological aspect of language regardless of lexical aspect as the poem consists mostly of pseudowords. After EEG registration, subjects were asked to evaluate each fragment on the scales "relaxing/activating" and

"distracting/concentrating". We analyzed spectral power (SP) of EEG during rest state and while listening to the poetry. α-rhythm's SP increased in parietal areas and decrease in θ-range while listening to meaningless and meaningful poetry in English and meaningless stimuli in Ukrainian language. This could be related to complexity of the verbal stimulus. β-rhythm's SP increased in the left temporal areas while listening to the meaningless Ukrainian and both English stimuli, which can be explained by the activation of language centers. The increase of β-rhythm's SP in the right hemisphere in response to the meaningful Ukrainian stimulus is consistent with the concept of linguistic stereotype formation that requires relatively fewer brain resources. In the subjects rating meaningful poetic passages as "activating", we observed a statistically significant higher θ and α SP, as compared to those rating the poetic passages as "relaxing". In the case of meaningless poetic passages, the SP of α and θ rhythms was higher in the group evaluating the poetic passages as "relaxing". This study proves that the perception of verbal information depends on the human emotional response, reflected upon the changes in the psychophysiological state of subjects.

Disclosures: **N. Plakhotnyk:** None. **S. Tukaiev:** None. **I. Zyma:** None.

Poster

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NSERC Grant 217309-2013

Title: Testing for transfer: does lexical training improve performance in a related but non-linguistic task?

Authors: S. VAN HEES, P. M. PEXMAN, I. S. HARGREAVES, L. ZDRAZILOVA, K. MYERS-STEWART, F. CORTESE, *A. B. PROTZNER;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Recently, the issue of whether task-specific training improves performance beyond the trained task has gained attention, likely due to the increasing popularity of brain training programs that claim to improve general cognitive function. However, studies investigating the validity of these claims suggest null or limited effects. It is possible that the lack of effects is a

consequence of the relatively small amount of time spent in training (days or weeks). We investigated training-related transfer in competitive Scrabble players, who dedicate years to training of Scrabble-related lexical skills and have shown enhanced performance in the lexical decision task. To test for transfer we developed a symbol-matching task, in which the letter strings of the lexical decision task were replaced with either a string of all unique non-letter symbols, or a string that included one non-letter symbol presented twice. Strings were presented in horizontal and vertical orientations, and yes/no decisions were required as to whether the string contained a pair of matching symbols. We collected behavioural and fMRI data from 12 Scrabble experts and 12 age matched controls. In terms of behaviour, we analyzed response times with a 2 (group) x 2 (orientation) ANOVA. We found no evidence of behavioural transfer, as Scrabble experts were no faster than controls at correctly identifying match/nomatch trials in either orientation. In terms of brain data, we used behavioural Partial Least Squares (PLS) analysis to examine group dependent differences in the neural networks that support symbol matching, and are associated with Scrabble skill. We used anagramming scores as our index of Scrabble skill, as these scores were correlated with official rankings among the Scrabble experts, and available for both Scrabble and control participants. We identified a common network across groups that correlated with anagramming scores, and included the left middle temporal gyrus and superior medial gyrus, as well as the right precentral gyrus and temporal pole. We also found group differences. Controls primarily engaged left hemisphere language regions (including the inferior frontal, middle temporal, and fusiform gyri) and right hemisphere homologues. In contrast, Scrabble experts engaged regions associated with visuo-spatial imagery, manipulation of information in working memory, and decision-making (including the precuneus, superior parietal lobule, and insula). Overall, the results suggest only domain-specific performance advantages for Scrabble experts, and the brain data have implications for understanding effects of cognitive training and neuro-rehabilitation.

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Poster

174. Language II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 174.05/Y10

Topic: F.01. Human Cognition and Behavior

Title: Can you see what I mean? Perceptual and conceptual semantics during words reading

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Abstract: One of the greatest intellectual abilities of the human kind is that of manipulating symbols by retrieving from long term memory the semantic representation that define what they stand for. Such semantic representations include both perceptual (i.e. prototypical size, shape) and conceptual (i.e. taxonomic category) properties. Do these different components of words meaning dissociate in the brain? Two competing cognitive theories support different predictions: the embodied cognition theory predicts that word meaning is resolved by the reactivation of perceptuosemantic properties are stored in primary sensory-motor cortices (Pulvermuller, 2013), while the abstract cognition theory predicts that word meaning is encoded in abstract format and represented in amodal cortices (Patterson et al., 2007). Behavioral, clinical, computational and neuroimaging investigations have provided indirect support to both theories, thus so far no conclusive results has been shown. To put to test the two theories, we acquired high resolution 3T fMRI images of adult subjects silently reading words referring to concrete entities. Words varied parametrically along four orthogonal dimensions: a purely physical dimension (i.e., the number of letters), a visuo-perceptual dimension (i.e., the average real-world size of the objects the words referred to), an audio-perceptual dimension (i.e., the strength of the association with a prototypical sound) and, finally, a conceptual dimension (i.e., the semantic category and the semantic cluster as derived from subjects' similarity ratings). By means of multivariate pattern analysis (Davis and Poldrack, 2013), we have isolated the contribution of the different dimensions of word meanings to the pattern of activation observed in different brain regions. We found that the visuo-perceptual dimension appears to be encoded in primary and secondary visual regions, while the audio-perceptual dimension in secondary auditory regions. This is in agreement with the embodied theory of cognition and can be hardly accommodated in a purely abstract theory of word meaning. However, conceptual dimensions such as the category or sub-categorical cluster appear to be encoded in anterior temporal regions, in agreement with the abstract theory of cognition, a result that cannot be explained by a purely embodied theory of word meaning. Thus, these findings reconcile contrasting theories of semantic representations as they indicate that both sensory and multimodal association areas play an important role by coding for specific and complementary dimensions of the semantic space.

Disclosures: V. Borghesani: None. **E. Eger:** None. **M. Buiatti:** None. **M. Piazza:** None.

Poster

174. Language II

Location: Hall A

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Program#/Poster#: 174.06/Y11

Topic: F.01. Human Cognition and Behavior

Title: Electrophysiological investigation of cross-language translation and morphological priming in different scripts

Authors: *W. CHUNG[1], S. KIM[2], M. PARK[1];
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Abstract: The Revised Hierarchical Model (Kroll & Stewart, 1994) assumes asymmetric lexical links between first language (L1) and second language (L2) (i.e., stronger links from L2 to L1 than those from L1 to L2). Previous behavioral studies supported the model showing significant masked priming effects when the target was L2 and the prime was L1, but not when the prime was L2 and the target was L1 (e.g., Jiang, 1999). However, recent ERP studies provided controversial evidence for either supporting (e.g., Hoshino et al., 2010) or countering (e.g., Midgley et al., 2009) the model. In addition, a previous study showed that cross-language morphological priming effect was found exclusively for cognate words in Spanish-English bilinguals (Duñabeitia et al., 2013). The current study examined if the pattern of cross-language translation priming is consistent with the asymmetric links between L1 and L2 and if it occurs via morphological decomposition, using event-related potentials (ERPs) and a masked priming lexical decision paradigm with unbalanced Korean-English bilinguals. In Experiment 1, targets were Korean (L1) compound word (e.g., 꿀벌, ″kkwupel,″ *honeybee*), and primes were English (L2) words, either 1) translated whole word (honeybee), 2) translated morphemic constituent (bee), or 3) an unrelated word (e.g., ear). Experiment 2 was the same as Experiment 1, except that the targets were in English (L2) and the primes were in Korean (L1). In behavioral results, the translation priming effect and the morphological priming effect were significant only for L1-L2 (Experiment 2), but not for L2-L1 (Experiment 1). In ERP results, the translation priming effect was found only for L1-L2 on the N150, N250, and reduced N400. The morphological priming effect was found both for L1-L2 and L2-L1 on the reduced N400. Taken together, the results suggest that both cross-language translation priming and morphological priming occurs even between different scripts (between noncognate words), and the effects are stronger when L1 primed L2 as compared to when L2 primed L1. In addition, different time-course between translation priming and morphological priming suggests that cross-language morphological decomposition occurs after translation in bilingual readers.

Disclosures: W. Chung: A. Employment/Salary (full or part-time):; Dongguk University. **S. Kim:** A. Employment/Salary (full or part-time):; Nanyang Technological University. **M. Park:** A. Employment/Salary (full or part-time):; Dongguk University.

Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Support: AG017586

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Title: Cognitive and anatomic double dissociation in the representation of concrete and abstract words

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Abstract: Here we examine the anatomic basis for representation of abstract and concrete concepts in semantic memory by assessing patients with focal neurodegenerative disease. Evidence from functional imaging studies in controls suggests that there may be an anatomical dissociation between abstract and concrete representations: abstract words more strongly activate left inferior frontal gyrus relative to concrete words, while concrete words more strongly activate left temporal regions than abstract words. We test this dissociation with two patient groups with atrophy specific to these regions, the behavioral variant of Frontotemporal Dementia (bvFTD) and the semantic variant of Primary Progressive Aphasia (svPPA). We administered an associativity judgment task for abstract and concrete words, where subjects select which of two words is best associated with a given target word. Both bvFTD and svPPA patients were significantly impaired for all words compared to controls. Further, controls treated concrete and abstract words equally, but we found a category-specific double dissociation in patients: svPPA patients showed reversal of the concreteness effect (CE), with significantly worse performance for concrete over abstract words, while bvFTD patients showed significantly worse performance for abstract compared to concrete words. We regressed the magnitude of the reversal of CE in svPPA and the CE in bvFTD, with regions of grey matter atrophy. Results demonstrated that reversal of CE is associated with left inferior temporal atrophy in svPPA, while the CE is associated with left inferior frontal atrophy in bvFTD. These results support a model of semantic

memory organization where abstract and concrete words representations are supported by partially dissociable neuroanatomic substrates.

Disclosures: K.A. Cousins: None. **M. Grossman:** None.

Poster

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Title: Functional network dynamics of the language system

Authors: *L. CHAI[1], M. MATTAR[2], I. BLANK[3], E. FEDORENKO[4], D. S. BASSETT[1]; [1]Dept. of Bioengineering, [2]Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA; [3]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; [4]Dept. of Psychiatry, Massachusetts Gen. Hosp., Boston, MA

Abstract: Functional Network Dynamics of the Language System Lucy Chai, Marcelo G. Mattar, Idan Asher Blank, Evelina Fedorenko, Danielle S. Bassett Key brain regions involved in processing language show gross similarity in functional responses to task paradigms and in activity profiles at rest. Conversely, they also display fine-scale dissimilarities that may underpin functional differentiation. However, a cohesive understanding of these functional similarities and dissimilarities has been hampered by the lack of quantitative assessment methods to track the dynamics of cognitive circuits during language processing. In recent promising advances, dynamic network methods have been used to divide large-scale networks into core and periphery regions, with the core regions displaying stable connectivity over time and the periphery regions displaying variable connectivity over time. Here, we apply these methods to the language network to test for the existence of the core-periphery structure and characterize it quantitatively if it is present. We define language-specific regions within healthy human subjects using a

functional localizer, and extract regional BOLD time series from functional magnetic resonance imaging (fMRI) data collected during two different language processing tasks: story comprehension and word/number judgment. We construct dynamic functional networks by computing the time-dependent functional connectivity between all pairs of brain regions in 40s time windows. We then employ dynamic community detection techniques to uncover time-evolving putative functional modules: groups of brain regions with similar BOLD time series. Our results reveal a robust two-module structure that divides the language network into a module composed of left hemisphere regions and a module composed of right hemisphere regions. We characterize the dynamics of this two-module structure with module allegiance, the probability that two regions engage in the same module over time. Via comparison to a null model, we uncover a rigid core composed mainly of left hemisphere regions, and flexible periphery composed mainly of right hemisphere regions. We speculate that these dynamic connectivity features support the specialization in language processing regions. These findings present initial insight into the dynamic activity and functional specificity of the language network.

Disclosures: **L. Chai:** None. **M. Mattar:** None. **I. Blank:** None. **E. Fedorenko:** None. **D.S. Bassett:** None.

Poster

174. Language II

Location: Hall A

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Program#/Poster#: 174.09/Y14

Topic: F.01. Human Cognition and Behavior

Title: Information-based connectivity - IBC: a functional connectivity approach using multivariate classification

Authors: *J. M. CORREIA, M. BONTE, L. HAUSFELD, B. M. JANSMA, G. VALENTE; Maastricht Univ., Maastricht, Netherlands

Abstract: Functional connectivity is essential to higher-order cognitive processes encompassing communication in highly connected brain networks. In fMRI signals, functional connectivity is typically investigated using measures of temporal similarities across brain regions. Beyond co-variation of activity, functional connectivity may be extended to include measures of information representation among activity patterns in distinct brain regions. MVPA (multivariate pattern analysis) permits single-trial predictions of experimental conditions based on multiple voxel responses. Combining functional connectivity with the benefits of MVPA is a current challenge in neuroimaging that promises to find information- instead of activation-based communication

between brain regions. Here, we propose a functional connectivity method that exploits MVPA - IBC (information-based connectivity). IBC investigates mutual information independent of activity correlations by assessing consistencies of single-trial predictions between regions. Using fMRI simulations we investigated inter-regional signal properties underlying the principles of IBC. The results showed that IBC is affected by information fluctuations across trials, but not by activity fluctuations. We first validated IBC using fMRI data from a previous MVPA speech perception experiment that highlighted the role of distributed brain regions in the perception of spoken syllables. The IBC method extended previous findings by highlighting communication within sub-networks of speech and language that may underlie transfer of information during speech perception, in particular between auditory, sensorimotor and motor regions. Secondly, we investigated the potential of IBC to find associations between information within simultaneously acquired fMRI and EEG recordings. Neuronal activity generates both synchronized electrical activity captured in high temporal resolution EEG recordings and hemodynamic changes captured by high spatial resolution fMRI recordings. Combining both modalities at the level of neuronal activity is however challenging due to the unknown coupling between these qualitatively different signals. Here, the IBC method was employed to seek associations, at the information level, between spatial fMRI clusters and temporal EEG intervals. IBC allowed us characterizing time-courses of regions of interest obtained by fMRI. Overall, IBC shows the ability to find modulation of information across single-trials, thus help characterizing mutual information within neural networks, such as between different regions of interest and different imaging modalities.

Disclosures: J.M. Correia: None. **M. Bonte:** None. **L. Hausfeld:** None. **B.M. Jansma:** None. **G. Valente:** None.

Poster

174. Language II

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Program#/Poster#: 174.10/Y15

Topic: F.01. Human Cognition and Behavior

Title: Cross-language associative priming in the bilingual brain

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Abstract: The neuronal organization of the mental lexicon in bilingual speakers is dependent on the age of acquisition of the second language. Hierarchical models propose a common conceptual store for both languages and separate lexical stores. Here we investigated whether the neuronal processing of nouns and verbs differs between the first (L1) and second language (L2) dependent on the age of acquisition. We used magnetoencephalography (MEG) to study large-scale neuronal processing in an associative priming paradigm within and across languages. Sixteen French-German participants with early acquisition of the second language (< 3 years) and sixteen French-German participants with late acquisition of the second language (> 7 years) were presented written prime-target word pairs in four language combinations (French-French, French-German, German-French, and German-German). Prime and target words were either associatively related or unrelated and were presented in close temporal proximity (SOA = 300 ms). Event-related fields revealed differences between early and late bilinguals in the priming task involving the superior parietal cortex and the cerebellum (250-450 ms), supporting the notion that the cerebellum is also involved in nonmotor language processes. An interaction between priming direction and language acquisition (early vs. late bilinguals) involved the left temporal cortex (middle and inferior temporal gyrus), the left inferior frontal cortex, the left fusiform gyrus, and the superior parietal cortex between 250 and 450 ms. In early bilinguals amplitudes were larger in the left inferior frontal gyrus and the left lateral temporal cortex especially when the prime word was presented in the non-dominant language. These results suggest that inhibition of the dominant language and/or language switching costs in early bilinguals were larger than in late bilinguals. Taken together, the results support the hierarchical models especially the conceptual mediation model in highly proficient speakers when control of the dominant language is required.

Disclosures: **T.R. Schneider:** None. **F. Isel:** None. **A.K. Engel:** None.

Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Support: Tamagawa Univ. Brain Sci. Inst., Machida, Tokyo, Japan

Tamagawa Univ. Engineering department, Tokyo, Japan

Title: Disparity between Language preference among different types of bilinguals and auditory attentional behavior toward languages with Event Related Potential (ERP)

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Abstract: Bilinguals frequently manipulate two languages in accordance with the circumstances, topics, and interlocutors. However, the definitions of bilingual vary depending on the ability of languages in relation to the knowledge of languages, frequency of use, and duration of exposure. Especially, the age of exposure to the second language is much more controversial due to the age partition. There is one concept of the early bilingual that no one can argue, which is called "Bilingual First Language Acquisition" (BFLA) (De Houwer, 2009). This type of bilingual is exposed to two languages since birth and both languages simultaneously take place in daily life. Consequently, there is no second language in chronological order. In this study, comparing different exposure time for Japanese and English, we investigated the language preference (10-point scaled score for subjective language favor of 3 categories; Conversation for listening & speaking, News for complicated listening, and Study for reading & writing) of 3 different subject groups (36 subjects-18 females & 18 males; mean age: 22; 12 subjects for each group): 1) Japanese monolingual with at least six years of English education from twelve years old (JM); 2) bilinguals, who have Japanese parents, are exposed to English after three years old and have continued bilingual education in Japan (LB); and 3) bilinguals, who have Japanese mother and American father, are exposed to Japanese and English since their birth (BFLA). All subjects were living in Japan at the time of this study. Auditory attentional behavior was also examined by ERP recorded at Fz, Cz and Pz. The result of language preference showed that all subject groups had significant differences between the languages: 1) JM: mean score English = 4.0 & Japanese=9.5, $p < .01$; 2) LB: mean score English=7.9 & Japanese=9.1, $p < .05$; and 3) BFLA: mean score English=8.9 & Japanese=6.8, $p < .01$. However, the ERP results of the attentional language behavior showed that JM and LB had a significant difference for N1 amplitude between two languages ($p<.01$). The significant differences of the amplitude comparison between groups were, JM vs. LB: English P1 $p < .01$; JM vs. BFLA: English P1 $p < .05$ & N1 $p < .05$, Japanese P1 $p < .05$; LB vs. BFLA: English N1 $p < .01$. ERP results showed that even though the English language favor of LB was higher than JM, they had similar N1 amplitude for English. Namely, auditory attentional behavior does not accord with subjective language preference for JM and LB. On the other hand, BFLA had very alike amplitude for both languages. This suggests that BFLA does not pay attention whichever the language comes.

Disclosures: C. Kameyama: None. **R. Saji:** None. **T. Omori:** None.

Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Support: MRC grant MR/J004146/1

Title: A flexible, adaptive bilateral neural system for semantic cognition: a combined cTBS/fMRI study

Authors: *J. JUNG, M. LAMBON RALPH;
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Abstract: Semantic cognition reflects a higher cognitive function that sustains semantically-derived behaviours. Although converging evidence implicates bilateral anterior temporal lobes (ATLs) as a crucial component in semantic cognition, the underlying neural interplay between ATLs remains unclear. In the current study, we investigated the bilateral ventral ATL (vATL) semantic system by implementing the combined cTBS/fMRI approach. First, we applied virtual lesions delivered by continuous theta-burst stimulation (cTBS) in healthy participants (10, 4 males, mean age, 22.8 ± 2.9yrs) to test whether cTBS over the left vATL induces similar inhibitory effect to 1Hz rTMS on semantic performance. Then, we applied a "perturb-and-measure" approach in the bilateral vATL system with healthy participants (25, 7 males, mean age, 21.9 ± 3.7yrs). In the behavioural experiment, we observed that compared to cTBS over the control site, the disturbance of the left vATL resulted in slowing effect on semantic processing selectively. In fMRI experiment, we observed that cTBS over the left vATL suppressed neural activity related to semantic processing at the target region and induced the up-regulation in the homologous right vATL as well as the other areas within semantic network. To test whether the up-regulation of the right vATL after a focal perturbation of the left ATL reflects an adaptive short-term plasticity, we used dynamic causal modelling (DCM). DCM analyses demonstrated that cTBS altered the intrinsic connection of bilateral vATL by increasing the facilitatory influence from the right vATL to the left vATL. Our findings support the hypothesis that the perturbation at the left vATL induced the compensatory up-regulation at the homologous right vATL, which feeds into increased facilitatory connectivity in order to adapt the disturbance in the semantic network. These findings imply that after a focal lesion the other brain regions within the task-related network take a more assertive role in its function particularly at the homologous, contralateral area. Thus, our data suggest a flexible, adaptive bilateral neural system for semantic cognition.

Disclosures: J. Jung: None. **M. Lambon Ralph:** None.

Poster

174. Language II

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National Sciences and Engineering Research Council of Canada (NSERC; grant #245327 to ES)

Title: School-aged children consolidate foreign language regularities overnight: behavioral evidence and cortical substrates

Authors: *A. NORA[1,2], L. KARVONEN[1,2], T. PARVIAINEN[3], H. RENVALL[1,2], E. SERVICE[4], R. SALMELIN[1,2];
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Abstract: Incidental learning of phonological structures through repeated exposure is an important component of foreign-language learning. Children seem to outdo adults as second-language learners, possibly due to a sensitive period. However, how children differ from adult learners on the neural level is not known. The present study addresses the neural correlates of incidental learning of new phonological forms and foreign language phonotactic regularities in school-aged children and compares the results to similar data collected from adults. Cortical dynamics during perception of novel words were tracked with magnetoencephalography (MEG) in 13 Finnish-speaking children, aged 6-7 years. Participants performed delayed repetition of four-syllable word forms that were presented either four times (80) or only once (80) during the first session, and again on the second day, along with new word forms. Comparison of novel phonological forms that adhered either to the native (Finnish) or to foreign (Korean) phonotactic system was included to explore whether the effects are related to acquisition of specific word forms (in both languages) or more general learning of the foreign language regularities. A separate behavioral experiment assessed repetition accuracy of foreign word forms over two days. Learning of the recurring words manifested as improved repetition both in children and adults. This item-level learning effect was seen cortically as reduced superior temporal activation for repeatedly encountered words at 600 - 1200 ms, in the left hemisphere in adults but in the right hemisphere in children. Notably, only children showed generalized learning of the foreign

language after overnight consolidation. This was seen as improved foreign-language repetition of new and recurring words while the sustained left temporal responses to foreign language stimuli began to resemble responses to native language on the second day. To conclude, children recruited markedly different cortical network populations than adults for learning specific word forms, and only children showed evidence of generalized learning of the foreign language regularities after a consolidation period. The differences in brain activation between age groups may reflect the neural underpinnings of a sensitive period in foreign language acquisition.

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Poster

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SPRITS from Kyoto University

Title: The timing of semantic coding in the anterior temporal lobe: temporal representational similarity analysis of electrocorticogram data

Authors: ***Y. CHEN**[1], A. SHIMOTAKE[2], R. MATSUMOTO[3], T. KUNIEDA[4], T. KIKUCHI[4], S. MIYAMOTO[4], H. FUKUYAMA[5], R. TAKAHASHI[2], A. IKEDA[3], M. A. LAMBON RALPH[1];
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Abstract: Electrocorticograms (ECoG) provide a unique opportunity to monitor neural activity directly at the cortical surface. Ten patients with subdural electrodes covering ventral and lateral

anterior temporal regions (ATL) performed a picture naming task. Temporal representational similarity analysis was used to compare spatio-temporal neural patterns from the ATL surface with pre-defined theoretical models. The results indicate that the neural activity in the ventral subregion of the ATL codes semantic representations from 250 ms after picture onset. The observed activation similarity was not related to the visual similarity of the pictures or the phonological similarity of their names. In keeping with convergent evidence for the importance of the ATL in semantic processing, these results provide the first direct evidence of semantic coding in the ventral ATL.

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Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Support: Grant-in-aid 15H01846

Title: The enhanced consolidation of foreign word learning by overt pronunciation

Authors: ***N. AOKI**[1,2], S. K. SUGAWARA[1], M. HIROTANI[3], S. OKAZAKI[1], H. YAMAZAKI-KINDAICHI[1,2], T. YOSHIMOTO[1,2], H. YOKOKAWA[4], H. YOSHIDA[5], N. SADATO[1,2];
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Abstract: Word learning is an essential part of learning foreign language (Service, 1992). Phonological rehearsal has been believed to be effective in word learning of foreign language (Seibert, 1927). Consolidation process, where encoded memory transforms into a more stable form (Diekelmann et al., 2009), is important for word knowledge maintained across long time scale. Sleep is known to consolidate foreign word learning (Gais & Born, 2006; Schreiner & Rasch, 2014). However, the role of the phonological rehearsal in the consolidation is not known. Here, we conducted the behavioral experiment to detect the effect of phonological rehearsal on the consolidation of word learning, by teasing out the sleep effect. Twenty-eight Japanese native-

speakers were randomly assigned into two experimental groups. A half of them participated the learning session in the evening and the retest session after 12 hours including sleep (Sleep group; n = 14). The others learned in the morning and retested after 12 hours of wakefulness (Wake group; n = 14). Participants learned 60 pairs of nonsense visual object and English monosyllabic auditory pseudo-word as a word with three experimental conditions: with overt repetition (Repeat), through just listening (Listen), and with numerical phonological suppression (Number). In the learning session, participants were asked to continue the learning task until their memory performance of each condition reached above 60%. Memory performance was measured by cued recall task. Group (Sleep and Wake)×Condition (Repeat, Listen, and Number) ANOVA showed no main effect of group nor condition immediately after the learning session. For the retention performance after 12 hours of interval, the main effects of Group and Condition were significant without their interaction effect. Post-hoc comparisons for Condition effect indicated that the words encoded with overt phonological rehearsal (Repeat) were better retained than other conditions (Listen and Number). Present data replicated the sleep consolidation of word learning (Gais & Born, 2006; Schreiner & Rasch, 2014). Independent of sleep, overt pronunciation during encoding enhanced memory retention. Word learning is the formation of audio-visual redintegration, the relationship between any constituent parts of a complex whole and the totality of the whole. A high conditional probability of recall of a ‘‘whole unit’’ given a part of the unit has been recalled is known as redintegrative recall (Tulving & Madigan, 1970). Thus phonological rehearsal conceivably enhances consolidation of a foreign word through adding motor component to the audio-visual redintegration.

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Poster

174. Language II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 174.16/Y21

Topic: F.01. Human Cognition and Behavior

Support: Grant-in-Aid for Scientific Research on Innovative Areas Grant-in-Aid for Scientific Research on Innovative Areas 26119523

Title: An electroencephalographic investigation on linguistic tense and psychological distance to past, present, and future

Authors: *S. TOKIMOTO[1], N. TOKIMOTO[2];
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Abstract: This study examines the neural representations of linguistic tenses and of the psychological distances to them. We manipulated Japanese discourses, in which an event was described in the former half in three ways as past, present, or future. The psychological distance to the event was manipulated by Japanese indexicals referring to the event time in three ways as 'kono-hi,' 'sono-hi,' and 'ano-hi.' 'Kono-hi' (this day) and 'sono-hi' (that day) indicate that a speaker is psychologically near and far to the day of the event respectively. 'Ano-hi' (that past day) refers to the day in the past. The discourses were visually presented, and EEG was recorded at the input of the indexicals. The participants were asked to judge the acceptability of the discourse. For the discourses in which a present event (today) was described, 'ano-hi' was less acceptable than 'sono-hi,' and 'sono-hi' than 'kono-hi.' The lowest acceptability of 'ano-hi' is its manifestation to refer to an event in the past. The less acceptability of 'sono-hi' than 'kono-hi' indicates the difficulty in describing the present event from a viewpoint psychologically far from the present. A negative ERP component was observed for 'ano-hi' and 'sono-hi' in the left frontal, and a positivity was observed for 'sono-hi' in the occipital in the latency of 300 to 450 ms in comparison to 'kono-hi.' For the discourses for past events, we found no significant effect in the acceptability judgments, but a negativity was observed for 'kono/sono-hi' in the left frontal from 300 to 500 ms in comparison to 'ano-hi.' Further, a negativity was observed for 'kono-hi' in the central from 500 to 700 ms in comparison to 'sono-hi.' For the future events, 'ano-hi' was less acceptable than 'kono/sono-hi' with no significant difference between 'kono-hi' and 'sono-hi,' which is again the manifestation of 'ano-hi' to refer to a future event. A positivity was observed for 'ano-hi' in the parietal from 400 to 700 ms in comparison to 'sono-hi.' 'Kono-hi' elicited a positivity in the parietal from 400 to 500 ms as an effect of psychological distance in comparison to 'sono-hi.' The ERP components elicited by anomalous 'sono-hi' for the present and 'ano-hi' for the present and the future coincide with the ERP components often observed for semantic and pragmatic anomaly in sentence processing. However, the distribution of our components differs between the present and the future. Further, the ERP effects of psychological distance between 'kono-hi' and 'sono-hi' were observed in different regions and latencies according to the tense. These results suggest that linguistic tenses are neurally coded differently and the psychological distance interact with them.

Disclosures: S. Tokimoto: None. **N. Tokimoto:** None.

Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant P50HD055784-08

Neuroimaging Training Program and Fellowship NIH NIDA T90-DA022768

Title: Neural correlates of implicit language learning in 9-month-old infants

Authors: *J. LIU, C. PONTING, T. TSANG, R. MCCARRON, M. DAPRETTO;
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Abstract: Word segmentation is a fundamental aspect of language learning, since the identification of word boundaries in continuous speech must occur before the acquisition of word meanings can take place. Behavioral and ERP studies in infants and adults have demonstrated that a continuous speech stream can be readily segmented based solely on the statistical or prosodic cues available in the input. By adapting a paradigm used in prior behavioral studies with infants, we previously used fMRI in children and adults to examine the neural network subserving the detection of word boundaries. Using the same word segmentation paradigm, here we report from an fMRI study we conducted in 9-month-old infants in order to identify the early signatures of language-related learning. Several studies have previously shown that neural activity related to language processing can be detected in infants as young as 2 days old; to date, however, very little is known about the neural mechanisms underlying language *learning* during infancy. During natural sleep, infants were exposed to three speech streams consisting of concatenated syllables where the statistical regularities and prosodic speech cues were manipulated across three conditions: the Stressed Language condition contained prosodic cues (i.e., stress) in addition to strong statistical regularities (i.e., transitional probabilities), the Unstressed Language condition contained solely strong statistical regularities, and the Random Syllables condition had weak statistical regularities and no prosodic cues. Reliable neural activation in temporal language areas was detected for each speech stream. In particular, the stream containing strong statistical regularities and prosodic cues (Stressed Language) elicited significantly greater activity in medial prefrontal cortex and right superior temporal regions as compared to listening to the speech stream containing only statistical regularities (Unstressed Language) or the speech stream containing minimal statistical cues to word boundaries (Random Syllables). In addition, learning-related signal increases (i.e., increased activity as a function of exposure to each speech stream) varied across conditions and were related to behavioral indices of language development. These findings indicate that the neural signatures of online language-related learning can be detected in 9-month-old infants even during natural sleep. Furthermore, these data show that by 9 months of age, infants are sensitive to both statistical and prosodic cues as reflected in different neural responsivity to the speech streams containing different types of cues.

Disclosures: **J. Liu:** None. **C. Ponting:** None. **T. Tsang:** None. **R. McCarron:** None. **M. Dapretto:** None.

Poster

174. Language II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 174.18/Y23

Topic: F.01. Human Cognition and Behavior

Support: NSFC (30870757)

Title: Cerebellar dysfunction in dyslexia

Authors: ***X. FENG**[1], W. XIE[2], X. MENG[2], M. TIAN[1], G. DING[1];
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Abstract: Introduction Dyslexia showed dysfunctional activation and disrupted functional connectivity between the cortical regions of reading network. These regions include temporoparietal, occipitotemporal and inferior frontal cortex, as well as the cerebellum which were found to activate in various language tasks. The cerebellum establishes close connections with the frontal, temporal and occipital lobes, forming a dense reciprocal network of crossed cerebro-cerebellar pathways. This neuroanatomical substrate was considered to subserve a recently acknowledged non-motor role of the cerebellum in reading. Neuroimaging studies have identified structural abnormalities and different activation of the cerebellum in dyslexia. To date few functional studies have specifically focused on or discussed the role of cerebellum in reading and the results were inconsistent. In this study we investigated the cerebellar dysfunction in dyslexia by adopting non-motor orthographical and phonological reading tasks. Considering cerebellum's functional topography and rich neural connections, we focused on both functional activation and functional connectivity of cerebellum, and compared them between typical children and those with dyslexia. To improve the alignment of infra-tentorial anatomical and functional areas, we did preprocessing using cerebellar template and suit-toolbox for SPM. Results In orthographical task, we found significant group difference in right cerebellum VI with greater activation in dyslexia than normal readers. We further found the activation in right cerebellum VI was significantly negative correlated with behavior scores in Reading efficiency test in dyslexic group(r=-0.7772), while the correlation with scores in Chinese phonological awareness test was not significant. However, there was no significant group difference in phonological task even the threshold was lowered. Whole-brain connectivity analysis revealed

that dyslexia showed decreased functional connectivity between right cerebellum VI and left thalamus. The functional connectivity between the two regions was positively correlated with behavior scores in Reading Efficiency test in both normal groups(r=0.464) and all groups(r=0.522).

Disclosures: **X. Feng:** None. **W. Xie:** None. **X. Meng:** None. **M. Tian:** None. **G. Ding:** None.

Poster

174. Language II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 174.19/Y24

Topic: F.01. Human Cognition and Behavior

Support: Ontario Trillium Scholarship

Title: N400 evidence for embodied processing of concrete words after a picture context

Authors: D. SCHMIDTKE, E. SERVICE, R. MAH, *J. F. CONNOLLY;
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Abstract: As a sentence unfolds, semantic information accumulated from the surrounding context facilitates the comprehension of upcoming speech. Previous neurolinguistic research has revealed that word recognition is sensitive to prior contextual information that is present in the wider linguistic discourse. The current research used ERPs to investigate whether context effects on word recognition are also influenced by contextual cues that are provided by non-linguistic information. In the experiment 25 participants (20 female) listened to sentences, such as *He could not hide his anger/delight upon hearing the news*, designed such that the alternative critical words were equally acceptable within the local sentence context. These sentences followed a context-providing image rendering one of the critical words semantically anomalous (e.g., an image of a man with a happy expression). Relative to the context-congruent alternative, context-anomalous words elicited an N400 effect that started at 300–330 ms post acoustic word onset. This effect had a typical centroparietal distribution, with a right hemisphere bias. Furthermore, linear-mixed effects regression models revealed that this contextual congruency N400 effect was modulated by the visual perceptibility (concreteness) of the critical word. In congruent contexts, we found that highly concrete critical words elicited more positive-going (smaller N400) waveforms compared to abstract critical words. However, the same effect of concreteness was not observed in context-anomalous trials. The overall results support the claim that an extra-sentence context can yield a strong expectation for a particular upcoming word. Moreover, we

present two extensions to this hypothesis by revealing that for the effect of contextual constraint to arise, the information source of the context need not be provided linguistically. Crucially, we demonstrate the novel finding that a visual-scene contextual constraint effect on word recognition interacts with lexical characteristics such as concreteness. Importantly, these effects hold even when other predictive cues such as word frequency and the semantic plausibility of the local sentence context are accounted for. Taken together, our results show contextual priming of embodied aspects of processing of concrete words.

Disclosures: D. Schmidtke: None. **E. Service:** None. **R. Mah:** None. **J.F. Connolly:** None.

Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant NS40596

NIH Grant NS091139

Title: Comparison of electrocorticography and electrocortical stimulation in mapping classical language areas: a region of interest approach

Authors: *Y. WANG[1], M. S. FIFER[2], A. FLINKER[4], A. KORZENIEWSKA[1], M. C. CERVENKA[1], D. BOATMAN-REICH[1], W. S. ANDERSON[3], N. E. CRONE[1]; [1]Neurol., [2]Biomed. Engin., [3]Neurosurg., Johns Hopkins Univ., Baltimore, MD; [4]Psychology, New York Univ., New York, NY

Abstract: Functional human brain mapping is commonly performed during or prior to invasive brain surgery for the treatment of drug-refractory epilepsy or brain tumors. The current gold standard, electrocortical stimulation mapping (ESM), is time-consuming and often induces afterdischarges or seizures, and it may overestimate eloquent areas due to propagated effects of stimulation. Spectral analysis of passive electrocorticographic (ECoG) signals has recently emerged as a potential alternative to ESM. Many aspects of passive ECoG mapping are attractive in a clinical setting, especially the ability to rapidly evaluate brain function at all recording sites simultaneously. However, investigators have observed less correspondence between ECoG and ESM maps of language than between their maps of motor function. This may be due to the complexity of speech perception and production and the brain dynamics that support them. We

evaluated seven patients who underwent invasive monitoring for seizure localization whose language and motor areas were identified using ESM. Additionally, all patients performed language tasks including visual object naming and auditory word repetition during passive ECoG recordings. The average sensitivity and specificity of ECoG relative to ESM were 69.9% and 83.5%, respectively, similar to previous studies of ECoG language mapping. Because of the potential inaccuracies of ESM for functional localization, we decided to evaluate the accuracy of both ECoG and ESM with respect to cortical regions of interest drawn from the rich literature on the effects of brain lesions affecting speech production and perception, as well as on regions activated on fMRI during language tasks. Using this approach we found that the sensitivity of ECoG was significantly greater than that of ESM (65.6% vs. 47.9%, respectively, $p = 0.0068$, McNemar's test), while the specificity of ECoG was greater than that of ESM, though not significantly so ($p = 0.066$, McNemar's test). In light of these findings, we believe that both ESM and passive ECoG mapping offer approximations of the patient's true functional anatomy and that more studies are needed to understand their comparative utilities in clinical practice.

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Poster

174. Language II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 174.21/Y26

Topic: F.01. Human Cognition and Behavior

Support: ESRC Studentship

Title: Stammering and synchronised speech

Authors: *S. MEEKINGS[1], K. JASMIN[2], S. K. SCOTT[2];
[1]UCL, London, United Kingdom; [2]Univ. Col. London, London, United Kingdom

Abstract: Speaking in synchrony with another person is usually central to activities that emphasise group cohesion- for example, praying or oath-taking. However, in many people who stutter, the most important consequence of synchronised or 'choral' speech is an immediate and often dramatic improvement in fluency. We used functional magnetic resonance imaging to investigate how synchronous speech is processed in fluent speakers and people who stutter (classified using Riley's Stuttering Severity Instrument, 4th ed). Participants heard either a live

speaker or a pre-recorded voice. They either listened without speaking, read the same sentence aloud (synchronous speech), or read a different sentence aloud (asynchronous speech). In these conditions, questioning determined that participants were not able to distinguish the live speaker from the pre-recorded voice. There was an additional control condition in which subjects spoke on their own with no second speaker. The stammering group were compared to the controls, in whom synchronous speech resulted in bilateral activation in superior temporal gyrus. The auditory suppression response associated with speech in quiet did not occur when typical speakers synchronised with a live speaker. We discuss the implications for various models of stuttering, such as the EXPLAN model and the theory that stuttering may result from an over-reliance on auditory feedback.

Disclosures: **S. Meekings:** None. **K. Jasmin:** None. **S.K. Scott:** None.

Poster

174. Language II

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 174.22/Y27

Topic: F.01. Human Cognition and Behavior

Title: Influence of transcranial direct current stimulation on semantic processing

Authors: S. BRÜCKNER, *M. SPITZER, T. KAMMER;
Dept. of Psychiatry, Univ. of Ulm, Ulm, Germany

Abstract: Recently, the investigation of transcranial direct current stimulation (tDCS) effects in the cognitive domain got a great deal of attention. Interestingly, the canonical assumption (anodal excitatory, cathodal inhibitory) from the motor cortex could not generally be transferred to other cortical regions. In this study, we investigated the effects of tDCS on semantic processing. In a between-group-design, subjects received either anodal (n=20), cathodal (to date n=13) or sham (n=20) tDCS. The active electrode was placed over CP5 and the reference electrode on the left shoulder. TDCS was applied for 15min with a stimulation intensity of 1mA. During stimulation, subjects had to perform a simple auditory oddball task to standardize cognitive activity. Two minutes after tDCS, they started to perform a lexical decision task. Since the electrodes and thus the stimulated cortex area is quite large (7x5cm), we controlled for motor cortex modulations using a choice reaction task in the middle and at the end of the lexical decision task. Mean reaction times (RTs) and error rates were analyzed for the anodal and sham group using t-tests. Subjects receiving anodal tDCS show a trend to decreased RTs on pseudowords ($t=2.01$, $p=0.058$), but not on words ($t=1.39$, $p=0.17$) compared to the sham group.

For error rates, there is no difference between the two groups, and no effect of tDCS on reaction times in the choice reaction task ($t=0.95$, $p=0.35$) were found. Since the cathodal group is not finished yet, we refrain from reporting inferential statistics. Visual inspection of the data reveals that cathodal tDCS decreases RTs on both word types, whereas error rates and motor responses are not affected. The effect of excitation seems to be even more pronounced as with anodal tDCS. Our preliminary data are in line with other studies showing that effects of tDCS on motor cortex excitability cannot be transferred to other cortical regions. Although anodal tDCS showed a trend to excitation as expected, cathodal tDCS seems to facilitate semantic processing even more. As a possible explanation, the initial state of the neurons stimulated may play a role for cathodal tDCS effects due to homeostatic metaplasticity, making it necessary to accurately control neuronal activity during stimulation. We will continue our measurements up to 20 subjects in the cathodal group and prepare comprehensive statistics including a split-half analysis to investigate the time course of the effects. Additionally, we will present the results in analogy to those published by our group, where effects of repetitive transcranial magnetic stimulation on semantic processing were shown using the very same lexical decision task.

Disclosures: **S. Brückner:** None. **M. Spitzer:** None. **T. Kammer:** None.

Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Support: Medical Research Council (UK) MR/J004146/1

Title: Establishing task- and modality-dependent dissociations between the semantic and default mode networks

Authors: *G. HUMPHREYS, M. LAMBON RALPH;
Univ. of Manchester, Manchester, United Kingdom

Abstract: The default mode network (DMN) and semantic network (SN) are two of the most extensively studied human neurobiological systems. The DMN includes those neural regions that shows task-related deactivation during many goal directed tasks (i.e., rest > task) in neuroimaging studies. The SN consists of regions that are sensitive to semantic tasks > rest/non-semantic control tasks. There are strong reason to assume a close relationship between the networks, both theoretically and anatomically. Theoretically, the two networks may share

cognitive processing, reflecting the semantic processing that occurs during rest. Anatomically, evidence suggests regions, such as the anterior temporal lobe (ATL) and angular gyrus (AG), are critical to both systems. Nevertheless, despite theoretical and anatomical motivations, the relationship between the SN and DMN has been little studied. Here we directly address this issue by comparing the SN and DMN using a large distortion-corrected fMRI dataset (N=69) including a wide range of semantic and non-semantic tasks varying in stimulus-type (pictures, words, non-semantic items), input modality (visual, auditory), and task difficulty. A distortion-corrected protocol is critical here to account for the signal distortion associated with the ATL. The results illustrated that both networks vary depending on the level of semantic involvement, stimulus-type, modality and task difficulty. Critically, the ATL and AG showed very different responses, despite claims that both AG and ATL are semantic-hubs. Specifically, the left ATL was positively activated relative to rest for all semantic tasks but deactivated during non-semantic task performance. In contrast, the left AG was deactivated for all tasks, with the level of deactivation related to task-difficulty. This finding supports the role of ATL but not AG in semantic representation. Furthermore, the result suggest that rather than sharing a common interest in semantic tasks, the AG and ATL share a common "disinterest" in non-semantic tasks. These data have implication regarding our understanding of task variability in the SN and DMN, and clarify the role of the ATL and AG in both networks, with the ATL as a semantic-hub and the AG showing more domain general sensitivity.

Disclosures: **G. Humphreys:** None. **M. Lambon Ralph:** None.

Poster

174. Language II

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Topic: F.01. Human Cognition and Behavior

Support: 973 Program 2013CB837300

973 Program 2014CB846100

NSFC 81030028

NSFC 81225012

Title: Dissociable intrinsic functional networks support noun and verb processing

Authors: *H. YANG[1,2], Z. HAN[2], L. SONG[3], H. LI[2], Q. LIN[2], Y. HE[2], Y. BI[2];
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Abstract: The neural circuits supporting noun (entity) and verb (event) processing are a central issue underlying language research, with robust evidence indicating widely distributed regions showing different preference for these two classes of items. We conducted two experiments to examine the intrinsic network organizations of these regions and their corresponding behavioral relevance. Experiment 1 explored whether the brain regions previously shown to be selectively activated by noun or verb are intrinsically organized into different functional networks using resting-state fMRI data of 146 healthy adults. Noun- and verb-preference nodes were identified by the activation likelihood estimate (ALE) meta-analyses from 22 imaging studies, resulting in 19 verb nodes and 15 noun nodes. The modularity analysis over these nodes showed that they could be reliably subdivided into three modules across a range of network sparsity (sparsity = 0.4, Q = 0.3, Z = 13.8). The majority (76%) of the nodes converged onto the distinct word-class preferences in the ALE analyses. Furthermore, when evaluating the functional connectivity strength (FCs) within the regions labeled for the two word classes based on the ALE analyses, the average within-class FCs was significantly greater than the average between-class FCs [$t(143) = 24.52$, $P < 0.01$; $t(143) = 18.51$; $P < 0.01$], suggestion that the regions showing activation differences for the two classes are indeed intrinsically organized into different functional networks. Experiment 2 examined the behavioral relevance of the intrinsic noun and verb networks using data from 88 brain-damaged patients. We found that across patients the relative mean FCs based on network identified in Experiment 1 significantly correlated with the relative behavioral performance in the picture associative matching task for verb (action) and noun (tool): modularity-analyses defined network: $r = 0.37$, $P < 0.01$; ALE-analyses defined network: $r = 0.31$, $P < 0.01$. The correlation remained significant even after controlling for the extent of the relative anatomical damage of the two networks, measured by number of voxels being lesioned in the nodes within each network ($r = 0.37$, $P < 0.01$). In summary, we found that noun and verb preference regions from prior studies are intrinsically organized into segregated functional networks and the integrity of such networks could significantly account for relative noun or verb selective deficits as consequences of brain lesion, indicating that noun and verb processing are supported by dissociable large-scale functional networks.

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Poster

175. Modulating Fear, Learning, and Memory

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 175.01/Y30

Topic: F.02. Animal Cognition and Behavior

Support: FAPESP

CNPq

Title: Fluoxetine facilitates consolidation of fear memory extinction through increased DH BDNF and through VH TrkB transactivation

Authors: *C. A. DINIZ[1], L. S. ANTERO[2], L. B. M. RESSTEL[2], S. R. L. JOCA[3];
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[2]Pharmachology, Sch. of Med. of Ribeirão Preto, Ribeirão Preto, Brazil; [3]Physics and chemistry, Sch. of Pharmaceut. Sci. of Ribeirão Preto, Ribeirão Preto, Brazil

Abstract: Purpose: Animal fear conditioning and PTSD depend on an association of the aversive stimulus with a previous neutral stimulus producing an intense conditioned fear response. PTSD patients have decreased hippocampus and impaired hippocampal-dependent cognitive functions, as well as impaired extinction of the conditioned fear response. Selective serotonin reuptake inhibitors are the first line pharmacological treatment for PTSD. In animals, chronic fluoxetine (FLX) treatment impairs renewal, reinstatement and spontaneous recovery of the conditioned response. These effects seem to depend on hipocampal BDNF. Besides, impaired extinction of fear-potentiated startle is observed in conditioned hipocampal BDNF knockout animals. The hippocampus can be divided into ventral hippocampus (VH), more closely related to anxiety and fear response, and dorsal hippocampus (DH), related to cognitive processes of learning and memory. Little is known about a possible distinct participation of DH and VH BDNF content in FLX-induced effects on fear extinction. Methods: Male Wistar rats (250 g) were used. Animals were submitted to conditioning procedure (day 1). Animals were treated with FLX (10mg/kg) or vehicle for twelve days. Chronic FLX, acute FLX and vehicle treatment groups were obtained. One day after the last injection animals were exposed to the extinction protocol (day 14) and in the next day to the extinction memory retention protocol (day 15). Independent groups of animals were submitted to the conditioning procedure and treatment as outlined above, but now the animals were sacrificed rather than exposed to extinction protocol in order to have their HD and VH portion prepared for BDNF protein analysis (ELISA). In another experiment, animals were submitted to stereotaxic surgery 5 days before extinction protocol and bilateral guide cannulas were allocated into DH or VH. K252 (TrkB functional blocker - 10pmol/0.5ul by side) or vehicle were infused into the DH or VH soon after extinction protocol in chronic FLX or vehicle i.p treatment. Results: Only chronic FLX treatment facilitated the extinction memory retention ($F_{2,29} = 2.881$; $p<0.05$). Chronic FLX treatment increased BDNF in DH ($F_{2,21} = 5,198$; $p<0.05$) while acute treatment increased BDNF in VH ($F_{2,16} = 5.796$; $p<0.05$). k252

administered into DH or VH prevented chronic FLX-induced effects on memory consolidation ($F_{3,41} = 4.166$; $p<0.05$) and ($F_{3,13} = 3.533$; $p=0.056$), respectively. Conclusion: Chronic FLX effect on sound extinction memory consolidation might occur through BDNF modulating learning and memory processes within the DH and probably occur also through fluoxetine TrkB transactivation in the VH.

Disclosures: C.A. Diniz: None. **L.S. Antero:** None. **L.B.M. Resstel:** None. **S.R.L. Joca:** None.

Poster

175. Modulating Fear, Learning, and Memory

Location: Hall A

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Program#/Poster#: 175.02/Y31

Topic: F.02. Animal Cognition and Behavior

Support: CU Denver start up fund

Title: Effect of DREADD-induced activation of the nigrostriatal dopamine pathway during auditory fear extinction on renewal of fear

Authors: *T. M. NICASTRO, N. M. GRAY, C. A. BOUCHET, E. C. LOETZ, B. N. GREENWOOD;
Psychology, Univ. of Colorado Denver, Denver, CO

Abstract: Fear renewal is the return of conditioned fear following extinction in contexts that differ from where fear extinction was learned. The contextual modulation of fear extinction memory contributes to the poor long-term efficacy of exposure therapy for the treatment of anxiety and trauma-related disorders. Prior work suggests that dopamine (DA) could be an important component of fear extinction memory formation; however, the specific neural pathways involved remain unclear, as does whether fear extinction facilitated by DA is resistant to renewal. Burst firing of DA neurons terminating in the dorsal and ventral striatum encode prediction errors which could be important for fear extinction learning. Of these DA target regions, the dorsal striatum could be a region in which DA acts to render fear extinction memory resistant to contextual modulation. Indeed, unlike the nucleus accumbens which receives strong hippocampal afferents, the dorsal striatum is free of contextual modulation from the hippocampus. Viral-mediated gene transfer of designer receptors exclusively activated by designer drugs (DREADD) was used to determine whether selective augmentation of DA signaling in the dorsal striatum during fear extinction can strengthen fear extinction memory and

reduce fear renewal. Male wild-type Long Evans or TH-Cre rats received micro-injections of a CRE-recombinase-dependent DREADD (AAV-hSyn-rM3Dq-mCherry) bilaterally into the substantia nigra pars compacta, which contains DA neurons projecting to the dorsal striatum. DREADDs are activated by the otherwise pharmacologically inert synthetic ligand clozapine-N-oxide (CNO). Therefore, this strategy produces transient augmentation of Gq-protein signaling in infected DA neurons when CNO is injected intraperitoneal (i.p.) and allows for selective activation of DA signaling during fear extinction. After 4 weeks to allow for viral gene expression, rats were exposed to auditory fear conditioning. The next day rats received CNO (1 mg/kg i.p.) 30 minutes before being exposed to auditory fear extinction in a novel context. Fear extinction in the presence of CNO was repeated 24 hours later. The next day rats were placed drug-free into either the familiar extinction context or a novel context and exposed to the auditory stimulus to assess fear renewal. The experiment is currently underway.

Disclosures: T.M. Nicastro: None. **N.M. Gray:** None. **C.A. Bouchet:** None. **E.C. Loetz:** None. **B.N. Greenwood:** None.

Poster

175. Modulating Fear, Learning, and Memory

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 175.03/Y32

Topic: F.02. Animal Cognition and Behavior

Support: UCD Start-up Funds

Title: Activation of dopamine 1 receptors in the dorsal striatum during fear extinction reduces fear renewal

Authors: *C. A. BOUCHET, T. M. NICASTRO, N. M. GRAY, E. C. LOETZ, F. EYAYOU, B. N. GREENWOOD;
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Abstract: Behavioral treatments for anxiety and trauma-related disorders focus on extinction-based exposure therapy, during which patients are exposed to an anxiety-eliciting stimulus in a safe environment in order to extinguish fear. Exposure therapy has low efficacy, and one factor contributing to the relapse of anxiety following treatment is the fact that extinction memory is context dependent. Thus, exposure to fear-inducing cues outside of the extinction context precipitates the return of fear; a phenomenon termed fear renewal. Identification of means to reduce fear renewal is of the utmost importance to mental health. Dopamine has been implicated

in fear learning and memory processes but the role of dopamine in extinction is not clear. Two terminal regions of dopaminergic signaling, the dorsal and ventral striatum, have been implicated in detection of prediction error which may be an important component of fear extinction learning. Neuroanatomical evidence suggests that the dorsal striatum, unlike the ventral striatum, does not receive strong hippocampal afferents. Therefore, potential manipulations that strengthen fear extinction learning in the dorsal striatum may do so in a manner that is independent of contextual modulation. Further, activation of dopamine 1 (D1) receptors within the dorsal striatum could influence well established fear extinction circuitry. We therefore hypothesize that activation of D1 receptors within the dorsal striatum during fear extinction will reduce fear renewal. To test this, male Long Evans rats were conditioned to fear an auditory stimulus. The next day, rats were exposed to fear extinction training in a novel context. Immediately prior to fear extinction training, rats received bilateral microinjections of the D1 receptor agonist SKF38393 (0.5µg/µl) or saline into the dorsal striatum. Drug- or saline-paired extinction training was repeated the following day. One day following the second fear extinction training session, rats were placed into either the same extinction context or a novel context drug-free and freezing was scored as an index of fear. Activation of D1 receptors in the dorsal striatum during fear extinction reduced freezing in the novel context during test. These data suggest that manipulations to activate the direct pathway neurons in the dorsal striatum during fear extinction can render extinction memory free of contextual modulation and prevent the renewal of fear.

Disclosures: **C.A. Bouchet:** None. **T.M. Nicastro:** None. **N.M. Gray:** None. **E.C. Loetz:** None. **F. Eyayou:** None. **B.N. Greenwood:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Program#/Poster#: 175.04/Y33

Topic: F.02. Animal Cognition and Behavior

Support: CU Denver Undergraduate research opportunity program

CU Denver Greenwood Start Up fund

Title: The effect of voluntary exercise during consolidation of auditory fear extinction on renewal of fear

Authors: *F. B. EYAYOU[1], C. A. BOUCHET[2], S. HOLMES[2], T. M. NICASTRO[2], E. C. LOETZ[2], N. M. GRAY[2], B. GREENWOOD[2];
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Abstract: Fear extinction learning forms the basis of exposure therapy for anxiety and trauma-related disorders. Unlike fear memories, fear extinction memories are labile and susceptible to relapse phenomenon such as fear renewal. Fear renewal is characterized by the return of conditioned fear responding in contexts outside of the extinction context, even following successful extinction. Prior work suggests that acute exercise either prior to or following the learning of extinction of contextual fear conditioning can enhance memory for fear extinction. Remaining unknown, however, is whether acute exercise can enhance the consolidation of auditory fear extinction memory, and whether fear extinction augmented by acute exercise is resistant to renewal. In order to explore these questions, male Long Evans rats were familiarized with wheel running by being exposed to either a running wheel or a locked wheel on alternating nights for a total of 6 nights. Forty eight hours after the third wheel running active cycle, rats were exposed to auditory fear conditioning which consisted of 4 pairings of an auditory conditioned stimulus (SC) with an aversive foot shock. The following day at the start of the active (dark) cycle, rats were placed into a novel context and were exposed to the CS tone multiple times in the absence of foot shock in order to extinguish fear. Immediately following fear extinction, rats were placed into either their familiar mobile or locked running wheels for 2 hours. Thus, rats in the acute exercise group were given two consecutive opportunities to run during the consolidation phase of fear extinction memory formation that occurs immediately after fear extinction training. This procedure was repeated the next day, so that rats had a total of two days of exposure to either a locked or mobile running wheel during the fear extinction memory consolidation phase. The morning after the second fear extinction session, rats were placed into either the familiar extinction context or a novel context and the extinguished CS was presented 4 times in order to evaluate fear extinction memory and fear renewal, respectively. Rats were sacrificed 90 minutes after the test session and brain circuits implicated in fear and extinction were processed with immunohistochemistry for the neural activation marker c-Fos. Behavioral and brain data are currently being analyzed

Disclosures: **F.B. Eyayou:** None. **C.A. Bouchet:** None. **S. Holmes:** None. **T.M. Nicastro:** None. **E.C. Loetz:** None. **N.M. Gray:** None. **B. Greenwood:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Support: JSPS KAKENHI 25116531

JSPS KAKENHI 25710003

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JSPS KAKENHI 15H04264

JSPS KAKENHI 15H01301

MEXT SRPBS

Brain/MINDS

Title: A neural circuit mechanism for calculating prediction errors in amygdala neurons to set adaptive level of fear memory

Authors: *T. OZAWA[1], E. A. YCU[1], T. AHMED[2], A. KUMAR[1,3], L.-F. YEH[1,4], J. KOIVUMAA[1], J. P. JOHANSEN[1,4];
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Abstract: For adaptive behavioral decision making, the strength of aversive learning needs to be appropriate to the level of danger and exaggerated fear learning is characteristic of anxiety disorders. During auditory fear conditioning, animals learn that an auditory tone predicts an aversive outcome (typically electric shock). Learning finally reaches a steady state at a certain memory strength (termed the learning asymptote) beyond which further training is ineffective at producing learning unless the strength of the aversive outcome is increased. We previously found that learning produces a reduction in shock-evoked responding in lateral nucleus of the amygdala (LA) neurons (termed prediction error coding) and that shock-evoked responding in LA neurons is required for fear learning to occur. Prediction error coding also occurs in periaqueductal gray (PAG) neurons which relay aversive shock signals to LA. PAG receives projections from the central nucleus of amygdala (CeA) which is activated by tones after fear learning. Here, we hypothesized that a CeA-PAG pathway provides a feedback signal to down-regulate aversive shock processing resulting in prediction error coding in PAG and LA. Furthermore, we hypothesized that this CeA-PAG pathway, and ultimately prediction error coding in LA, functions to set behavioral fear learning asymptotes. To address these questions, we first developed a 4-day fear conditioning paradigm in which rats were trained (days 1, 3) and tested (days 2, 4) twice. In this paradigm, rats reached learning asymptote after initial training (day 1)

as learning was not enhanced by overtraining (day 3). We found that: (1) optogenetic inactivation of CeA to ventrolateral PAG (vlPAG) afferents at learning asymptote disinhibited predicted shock-evoked responses (i.e. reengaged prediction error coding) in dorsolateral PAG (dlPAG) and LA neurons. (2) optogenetic inactivation of dlPAG cell bodies during the shock period in training impaired acquisition of fear conditioning. (3) optogenetic inactivation of the CeA-vlPAG circuit increased learning levels beyond the original asymptote through activation of LA neurons. These results demonstrate that a CeA-vlPAG pathway generates prediction error coding in dlPAG and LA to set fear learning asymptotes. This suggests a distributed circuit mechanism for setting adaptive levels of fear memory strength and shows that disrupting this circuit produces exaggerated levels of fear.

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Poster

175. Modulating Fear, Learning, and Memory

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RIKEN SPDR program

Title: The functional role of locus coeruleus noradrenaline neurons in fear learning and extinction

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Abstract: Noradrenaline neurons in the locus coeruleus (LC) play an important role in mood, attention, cognitive processes, and learning through their projections to a diverse set of brain regions. Recently, we showed that noradrenaline in the lateral amygdala modulates Hebbian plasticity mechanisms to facilitate the formation of auditory fear memories. Noradrenaline is also required for the extinction of fear memories, a process which involves the lateral amygdala as well as medial prefrontal cortex regions. While the LC sends projections to these brain areas the

functional role of LC noradrenaline neurons in fear learning and extinction is not clear. To understand these questions we used optogenetic and electrophysiological approaches in a transgenic rat expressing cre-recombinase under the control of the tyrosine hydroxylase promoter (TH-cre rat). We injected the LC of TH-cre rats with adeno-associated virus expressing a cre-dependent light responsive opsin protein archaerhodopsinT (ArchT)-GFP. This produced specific expression of ArchT in noradrenaline neurons (>95% of ArchT-GFP positive cells were TH positive) and could be used to inhibit these cells in response to orange light. This allowed for optogenetic identification and subsequent analysis of task related neuronal coding of LC noradrenergic neurons and an determination of the function role of these neurons in fear and extinction learning. Rats were trained with 3 pairings of an auditory tone (conditioned stimulus, CS) and foot shock (unconditioned stimulus, US) followed by CS alone presentations. We found that inhibition of noradrenaline neurons during the US period of fear conditioning significantly reduced long-term fear memory while inactivation during the CS period had no effect. Consistently, we found that many optogenetically identified LC noradrenaline neurons showed phasic responses to the US during fear conditioning. We next examined the role of LC neurons in fear extinction, where the tone is repeatedly presented without the aversive US following fear learning. Optogenetic inhibition of noradrenaline neurons during the CS period blocked long-term extinction memory, whereas inactivation during the shock US omission period had no effect. In electrophysiological experiments, LC noradrenaline neurons showed CS-evoked responses after fear learning but no change in response to shock omission. These results demonstrate a temporally precise, but differential role for activity in LC noradrenaline neurons during fear memory formation and extinction of these fear memories when they are no longer appropriate. Further results will be discussed in the poster.

Disclosures: **A. Uematsu:** None. **E. Ycu:** None. **J. Johansen:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01MH065961 to SM

College of Liberal Arts, Texas A&M University to SM and NN

Heep Fellowship to GMA

Title: Differential effects of allopregnanolone in the basolateral amygdala and bed nucleus of the stria terminalis on Pavlovian fear conditioning in rats

Authors: *G. M. ACCA[1], B. TSAO[2], J. JIN[1], C. FU[2], S. MAREN[1,2], N. NAGAYA[1,2];
[1]Inst. for Neurosci., [2]Psychology, Texas A&M Univ., College Station, TX

Abstract: Considerable evidence indicates that gonadal hormones and their metabolites regulate emotional behavior. We have previously demonstrated that allopregnanolone (ALLO), a progesterone metabolite and $GABA_A$ receptor modulator, inhibits the expression of conditional freezing to an aversive context when infused into the bed nucleus of the stria terminalis (BNST) in rats. Intra-BNST infusions of ALLO did not affect the expression of fear to an auditory conditioned stimulus (CS), consistent with a selective role for the BNST in contextual fear. In addition to the BNST, the basolateral amygdala (BLA) is a potential target for neurosteroid modulation of conditioned fear. Here we compare the effects of ALLO infusions into the BNST (Expt 1) or BLA (Expt 2) on the acquisition and expression of Pavlovian fear conditioning. After cannulae implantation and recovery, adult male rats were given infusions of either ALLO (8 μg/μl) or vehicle (VEH; 30% β-cyclodextrin) into the BNST or BLA before either conditioning or retention testing in a factorial design. Conditioning consisted of 5 CS (2 kHz, 10 s, 80 dB)-footshock (2 s, 1 mA) pairings and the retention test consisted of a context test (10 min) followed by a tone test (4 CS-alone trials) in a novel context. ALLO infused into the BNST disrupted both the acquisition and expression of contextual freezing, however, this effect was entirely state-dependent. That is, rats infused with either VEH or ALLO before both the conditioning and testing sessions exhibited similar and high levels of conditioned freezing. Conditioned freezing to the auditory CS was not affected by intra-BNST ALLO. In contrast, intra-BLA ALLO infusions attenuated the expression of both context and CS freezing, without affecting acquisition. These findings reveal that infusion of ALLO into the BNST modulates conditioned fear in a state-dependent manner, perhaps by altering the animal's representation of interoceptive context. This contrasts with the effects of ALLO in the BLA, which appear to produce a global attenuation in the expression of conditioned fear.

Disclosures: G.M. Acca: None. **B. Tsao:** None. **J. Jin:** None. **C. Fu:** None. **S. Maren:** None. **N. Nagaya:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH065961

Title: GABAA receptors in the infralimbic cortex regulate both the expression of extinction and renewal of fear in rats

Authors: *Q. WANG[1], G. M. ACCA[2,3], D. J. NINAN[2,3], S. MAREN[2,3];
[1]Biol., Texas A&M Univ., College Station, TX; [2]Inst. for Neurosci., [3]Dept. of Psychology, Texas A&M Univ., College station, TX

Abstract: There is considerable interest in the role of the infralimbic (IL) region of the medial prefrontal cortex in the regulation of conditioned fear. We have previously shown that infusion of the GABAA receptor antagonist, picrotoxin, into IL impairs the expression of freezing to an auditory conditioned stimulus (CS) (Chang and Maren, 2011). This suggests that GABAergic inhibition in IL is involved in fear regulation and may have a critical role in the regulation of extinguished fear. To examine this issue, we conducted two experiments in which rats received either muscimol (0.2 ug in 0.2 ul) or picrotoxin (10 ng in 0.5 ul) infusions into the IL prior to an extinction recall test or fear renewal test, respectively; freezing served as the index of conditional fear. Rats were implanted with a single guide cannulae targeting IL at the midline. After recovery from surgery, the rats underwent a standard fear conditioning procedure consisting of five tone (2kHz, 10sec, 80dB)-footshock (2s, 1mA) trials. Twenty-four hours later they were returned to either the conditioning context or a novel context and were presented 45 extinction (CS-alone) trials. Twenty-four hours after extinction, the rats were infused with drug or vehicle (0.9% saline) and, 15 minutes after infusion, placed in either the extinction context (Exp 1) or a context different from extinction (Exp 2) where they received 45 CS-alone trials to test retention of fear to the extinguished CS. Infusions of muscimol into the IL impaired the expression of extinction (Exp 1) and resulted in a relapse of conditioned freezing, whereas infusions of picrotoxin into the IL (Exp 2) yielded low levels of conditioned freezing and prevented fear renewal. These data suggest that GABAA receptors in IL bidirectionally regulate the expression of fear after extinction. Importantly, the IL is required for the retrieval of fear and safety memories after extinction. Supported by a grant from the NIH (R01MH065961).

Disclosures: Q. Wang: None. **G.M. Acca:** None. **D.J. Ninan:** None. **S. Maren:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Memory and Cognitive Disorders Award to S.M.

NIH R01MH065961 to S.M.

Title: Reversible inactivation of the nucleus reuniens of the midline thalamus disrupts fear suppression after extinction

Authors: *J. JIN[1,2], Q. WANG[3], S. MAREN[1,2];
[1]Inst. for Neurosci., Texas A&M Univ., College Station, TX; [2]Psychology Dept., College Station, TX; [3]Biol. Dept., College Station, TX

Abstract: The extinction of conditioned fear is context-specific: fear to an extinguished conditioned stimulus (CS) is suppressed in the extinction context but 'renews' in any other context. Recent work indicates that projections from the ventral hippocampus (VH) to the medial prefrontal cortex (mPFC) are involved in this process. In addition, indirect projections from the mPFC to the hippocampus via the thalamic nucleus reuniens (RE) have recently been implicated in context processing. As a first step to exploring the contribution of this pathway to contextual memory retrieval, we examined whether pharmacological (muscimol) or chemogenetic ('designer receptors exclusively activated by designer drugs', DREADDs) inactivation of RE would disrupt the context-specific expression of extinction memories. In Experiment 1, rats were implanted with a cannula aimed at RE. Seven days after surgery, rats underwent fear conditioning (5 tone-shock pairings) in context A and, twenty-four hours later, extinction (45 tone-alone) in context B. On days 3 and 4, rats were microinjected with either muscimol or vehicle and immediately placed in either the extinction context (ABB, extinction test) or the conditioning context (ABA, renewal test) and presented with the extinguished CS; drug condition and contexts were counterbalanced. Pharmacological inactivation of RE increased freezing in the extinction context, but did not affect the high levels of freezing expressed during the renewal of fear in a different context. In Experiment 2, rats received unilateral infusions of an adeno-associated virus (AAV) expressing an inhibitory DREADD (AAV5-CaMKIIα-hM4D[Gi]-mCherry) into the RE. Three weeks later, rats underwent conditioning, extinction and retrieval testing in a within-subjects design. Each rat received clozapine N-oxide (CNO, a synthetic DREADD agonist; 1 mg/kg, i.p.) or VEH prior to testing in both the extinction and conditioning contexts (for a total of four tests). CNO administration increased freezing to the extinguished CS in both test contexts. Together, these experiments suggest that RE inactivation impairs the retrieval of extinction memories. The RE may be an essential bridge in mPFC-HPC top-down inhibition of context-dependent fear expression after extinction.

Disclosures: **J. Jin:** None. **Q. Wang:** None. **S. Maren:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: R01MH065961

Title: Propranolol modulates medial prefrontal cortical activity and enhances extinction after recent fear

Authors: *T. F. GIUSTINO[1,2], P. J. FITZGERALD[2], S. MAREN[1,2];
[1]Texas A&M Inst. for Neurosci., College Station, TX; [2]Psychology, Texas A&M Univ., College Station, TX

Abstract: It is well known that psychological stress impairs extinction learning. This is exemplified by the immediate extinction deficit, a phenomenon in which extinction trials administered soon after fear conditioning fail to produce long-term fear reduction. We have recently shown that fear conditioning produces dramatic changes in medial prefrontal cortical (mPFC) single-unit firing, including sustained decreases in infralimbic cortical (IL) firing rates, up to an hour after conditioning. Interestingly, this effect was mitigated by systemic administration of propranolol (10 mg/kg, i.p.), a β-adrenergic receptor (β-AR) antagonist. This suggests that propranolol might facilitate extinction under stress by stabilizing shock-induced changes in mPFC firing. To address this question, we examined the consequences of post-conditioning propranolol administration on both mPFC activity and the immediate extinction deficit. On Day 1, rats underwent a standard auditory fear conditioning procedure, immediately followed by vehicle or propranolol administration. Thirty minutes after drug administration, animals received 45 extinction trials. Propranolol treatment reduced freezing throughout the session. Vehicle-treated rats exhibited post-shock increases and decreases in prelimbic (PL) and IL spontaneous single-unit activity, and a decline in conditioned stimulus (CS)-evoked activity throughout the extinction trials. Propranolol treatment counteracted post-shock suppression of spontaneous firing in IL, and suppressed CS-evoked firing in IL during extinction. Drug treatment also rescued shock-induced suppression of IL bursting. Fear conditioning was associated with changes in the local field potential in both IL and PL of vehicle-treated rats, with decreases in both gamma and theta power that persisted through the extinction period. Propranolol administration enhanced PL and IL theta power during the immediate extinction trials. In a drug-free retention test given 48 hours after extinction (Day 2), prior treatment with propranolol reduced freezing and also suppressed CS-induced increases in spontaneous firing rate. Prior drug treatment also enhanced PL gamma power and suppressed CS-evoked IL

responses. Collectively, these data show that systemic β-AR blockade produces marked alterations in mPFC neural activity during and after immediate extinction. Propranolol may be a useful adjunct to therapeutic interventions in recently traumatized individuals and patients suffering from stress- and trauma-related disorders.

Disclosures: T.F. Giustino: None. **P.J. Fitzgerald:** None. **S. Maren:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH065961

Title: Nonassociative inhibition of conditional fear engages the medial prefrontal cortex in rats

Authors: *P. J. FITZGERALD[1], T. GIUSTINO[1,2], S. MAREN[1,2];
[1]Dept. of Psychology, Texas A&M Univ., College Station, TX; [2]Texas A&M Inst. For Neurosci., College Station, TX

Abstract: Considerable evidence suggests the medial prefrontal cortex (mPFC) plays a crucial role in emotional regulation in both rats and humans. For example, the infralimbic (IL) region of the mPFC has an important role in both the acquisition and expression of extinction, a form of learning that results in the inhibition of conditioned fear responses such as freezing. Whether IL plays a specific role in extinction or a more general role in fear inhibition is not known. To investigate this possibility, we examined single-unit activity in the mPFC during the nonassociative inhibition of fear produced by a novel stimulus (i.e., external inhibition). On Day 1, animals received either signaled or unsignaled footshocks in the recording chamber; the conditioned stimulus (CS) (in signaled rats) consisted of a 2 sec, 80 dB, 2 kHz tone and the unconditioned stimulus (US) was a 0.5 s, 1 mA footshock. Twenty-four hours later, the animals were returned to the recording chamber (modified to create a novel context) where they received 5 tone-alone trials (60 sec ISI). During this test session, all rats exhibited a moderate level of generalized fear to the recording chamber. After tone presentation, freezing behavior increased in rats that had received signaled shocks, but decreased in animals in the unsignaled condition. Hence, a novel auditory stimulus caused the external inhibition of freezing behavior. Interestingly, single-unit responses in mPFC mirrored the differential behavioral responses to the auditory stimuli in signaled and unsignaled rats. In particular, the spontaneous firing rate of both

IL and PL neurons in signaled rats was reliably lower than that in unsignaled rats after presentation of the auditory stimuli. These changes in spontaneous firing rate returned to baseline within 4 minutes of the last tone in the unsignaled rats. In contrast to the spontaneous firing rate changes, short-latency tone-evoked responses (<200 ms after onset of the tone) were only observed in signaled rats. Collectively, these data suggest that increases in the spontaneous firing rate of mPFC neurons are associated with the external inhibition of fear to a novel stimulus, whereas decreases in this activity are associated with the expression of fear to a CS. In contrast to other reports, the neural correlates of fear expression and inhibition were similar in PL and IL. These data reveal that the mPFC is involved the regulation of fear by both associative and nonassociative stimuli.

Disclosures: P.J. Fitzgerald: None. **T. Giustino:** None. **S. Maren:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH065961 to S.M.

Title: Combinatorial DREADD silencing of ventral hippocampal neurons projecting to infralimbic cortex prevents fear renewal

Authors: *T. D. GOODE[1], J. JIN[1,2], R. HOLEHONNUR[3], J. E. PLOSKI[3], S. MAREN[1,2]; [1]Inst. for Neurosci., [2]Psychology Dept., Texas A&M Univ., College Station, TX; [3]Dept. of Mol. & Cell Biol., Univ. of Texas, Dallas, TX

Abstract: Extinguished fear to a conditioned stimulus (CS) renews outside of the place or context in which extinction training occurred. Here we examined the hypothesis that projections from the ventral hippocampus (VH) to the infralimbic region (IL) of the medial prefrontal cortex mediate fear renewal. In two experiments, we used 'designer receptors exclusively activated by designer drugs' (DREADDs) to either non-selectively inhibit VH neurons (Experiment 1) or selectively silence VH neurons projecting to IL (Experiment 2) during fear renewal. In Experiment 1, rats received bilateral infusions of an adeno-associated virus (AAV) expressing inhibitory DREADDs (AAV-CaMKIIα-hM4D[Gi]-mCherry) or a GFP control (AAV-CaMKIIα-GFP) into the VH. Two weeks later, in a between-subjects design, rats were subjected to an ABA renewal procedure. First, rats were conditioned in Context A, consisting of 5 CS (10 s, 2 kHz, 80

dB tone)-unconditioned stimulus (US; 2 s, 1 mA footshock) pairings. Freezing behavior served as the index of fear. Twenty-four hours later, rats were extinguished to the CS in Context B (45 nonreinforced CS-only presentations). Twenty-four hours after extinction, rats were injected with either clozapine N-oxide (CNO, a synthetic DREADD agonist; 3 mg/kg, i.p.) or vehicle at 30 min prior to a renewal test in Context A. In Experiment 2, the VH was infused with an inhibitory DREADD-expressing virus under the control of Cre (AAV-hSyn-DIO-hM4D[Gi]-mCherry). In these same animals, the IL was infused with a canine adenovirus (CAV) to retrogradely express Cre and inhibitory DREADDs in VH neurons projecting to IL. Four weeks later, rats underwent ABA renewal (as in Exp. 1) in a counterbalanced within-subjects design. Each rat was tested to the CS outside of the extinction context on CNO (1 mg/kg, i.p.) and vehicle. As predicted, DREADD-mediated inactivation of either VH or VH neurons projecting to IL disrupted fear renewal as compared to control animals. The data suggest that VH projections to the infralimbic cortex are necessary for renewal. These data are consistent with the possibility that, during fear renewal, the VH inhibits the IL to limit the suppression of fear to an extinguished CS.

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Poster

175. Modulating Fear, Learning, and Memory

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Support: NIH Grant NS034007

NIH Grant NS047384

Title: Differential control mechanisms mediating the acquisition and consolidation of cued fear extinction

Authors: *T. N. HUYNH, E. SANTINI, E. KLANN;
Ctr. for Neural Science, New York Univ., New York, NY

Abstract: De novo protein synthesis is required for the extinction of cued fear memory. Central to the regulation of translation is the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway, which stimulates cap-dependent translation through the phosphorylation of two downstream effectors: eIF4E-binding proteins (4E-BPs) and p70 S6 kinase 1 (S6K1).

Phosphorylation of 4E-BPs permits the binding of eIF4E to eIF4G to form eIF4F and phosphorylation of S6K1 leads to the phosphorylation of additional translational control molecules that stimulate protein synthesis. We first sought to determine the role of mTORC1 in the extinction of cued fear memory. We found that rapamycin, an mTORC1 inhibitor, had no effect on the acquisition of extinction, but blocked extinction memory. Similarly, inhibition of eIF4E-eIF4G interactions blocked the consolidation of extinction. We then examined the role of S6K1 in extinction. We found that both pharmacological inhibition and genetic ablation of S6K1 impaired acquisition of extinction. In addition, our biochemical studies indicate that S6K1 is regulated by extracellular signal-regulated kinase (ERK), but not mTORC1, during the acquisition of extinction. Moreover, we found that S6K1, via ERK activation, phosphorylates the GluA1 subunit the AMPA receptor during extinction. Overall, the results of our studies indicate that mTORC1 and its downstream effectors are critically involved in the acquisition and consolidation of cued fear extinction.

Disclosures: **T.N. Huynh:** None. **E. Santini:** None. **E. Klann:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Howard Hughes Medical Institute (KJR)

Title: Subcellular localization of HDAC4 affects amygdala-dependent fear memory

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Abstract: Epigenetic alterations have recently been implicated in the initial consolidation of amygdala-dependent fear memories. While much work has noted the regulation of histone acetylation in fear memory processes, little is known about the specific role that histone deacetylases (HDACs), which mediate the deacetylation of histone tails, might have in fear memory consolidation. Motivated to understand the epigenetic alterations that may be associated with human clinical PTSD, our group has previously noted that CpG methylation in HDAC4 is associated with PTSD. While much work has examined the role of HDACs in learning and memory employing pan HDAC inhibitors in rodent models, very little is known about the role that specific HDACs play in mediating the initial formation of fear memories. HDAC4 is a class IIa HDAC whose subcellular localization can be cytoplasmic or nuclear and is regulated by Ca2+ and NMDAR-mediated signaling cascades; a level of regulation not yet explored *in vivo* with fear memory formation. Recent work in rodent models has noted that repression of HDAC4 is associated with deficits in spatial learning and hippocampal-dependent contextual fear memory consolidation (Sando et al 2012; Kim et al 2012) suggesting a role for HDAC4 in memory. Despite this progress, the role of HDAC4 in amygdala-dependent auditory fear memory has not been elucidated. Using auditory fear conditioning, we examined the regulation of HDAC4 mRNA in the amygdala 2h following tone-shock pairings and revealed training-related regulation of HDAC4 mRNA. Next, using cytoplasmic or nuclear restricted HDAC4 viral constructs in the amygdala we examined if the subcellular localization of HDAC4 impacts fear memory formation. While we found no difference in freezing during fear acquisition, 24h later mice expressing nuclear-restricted HDAC4 show impaired fear memory consolidation compared to the cytoplasmic-restricted and GFP-control animals. Given the role of HDACs in regulating transcriptional processes we examined how HDAC4 may mediate the transcriptional processes underlying fear learning and memory by examining HDAC4 occupancy at gene loci using ChIP. We found a reduction in HDAC4 occupancy at the BDNF locus following conditioning, at a timepoint when the mRNA expression of BDNF has been shown to increase with fear conditioning. These data suggest that nuclear accumulation of HDAC4 likely functions as a negative regulator of fear memory-related plasticity through impeding training-related transcriptional processes, such as the transcription of BDNF. In sum, these findings strongly suggest that HDAC4 and its subcellular localization impacts fear memory consolidation.

Disclosures: **S.A. Maddox:** None. **R.D. Penrod:** None. **C.W. Cowan:** None. **K.J. Ressler:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: National Science Centre Grant DEC 2011/01/D/NZ3/02149

Title: Neuronal activation underlying recent and remote fear extinction memory

Authors: *W. A. SZADZINSKA, J. BUKOWCZAN, M. MIKOSZ, K. ROKOSZ, E. KNAPSKA;
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Abstract: Fear extinction memory is a susceptible memory that becomes weaker with the passage of time and is liable to the environmental changes. To understand the neuronal basis of this phenomenon, we subjected rats to Pavlovian fear conditioning with a tone (CS) co-terminated with a foot-shock (US) on the first day. On the following day rats were subjected to fear extinction - in a changed context. The test of the extinction memory was performed 1 or 28 days after extinction, in the original or changed context. Behavioral results (measured as the levels of freezing during CS) indicated that successful retrieval of extinction memory (low levels of freezing) occurred only in the group tested 1 day after extinction in the extinction context. c-Fos expression analysis in the prefrontal cortex, amygdala and hippocampus revealed distinguishable patterns of activation in different groups. Different patterns of activation of amygdala inhibitory neurons (GAD67 positive) were also observed. 28 days after extinction, we observed different, context-dependent patterns of the activation of prefrontal cortex layers. Further, we investigated active projections from the basolateral amygdala and ventral hippocampus to the prelimbic part of prefrontal cortex with the use of anterograde tracing in a transgenic rat in which neurons express a dendritically-targeted PSD-95:Venus fusion protein under the control of a c-fos promoter. Co-localization of projections and construct-positive neurons revealed that the basolateral inputs are highly activated in the groups expressing high levels of fear. The results show different patterns of brain activation underlying recent and remote fear extinction memory, and suggest that the basolateral inputs to the prelimbic cortex may drive retrieval of fear memory.

Disclosures: W.A. Szadzinska: None. **J. Bukowczan:** None. **M. Mikosz:** None. **K. Rokosz:** None. **E. Knapska:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: Australian National Health and Medical Research Council Project Grant 1061705

Title: Distinct roles of the basolateral amygdala and infralimbic cortex in the extinction of restored fear

Authors: *N. LINGAWI[1], V. LAURENT[2], R. WESTBROOK[1];
[1]Psychology, Univ. of New South Wales, Sydney, Australia; [2]Psychology, Univ. of Sydney, Sydney, Australia

Abstract: The basolateral amygdala (BLA) has been consistently implicated in fear extinction. However, fear spontaneously recovers after extinction with the passage of time, and is renewed by a change in context. Previous research in our lab suggests that the mechanisms underlying initial and subsequent extinction in fact differ. For example, the BLA is not required to re-learn inhibition of fear after reconditioning. It has been proposed that compensatory mechanisms in the infralimbic prefrontal cortex (IL) are recruited to relearn fear inhibition during times when the BLA is inactive. In a series of experiments, we examined the roles of the BLA and IL in extinction of recovered and renewed fear. These behavioral paradigms directly mimic the circumstances under which relapse of anxiety disorders are likely to occur in patient populations. We confirmed that inactivation of the BLA impaired long-term extinction learning. However, inactivation of the BLA during extinction of recovered and renewed fear spared long-term extinction learning. These results suggest that the BLA is not required to learn extinction of recovered fear if initial extinction occurred while the BLA was intact. We also found that pharmacological stimulation of the IL facilitated long-term extinction of renewed fear. Together, these results suggest that the BLA and IL work in concert to learn inhibition of fear after restoration, and provide insight into the neural mechanisms of fear inhibition after relapse.

Disclosures: **N. Lingawi:** None. **V. Laurent:** None. **R. Westbrook:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MHO86591

Title: Small conductance calcium-activated K+ channels in the prelimbic cortex modulate extinction of fear memory in male C57BL/6J mice

Authors: *J. C. LORA, L. SHARVIT, R. W. STACKMAN, Jr.;
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Abstract: Post-traumatic stress disorder (PTSD) is characterized by the resurgence of a memory of a traumatic event by an external stimulus. Common behavioral symptoms of PTSD are aggressiveness, irritability, and decrease in attention among many others. Understanding the underlying brain mechanisms of PTSD and the ways to attenuate aberrant behavioral manifestations is of great interest. One current project focuses on small conductance calcium-activated potassium (SK) ion channels, which are expressed in brain regions important for long-term memory. SK1, SK2 and SK3 channels limit the development of learning-dependent synaptic plasticity, and memory for spatial, non-spatial and emotional information. We have previously reported that drugs that block SK channels facilitate learning and memory, while drugs that activate SK channels impair learning and memory. In the present fear extinction study, adult male mice received three pairings of a 30-s tone (CS) that co-terminated with a 1-s mild foot-shock (US). Twenty-four hr after training, mice received local bilateral microinfusions of an SK2 channel activator, CyPPA or vehicle control, into the prelimbic cortex, and then were presented with 20 CS-alone extinction trials. Freezing, a measure of fear elicited by the CS, was found to decline significantly in the vehicle control mice over the course of the extinction trials. In contrast, CyPPA-treated mice exhibited a significant impairment in the acquisition of fear extinction. These results indicate that activation of prelimbic SK2 channels delays extinction of fear memory. We are currently testing whether blocking prelimbic SK2 channels can facilitate fear extinction in mice. This work represents a first step in determining the efficacy of SK channels to modulate the manifestation of behaviors that may relate to symptoms expressed in PTSD. SK channels may prove to be an effective target to include in an effort to reduce the PTSD symptomology in order to improve cognitive recovery.

Disclosures: **J.C. Lora:** None. **L. Sharvit:** None. **R.W. Stackman:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: DA017949

Title: High-affinity α4β2 nicotinic receptors are required for the impairing effects of acute nicotine on contextual fear extinction

Authors: *M. G. KUTLU, E. HOLLIDAY, T. J. GOULD;
Temple Univ., Philadelphia, PA

Abstract: Previously, studies from our lab have shown that while acute nicotine administered prior to training and testing enhances contextual fear conditioning (e.g. Gould & Higgins, 2003), acute nicotine injections prior to extinction sessions impair extinction of contextual fear (Kutlu & Gould, 2014). There is also strong evidence showing that the acute nicotine's enhancing effects on contextual fear conditioning require high-affinity α4β2 nicotinic acetylcholine receptors (nAChRs) but not low-affinity α7 nAChRs (Davis Gould, 2006; Davis et al., 2007; Gould et al., 2012). However, it is unknown which nAChR subtypes are involved in the acute nicotine-induced impairment of contextual fear extinction. In this study, we investigated the effects of acute nicotine administration on contextual fear extinction in knock-out (KO) mice lacking α4, β2 or α7 subtypes of nAChRs and their wild-type (WT) littermates. Both KO and WT mice were first trained and tested for contextual fear conditioning and received a daily contextual extinction session for 4 days. Subjects received intraperitoneal injections of nicotine (0.18 mg/kg) or saline 2-4 mins prior to each extinction session. Our results showed that similar to the acute nicotine's enhancing effects on contextual fear conditioning, the mice lack α4 and β2 subtypes of nAChRs showed normal contextual fear extinction but not the acute nicotine-induced impairment while the mice that lack α7 subtype showed both normal contextual extinction and nicotine-induced impairment of contextual extinction. These results clearly demonstrate that activation of high-affinity α4β2 are necessary for the acute nicotine's impairing effects on contextual fear extinction.

Disclosures: **M.G. Kutlu:** None. **E. Holliday:** None. **T.J. Gould:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: Austrian Science Fund FWF: SFB F4410

Signal Processing In Neurons (SPIN)

Title: Rescuing impaired fear extinction is associated with the regulation of miRNAs in the amygdala

Authors: *C. MURPHY[1], V. MAURER[1], R. GSTIR[2], S. SCHAFFERER[2], N. WHITTLE[3], A. HÜTTENHOFER[2], N. SINGEWALD[1];
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Abstract: A particular challenge in the treatment of anxiety disorders, including exposure-based therapy (EBT), is to obtain long-lasting benefits. Mimicking anxiety patients which show delayed and/or reduced extinction of fear (Duits et al, 2015), 129S1/SvImJ (S1) mice exhibit profound resistance to induce fear extinction. This impaired fear extinction in S1was shown to be rescued by dietary Zn2+ restriction (ZnR), facilitating long-lasting and context-independent fear inhibitory memories. Here we aim to reveal mechanisms involved in the formation of these fear inhibiting memories including the regulation of de novo gene expression by microRNAs (miRNAs) which control gene expression by targeted translational repression and/or mRNA degradation. We assessed the regulation of miRNAs in an extinction relevant brain area, the amygdala (AMY) of S1 mice after ZnR-induced rescue of deficient fear extinction. Microarray-based analysis revealed the regulation of a number of miRNAs after the rescue of impaired fear extinction. Using diverse bioinformatic platforms we identified promising candidate miRNAs for further investigation. We focused on a candidate miRNA that has been previously linked with the positive regulation of the signalling cascades MAPK/ERK and PI3/ARK. MAPK/ERK activation in the basolateral amygdala (BLA) is required for the consolidation of fear extinction. Indeed, fluorescent *in situ* hybridization (FISH) revealed an increased expression of this miRNA in the BLA of S1 mice after rescuing impaired fear extinction. Using Immuno-staining coupled with FISH, we then assessed the role of miRNA mediated activation of the MAPK/ERK signalling cascade by examining the levels of phosphorylation of p44/42 (MAPK(ERK1/2). Understanding the role of miRNAs in the formation of long-lasting fear inhibitory memories could provide novel targets for the treatment of anxiety disorders such as post-traumatic stress disorder. Supported by the Austrian Science Fund FWF: SFB F4410, Signal Processing In Neurons (SPIN).

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Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: NIH COBRE, 1P20GM103653

University of Delaware Research Foundation Award

Title: Using measures of transcriptional activity to determine mnemonic processes through which SPS exposure leads to extinction retention deficits

Authors: *D. K. KNOX[1,2], B. R. STANFIELD[2], J. M. STAIB[2], S. M. KELLER[2], S. P. ALBANESE[2];
[1]Psychology, [2]Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Previous research has demonstrated that single prolonged stress (SPS), a rodent model of post traumatic stress disorder (PTSD), induces deficits in extinction retention (i.e. the ability to inhibit conditioned fear responding to a previously extinguished fear conditioned stimulus (CS)). While this effect has been consistently observed, it is unknown if SPS disrupts extinction retention by enhancing fear memory, disrupting extinction memory, or some combination of both processes. We previously attempted to use behavioral manipulations to address this question, but the results of these experiments were inconclusive. In this study, we examined mnemonic processes through which SPS exposure results in extinction retention deficits by assaying c-Fos levels in fear and extinction circuits after fear conditioning, extinction training, and extinction testing in SPS and non-stressed rats. If SPS disrupts extinction retention by enhancing fear memory or disrupting extinction memory, then relevant memory-specific changes in c-Fos levels should be observed with SPS exposure after fear and/or extinction memory formation. Groups of SPS and non-stressed rats were either fear conditioned (CS-fear) or presented with CSs in the absence of any footshock (CS-only). Subsets of SPS and non-stressed rats were then euthanized after fear conditioning, extinction training, or extinction testing. A separate set of SPS and non-stressed rats were euthanized after removal from their housing colony in order to establish basal levels of c-Fos in each brain region. Semi quantitative measures of c-Fos levels were measured in the medial prefrontal cortex (mPFC), dorsal hippocampus (dHipp), ventral hippocampus (vHipp), lateral amygdala (LA), and basal amygdala (BA). In replication of previous studies, SPS induced extinction retention deficits. Preliminary findings suggest that SPS exposure decreases baseline c-Fos levels in the vHipp, LA, and BA. Furthermore, enhanced c-Fos expression in the mPFC, LA, BA, and vHipp were observed with CS-fear and CS-only presentations in SPS rats during fear conditioning. While the study is ongoing, the results support the hypothesis that SPS-induced changes in transcriptional activity prior to and during CS memory formation contribute to extinction retention deficits in the SPS model.

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Poster

175. Modulating Fear, Learning, and Memory

Location: Hall A

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Program#/Poster#: 175.21/Z6

Topic: F.02. Animal Cognition and Behavior

Title: Altered fear extinction in a rodent model of mild traumatic brain injury and posttraumatic stress disorder

Authors: *J. A. GRECO[1,2], I. LIBERZON[1,2];
[1]Univ. of Michigan, Ann Arbor, MI; [2]VA Ann Arbor Healthcare Syst., Ann Arbor, MI

Abstract: Background: Mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) are common, debilitating conditions that often co-occur following trauma. Changes in fear-associated learning have been found in rodent models of PTSD and mTBI, and in PTSD patients. However, limited work has explored changes in fear-associated learning following both PTSD and mTBI. In this study, we explored the effect of controlled cortical impact (CCI) and single prolonged stress (SPS), models of mTBI and PTSD, on fear-associated learning and extinction in rodents. Methods: Experiment 1: 12 Sprague-Dawley rats underwent CCI (5 mm impact, 1.5 mm depth, 3.0 m/s velocity, 100 ms dwell time). An additional 12 rats received craniotomies without receiving impact. Seven days after CCI or sham injury, all rats underwent a 3-day fear learning and extinction paradigm in individual observation chambers. Freezing during the acquisition, extinction and extinction recall phase was scored and analyzed. Experiment 2: 24 Sprague-Dawley rats underwent CCI, and 24 rats received craniotomies without receiving impact. Seven days after CCI or sham injury, 12 rats from each group underwent SPS, while another 12 rats from each group remained in their cages. Seven days later, all rats underwent the 3-day fear learning and extinction paradigm. Freezing during all phases was scored and analyzed. Results: No significant results were seen during fear acquisition. During fear extinction, repeated measures ANOVA found a significant interaction of injury x time in both Experiment 1 ($F(8.486, 93.351) = 2.464$, $p = .016$) and Experiment 2 ($F(3.149, 100.756)=3.699$, $p=.013$). During fear extinction recall in Experiment 2, there was an interaction approaching significance of injury x time (Block*Injury: $F(4,128)=2.187$, $p=.074$). Conclusions: These results suggest that the rate of fear extinction is impaired in a model of mTBI in rodents. This impairment was seen in both experiments, with extinction occurring 8 days after CCI in

Experiment 1, and 15 days after CCI in Experiment 2. Immunohistochemistry will characterize the inflammation and damage following the injury to further examine the effect of mTBI and PTSD together on fear learning.

Disclosures: J.A. Greco: None. **I. Liberzon:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Title: Reconsolidation promotes the strengthening of extinction memory

Authors: *J. HAUBRICH, A. MACHADO, F. ZACOUTEGUY BOOS, A. P. CRESTANI, F. SANTANA, R. ORDONEZ SIERRA, L. DE OLIVEIRA ALVARES, J. A. QUILLFELDT; Federal Univ. of Rio Grande Do Sul, Porto Alegre, Brazil

Abstract: Brief reactivation of a previously stored memory can render it into a labile state that requires de novo protein synthesis in order to be re-stabilized and persist, a process called reconsolidation. Previous works have shown that this procedure can lead to memory strengthening. On the other hand, when an associative memory is persistently retrieved in the absence of the unconditioned stimulus, the consolidation of an extinction memory takes place. Although effective in suppressing fear at short delays, extinction fails to do so permanently since several relapse processes can take place, such as spontaneous recovery. In this work we evaluated whether the natural decay of an extinction memory can be counteracted by a reconsolidation process able to strengthen the trace. In all experiments, male wistar rats were trained in the contextual fear conditioning task and 24h later were re-exposed to the training context during 30 minutes without the US/footshock (extinction session). On experiment 1, rats were tested on day 3 and retested either on days 10, or 17, or 24, or 31, in order to assess the time-point when fear spontaneous recovery occur. On experiment 2, rats were again re-esposed to the context without the US on day 3, but only for 3 minutes (reactivation session), being injected with the protein synthesis inhibitor cyclohexemide (2 mg/kg i.p.) immediately after; on day 4, test was conducted in order to verify if the extinction memory did undergo reactivation-induced reconsolidation. On experiment 3, rats where tested on day 3 and a spontaneous recovery test was conducted on day 31 in order to confirm if the procedure can strengthen the memory trace; in between these two tests, on days 10, 17 and 24, one group of animals was periodically exposed to 3-min reactivation sessions while controls remained in their homecages.

First, we confirmed that the extinction memory undergoes a time-dependent decay, and spontaneous recovery of fear response is observable already after 21 days. The observed disruptive effect of cyclohexemide shows that, whatever takes place after a brief reactivation session, it requires a de novo protein synthesis in order to persist, suggesting that the extinction memory undergoes a labilization-reconsolidation process. When periodically reactivated, the time-dependent decay of the extinction memory was interrupted and no spontaneous recovery was observed for at least 4 weeks. Taken together, our results show not only that an extinction memory can be modified by reconsolidation, but that this procedure may allow for a long-lasting persistence of fear suppression.

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Poster

175. Modulating Fear, Learning, and Memory

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Program#/Poster#: 175.23/Z8

Topic: F.02. Animal Cognition and Behavior

Title: Dnmt3a2 is required for fear memory extinction

Authors: *H. BADING, T. J. HEMSTEDT, A. M. M. OLIVEIRA, H. E. FREITAG;
IZN At the Univ. of Heidelberg, Heidelberg, Germany

Abstract: During the past years, the regulation of epigenetic processes in memory formation and fear extinction have been shown to be crucial in a variety of studies (Oliveira et al., 2012, Bahari-Javan et al.,2012). However, the underlying molecular and cellular mechanisms are far from being understood. The expression level of the de novo DNA methyltransferase Dnmt3a2 has been previously shown to influence memory performance in young and aged mice (Oliveira et al., 2012). Here, we demonstrate that memory formation can be enhanced when Dnmt3a2 is overexpressed in the hippocampus of young adult mice. In addition, by using an inducible CreERT2/loxP system in combination with rAAV-mediated gene transfer, we show that overexpression of Dnmt3a2 leads to facilitated fear memory extinction and is further associated with an increase in expression levels of plasticity-related genes. Conversely, mice show an impairment in fear memory extinction when the level of Dnmt3a2 is decreased. Thus, besides of its impact on memory formation the level of Dnmt3a2 is further critical for fear memory

extinction. Therefore, regulating Dnmt3a2 expression may serve as novel strategy to treat anxiety and fear disorders, such as post-traumatic stress disorder.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: HHMI grant to Swarthmore College

Title: Preventing recovery of learned fear: A novel extinction procedure

Authors: *A. M. SCHNEIDER[1], P. E. SIMSON[3], M. LICHTEN[1], J. WALSH[1], D. KALAMARIDES[4], C. EVERBACH[2], L. G. KIRBY[4];
[1]Dept Psychol, [2]Dept Engin., Swarthmore Col., Swarthmore, PA; [3]Dept Psychology and Ctr. for Neurosci., Miami Univ., Oxford, OH; [4]Ctr. for Substance Abuse Res., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Following fear conditioning in a typical extinction experiment, animals are returned to and remain in the apparatus in the absence of shock until fear is extinguished. The effect of extinction is often temporary: with passage of time fear recovers. This recovery is thought to result from an inhibitory memory acquired during extinction that temporarily suppresses the otherwise intact fear memory. In contrast to the typical extinction procedure, we recently used a modified extinction procedure in which animals, following contextual fear conditioning, were exposed to the conditioning apparatus in the absence of shock for a brief period (30-sec); thus, animals were removed from the apparatus before an opportunity developed to recall fear to a significant degree. A retention test the next day revealed that the brief-exposure was effective in weakening retention of fear. What remained to be determined were the endurance of the brief-exposure effect and the degree to which it could be manipulated. To this end, in the present study, male Long-Evans rats underwent contextual fear conditioning followed 24 hr later by the brief-exposure procedure. Fear conditioning consisted of placing rats in a dark compartment for 120 sec followed by a single footshock (0.8 mA, 0.5 sec); the brief-exposure procedure consisted of confining the animals to the dark compartment in the absence of shock for 0 sec, 30 sec, 60 sec or 180 sec or for two 30-sec exposures separated by 10 min (the 30-sec/10 min/30-sec

condition). Level of fear (freezing behavior) was monitored during the exposure conditions and a retention test to assess recovery of fear was administered either 1 or 13 days later. The results indicated that fear increased during exposure in all conditions with one exception: fear decreased in the 30-sec/10 min/30-sec condition. In addition, retention of fear decreased in the short term (retention test 1 day later) and recovered in the long term (retention test 13 days later) in all conditions with one exception: retention of fear did not recover in the 30-sec/10 min/30-sec condition. To account for these results, we propose that learning occurs during the exposure conditions, but the type of learning is dependent on the level of fear during the exposure. If fear is relatively high (as a result of 30, 60 or 180 sec exposure) inhibitory learning (or equivalently prediction error) occurs, the original fear memory is temporarily suppressed and retention of fear recovers. If fear is relatively low (as a result of the 30-sec/10 min/30-sec condition) the animal learns to associate weak fear with the apparatus, the original fear memory is displaced and retention of fear does not recover.

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Poster

175. Modulating Fear, Learning, and Memory

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Support: EMBO Long-Term Fellowship

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Title: Sub-region specific prefrontal-amygdala interactions during rapid eye movement (REM) sleep underlies consolidation of extinction memory

Authors: *S. DUVARCI[1], D. POPA[2], T. SIGURDSSON[1], D. PARE[3];
[1]Goethe-University Frankfurt, Frankfurt, Germany; [2]IBENS, Paris, France; [3]CMBN, Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Animals must identify and react appropriately to stimuli that signal danger in their environment but they must also adapt their behavior when those stimuli no longer predict danger. A well-studied example of this process is fear extinction, during which animals decrease their

fear responses to a conditioned stimulus (CS) previously associated with an aversive unconditioned stimulus (US). The available evidence suggests that fear extinction represents a new form of learning rather than the erasure of the original CS-US association. There is also evidence to suggest that extinction learning, like other forms of learning, requires consolidation in order to stabilize the extinction memory. Sleep has been shown to play an important role in memory consolidation and REM sleep in particular has recently been shown to be important for the consolidation of extinction learning. However, the neural network mechanisms underlying the consolidation of extinction memories during sleep remain unknown. The prefrontal cortex (PFC) plays a key role in extinction, likely via its interactions with the basolateral amygdala (BLA). Notably, different PFC sub-regions play opposing roles in this process, with the IL mediating fear extinction and the PL promoting fear expression. We therefore recorded neural activity in BLA, PL and IL in rats during sleep after fear extinction learning. We quantified BLA-PFC interactions by measuring correlations in the power of theta oscillations in the two structures, which recent studies have associated with the regulation of fear and anxiety. We found a selective increase in IL-BLA theta power correlations during REM sleep following fear extinction learning. In contrast, PL-BLA theta power correlations did not change. Although all animals displayed extinction learning, freezing levels varied considerably during the extinction recall test the next day, reflecting variability in extinction memory consolidation. Strikingly, theta power correlations between BLA and IL during REM sleep negatively correlated with freezing levels during extinction recall. In contrast, theta power correlations between BLA and PL showed the opposite relationship. Our results suggest that theta coordination in the prefrontal-amygdala circuit during REM sleep underlies consolidation of fear extinction in a prefrontal sub-region specific manner.

Disclosures: **S. Duvarci:** None. **D. Popa:** None. **T. Sigurdsson:** None. **D. Pare:** None.

Poster

175. Modulating Fear, Learning, and Memory

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 175.26/Z11

Topic: F.02. Animal Cognition and Behavior

Support: FAEPA

CNPQ

CAPES

FAPESP

Title: Medial prefrontal cortex NMDA and CB1 receptors interaction modulating reconsolidation and extinction contextual fear conditioning memory

Authors: *D. G. REIS, A. FASSINI, D. C. LAGATTA, S. F. LISBOA, L. B. M. RESSTEL; Univ. Sao Paulo, Ribeirao Preto, Brazil

Abstract: Introduction: The ventral portion of medial prefrontal cortex (vMPFC) is involved in the modulation of processes involving learning and memory, especially in processes of expression and extinction of conditioned emotional response (CER). Studies showed that vMPFC NMDA and CB1 receptors present opposing roles on behavioral and autonomic modulation during expression of the CER. However, the precise role of a possible interaction between these receptors on autonomic and behavior responses associated to reconsolidation and extinction of contextual fear memories has not be fully studied. The aim of the present study was to investigate the effect of simultaneous antagonism of NMDA and CB1 receptors in vMPFC on reconsolidation and extinction processes, by CER responses evoked by re-exposure to an aversively conditioned context. Methods: Male Wistar rats (250g) had a radio-telemetry probe implanted for recording autonomic activity and guide cannulas in vMPFC for drug administration. Day 1: Preconditioning started 1 wk after guide cannula implantation and consisted of one 10-min-long pre-exposure (habituation) in the footshock box. After 4h, the animals received 4 electrical footshock (FS) (0.8 mA, 1s). Day 2: Two groups were tested, reconsolidation group (5 min session) or extinction group (30 min session) re-exposure without FS presentation. Each group received two intra vMPFC injections (1st vehicle (V) or CB1 antagonist AM251 30 pmol (AM) / 2nd vehicle or NMDA antagonist LY235959 0.4 nmol (LY) 15 or 10 min before reconsolidation or extinction sessions forming 4 groups (V x V / V x LY / AM x V / AM x LY). Day 3: The test session take place in the next day consisting of a 10-min re-exposure to the FS box without any shock or injection given. Results: Reconsolidation Day 2: No significant differences were observed between all groups on CER (Freezing: $F(3, 19)=0.4$; $P>0.05$ ΔMAP: $F(3, 85) = 1.386$ $P<0.05$ ΔHR: $F(3, 85) = 0.7$ $P<0.05$ ΔCT: $F(3, 85)=22$; $P<0.05$). Day 3: Group AM x V presented an elevated expression of CER compared to all other groups (Freezing: $F(3,19)=8.9$; $P<0.05$ ΔMAP: $F3,85=13$ $P<0.05$ ΔHR: $F3,85=42$; $P<0.05$ ΔCT: $F3,85=22$; P0.05 ΔMAP: $F (3, 102) = 1,1$ $P>0.05$ ΔHR: $F (3, 102) = 0.6$ $P>0.05$ ΔCT: $F (3, 102) = 0.3$ $P>0.05$. Day 3: Group AM x V presented an elevated expression of compared to all other groups (Freezing: $F(3,19)=8.9$; $P<0.05$ ΔMAP: $F3,85=6.37$ $P<0.05$ ΔHR: $F3,85=19$; $P<0.05$ ΔCT: $F3,85=31$; $P<0.05$). Conclusion: The interaction between NMDA and CB1 receptors in vMPFC modulates both reconsolidation and extinction process associated to contextual fear memories.

Disclosures: D.G. Reis: None. **A. Fassini:** None. **D.C. Lagatta:** None. **S.F. Lisboa:** None. **L.B.M. Resstel:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: FAPESP Grant 2013/04741-5

Title: Pharmacologically-induced grooming behavior: grooming patterning and its relation with fear extinction

Authors: *A. E. REIMER[1,4], A. R. DE OLIVEIRA[5,4], J. B. DINIZ[2], M. Q. HOEXTER[2], S. CHIAVEGATTO[3], R. G. SHAVITT[2], E. C. MIGUEL[2], M. L. BRANDÃO[1,4];
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Abstract: Self-grooming consists of complex sequences of movements directed to clean and maintain the fur and skin of the head and body. The complex patterning of grooming in rodents is highly sensitive to stressors, which can alter the quantity, the regional distribution and the transitions between grooming stages. Additionally, grooming behavior can be induced pharmacologically by compounds that alter serotonergic or oxytocinergic neurotransmissions, both supposed to be involved in obsessive-compulsive disorder (OCD) physiopathology. Since recent evidence have indicated that OCD patients show important impairments in fear extinction, in the present study we evaluated pharmacologically-induced grooming patterns and its relation with conditioned fear expression and extinction. For this, male Wistar rats were submitted to a contextual fear conditioning training and re-exposed to the aversive context for three consecutive days (extinction sessions). Before the first extinction session or before each extinction sessions, rats received intra-central amygdala administration of oxytocin (OXT) or intraperitoneal administration of mCPP (a nonspecific serotonergic agonist). Grooming behavior was evaluated before first extinction session, immediately after drug treatments. Freezing behavior was evaluated for 10 min, in each one of the three extinction sessions. Both OXT and mCPP increased grooming expression but, OXT-treated rats presented an altered grooming regional distribution (increased caudal grooming), when compared with control group. When single administered, OXT and mCPP did not produce effects on fear extinction. However, when administered before each extinction session, OXT enhanced fear extinction throughout the sessions while mCPP impaired extinction. Present data indicate that grooming can be modified by different pharmacological approaches and it is possible that these different grooming patterns

could be associated with distinct emotional states. Besides, the grooming induced by OXT, which has been previously proposed as an OCD animal model, seems not to be associated with impaired fear extinction, a deficit observed in OCD patients. Further analyses on grooming syntactic chain could help to examine other possible relations between grooming behavior and anxiety disorders.

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Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA034010

Title: Early adversity disrupts the adult use of aversive prediction errors to reduce fear in uncertainty

Authors: *K. M. WRIGHT, A. DILEO, M. MCDANNALD;
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Abstract: Early adversity increases anxiety in adult rodents and primates, and increases the risk for post-traumatic disorder (PTSD) in humans. Long-studied in the context of reward, recent work highlights the role of prediction errors in aversive learning. Increased anxiety in adverse-experienced individuals may partially result from an inability to utilize negative aversive prediction errors (−APEs) to reduce fear. To test this hypothesis, we gave adolescent rats a battery of adverse experiences from postnatal day 26 to 35, then assessed their ability to reduce fear in a probabilistic Pavlovian fear conditioning task during adulthood (beginning postnatal day 75). In the task, rats were confronted with three cues associated with different probabilities of foot shock: one cue (A1) never predicted shock - 0.00, another cue (A2) predicted shock with uncertainty - 0.25, and a final cue (A3) always predicted shock - 1.00. Control rats initially acquired fear to all three cues, but reduced fear to both the A2 and A3 cue halfway through discrimination trials, maintaining these levels for the remainder of the task. Because A2 predicts shock with uncertainty, the use of -APEs is most likely required for A2 fear reduction. The low level of fear to the uncertain cue suggests Controls successfully used -APEs to reduce fear. Adverse-experienced rats (EA) were impaired in the reduction of fear to the uncertain cue and

the cue not predicting shock. While they eventually showed little or no fear to the non-predictive cue, by the end of discrimination trials, EA rats continued to show elevated fear to the uncertain cue. These results suggest early adversity impairs the use of -APEs to reduce fear, especially in situations of uncertainty.

Disclosures: K.M. Wright: None. **A. DiLeo:** None. **M. McDannald:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Topic: F.02. Animal Cognition and Behavior

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Title: Fear extinction combined with chronic treatment of fluoxetine in pyramidal neuron- and interneuron-specific TrkB heterozygous knockout mice

Authors: *J. UMEMORI[1], F. WINKEL[1,2], C. BUJ[3], E. CASTRÉN[1];
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Abstract: Major neuronal network plasticity is normally limited to the critical periods of early postnatal life. We have previously reported that antidepressant drug treatment induces a juvenile critical period-like plasticity (iPlasticity) in the adult brain. This process allows brain networks to better adapt to the internal and external milieu (1-3). We hypothesize that, in the context of neuropsychiatric disorders, iPlasticity allows psychotherapy to guide plastic networks resulting in a more effective treatment. In fact, combination of extinction training and chronic fluoxetine (Flx) treatment, but neither treatment alone, was demonstrated to induce an enduring loss of conditioned fear memory in adult mice (2) similar to the permanent fear extinction found in early postnatal mice (4,5). Chronic Flx treatment increases expression levels of Brain-derived neurotrophic factor (BDNF), and our laboratory showed that its expression is necessary and sufficient for iPlasticity in the visual cortex and amygdala (1, 2). BDNF binding to TrkB receptors causes dimerization and autophosphorylation of TrkB and results in transcription of genes that stimulate neuronal survival, outgrowth and neurogenesis (6). However, it is not clear whether TrkB activation is necessary for iPlasticity or in which neuronal subpopulations expression of TrkB is required. In this study, we used mice lacking TrkB in Calcium/Calmodulin-Dependent Protein Kinase II Alpha (aCaMKII) and Parvalbumin (PV)

expressing neurons to test fear extinction with chronic Flx treatment. We used heterozygotes since homozygous aCaMKII- and PV- TrkB KO mice had hyperactive phenotype and vestibular dysfunction respectively (7,8), which disturbs further analysis. Our preliminary experiments show that PV-TrkB KO mice treated with Fluoxetine have lower extinction compared to control mice, suggesting that TrkB expression in PV neurons are involved in erasure of fear memories. We will also discuss the possible roles of TrkB expressed in pyramidal neurons and interneurons for fear extinction. 1. Maya Vetencourt JF et al., *Science* 320, 385 (2008) 2. Karpova NN et al., *Science* 334, 1731 (2011) 3. Sale A et al., *Trends Neurosci* 32, 233 (2009) 4. Kim JH and Richardson R, *Biol. Psychiatry* 67, 297 (2010) 5. Gogolla N, et al., *Science* 325, 1258 (2009) 6. Castrén E, *Nat Rev Neurosci* 6, 241 (2005) 7. Zorner B et al., *Biol Psychiatry* 54, 972 (2003) 8. Lucas EK et al., *Behav Brain Res* 274, 219 (2014)

Disclosures: J. Umemori: None. **F. Winkel:** None. **C. Buj:** None. **E. Castrén:** None.

Poster

175. Modulating Fear, Learning, and Memory

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Support: NIMH (R01 MH084966)

U.S. Army Research Office and the Defense Advanced Research Projects Agency (grant W911NF-10-1-0059)

Title: Fear extinction modulates AMPA receptor expression in the nucleus accumbens

Authors: *A. MCGRATH[1], S. S. CORREIA[2], K. A. GOOSENS[2];
[2]Dept. of Brain and Cognitive Sci. and McGovern Inst. for Brain Res., [1]MIT, Cambridge, MA

Abstract: Fear extinction is a process in which fear responses are weakened through repeated exposure to the fearful stimulus in a safe environment. It has been used in clinical settings to treat fear-based psychiatric illnesses such as phobias, panic disorder, anxiety and post-traumatic stress disorder. The nucleus accumbens (NAc), a striatal region well-known for its involvement in reward processing, is thought to also participate in aversive memory. In addition, the NAc is believed to be important for fear extinction. It has been previously demonstrated that antagonism of the AMPA receptor in the NAc can modulate both appetitive and avoidance behavior, suggesting that AMPA receptors in the NAc are critical for responding to motivational stimuli.

Given that the NAc has been implicated in fear extinction, and that AMPA receptors in the NAc impact emotional behavior, we hypothesized that fear extinction would change the expression of AMPA receptors in the NAc. In this experiment, rats underwent fear conditioning and varying degrees of extinction training. One hour after the final training day, the rostral and caudal NAc were harvested, and Western blot analysis was used to determine the levels of the AMPA receptor subunits GluA1 and GluA2. We found that both fear conditioning and extinction modulate expression of AMPA receptors in the NAc, and that AMPA receptor levels correlated with freezing levels during extinction. A better understanding of the molecular basis of fear extinction can contribute to the improvement of treatments for patients with fear-based psychiatric diseases.

Disclosures: **A. McGrath:** None. **S.S. Correia:** None. **K.A. Goosens:** None.

Poster

176. Decision Making: Primates

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust 095495

Michael Foster PhD studentship in Physiology

Title: Performance of formal economic coordination and cooperation games by macaques

Authors: *C. R. VAN COEVERDEN[1], W. SCHULTZ[2];
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Abstract: Coordination and cooperation are necessary for the survival of social animals. Coordination merely requires the organisation of actions to work together. In addition to this, cooperation also requires an actor to forgo some of her immediate payoff to obtain a later, higher mutual outcome. A well validated economic game for investigating cooperation is the prisoner's dilemma (PD). In PD the highest individual reward can be obtained by defection when the opponent chooses to cooperate. This highest reward is also commonly referred to as the temptation value. Recent studies show that macaques cooperate in a significant proportion of trials when playing an iterated PD. However, it is not clear to what extent the temptation value in otherwise similar games drives differences in levels of coordination and cooperation.

Furthermore, it is currently unclear whether and how the animals use information from other animals' choices to inform their own. In our experiment two macaques indicated choices (cooperation or defection) on a touchscreen to obtain rewards. The reward magnitudes were dependent on the choices of both animals. In different versions of the task we varied the temptation value. This resulted in different games varying from a simple coordination game to more complex prisoner's dilemmas. Analyses of both macaques' choices revealed that there was a significant decline in the amount of coordination as a function of the temptation value (binary logistic regression: b=-.255,Wald=390, $p<0.0001$). In addition, there was an even sharper decline in cooperation in games with higher temptation values (b=-.440,Wald=1162, $p<0.0001$). Even though levels of defection in the prisoner's dilemmas where higher, macaques mutually cooperated on a significant number of trials (26.3%, Z=2.44p=0.0015). Interestingly, there was a significant increase in response time of the second chooser when the first chooser chose the cooperative option across all games. To summarize, our results suggest that macaques can coordinate their actions and cooperate in economic games. However, coordinating on a higher payoff becomes less frequent in prisoner's dilemmas where cooperation is required to overcome temptation. Macaques also seem to use the information from seeing the other's actions to adapt their behaviour when playing social economic games. This suggests that, in our experiment, macaques do not merely make choices based on their trial history but show a social component to their behaviour.

Disclosures: C.R. Van Coeverden: None. **W. Schultz:** None.

Poster

176. Decision Making: Primates

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Topic: F.02. Animal Cognition and Behavior

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National Institutes of Health Caltech Conte Center

Title: Role of primate amygdala neurons in decision-making

Authors: *F. GRABENHORST, W. SCHULTZ;
Univ. of Cambridge, Cambridge, United Kingdom

Abstract: The amygdala is considered a key system for reward and emotion with a classic function in fear; however, recent evidence suggests that it also participates in decision-making beyond reward processing. We recently showed that primate amygdala neurons signal economic choices (Grabenhorst et al., 2012) and plans for internal, distant reward goals (Hernadi et al., 2015). Despite these advances, the amygdala's role in decisions is poorly understood. Because the amygdala is implicated in a wide spectrum of behaviors and pathological conditions, it is critical to know whether its neurons express a decision function. Here we recorded the activity of 180 amygdala neurons as a monkey chose between sequentially presented visual objects based on learned reward probability and cued reward magnitude. On each trial, two objects appeared sequentially in a central position, separated by a delay, before reappearing in randomized left-right positions as saccade targets. Object-specific reward probabilities changed over trial blocks and had to be learned through experience; reward magnitudes changed trial-by-trial and were cued sequentially with the objects, thus requiring flexible computation of expected value. Our design separated valuation, decision-making and action planning: the monkey could not make its choice until seeing the second object and could not plan its action until seeing the final object positions. We found evidence for gradually unfolding decisions in amygdala neurons. At first object presentation, a significant number of neurons (18%) encoded object-specific values reflecting both reward probability and magnitude. Such 'object values' seem suited as inputs for a competitive decision process. At second object presentation, neurons encoded the value of the second object relative to the first object (value comparison, 17%) and upcoming choice for first or second object, irrespective of its identity (11%). At left-right object presentation, many neurons encoded the identity of the chosen object (21%), thus signalling the output of an object-based decision process. These results indicate that amygdala neurons encode the principal stages of reward-based decision-making: converting object value input via value comparison to object choice output. The data are consistent with a decision function for this key reward structure.
Grabenhorst, F., Hernadi, I., and Schultz, W. (2012). Prediction of economic choice by primate amygdala neurons. Proc Natl Acad Sci U S A 109, 18950-18955. Hernadi, I., Grabenhorst, F., and Schultz, W. (2015). Planning activity for internally generated reward goals in monkey amygdala neurons. Nature Neuroscience 18, 461-469.

Disclosures: F. Grabenhorst: None. **W. Schultz:** None.

Poster

176. Decision Making: Primates

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Title: Utility functions predict skewness preferences in monkeys

Authors: W. GENEST[1], *W. R. STAUFFER[2], W. SCHULTZ[1];
[1]Physiology, Develop. and Neurosci., [2]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Individuals must often choose between risky alternatives, and it has long been recognized that risk influences subjective value (utility). Risk modulated by the variance of outcome distributions is commonly studied in the lab. However, few real-world gambles are normally distributed, thus variance alone does not provide a full description of the risk for these gambles. Indeed, a large body of evidence suggests that individuals are also sensitive to risk modulated by skewness - the asymmetry of the outcome distribution. Moreover, skewness attitudes and variance attitudes stem from different properties of the utility function. They are therefore formally independent of each other. Here we sought to characterize skewness attitudes and investigate the relationship between skewness attitudes and utility functions. We derived utility functions from the choices two monkeys made between safe rewards and binary, equiprobable gambles. Such gambles are not skewed; hence the risk was modulated solely by the variance. The utility functions obtained from those choices predicted the subject value of eight new binary gambles, providing evidence for their numerical validity (R^2=0.734, P=10^{-12}). Next, we introduced skewness using gambles with three equiprobable outcomes. We measured the subjective values of these gambles and tested how well they could be predicted by our utility functions. Linear regression analysis revealed that these utility functions (derived using only variance) predicted well the subjective value of four skewed gambles (R^2=0.232, P=10^{-8}). The subjective value rankings of the skewed gambles suggested a preference for positively skewed gambles over negatively skewed ones. To test this we presented the animals with choices between positively and negatively skewed three outcome gambles (all with the same expected value and variance). They consistently preferred positively skewed gambles over negatively skewed ones (P = 10^{-6} in both animals). Thus, their choices were consistent with third order stochastic dominance (a measure of risk sensitivity that demonstrates meaningful incorporation of skewness into subjective value). Empirically derived utility functions were therefore useful behavioural measures for inferring risk preferences modulated by both variance and skewness. This lays the foundation for further investigations into the neural origins of risk and skewness attitudes, and the mechanism by which the brain computes economic utility.

Disclosures: W. Genest: None. **W.R. Stauffer:** None. **W. Schultz:** None.

Poster

176. Decision Making: Primates

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Title: Evaluating the neurobiology of strategic coordination in non-human primates

Authors: *W. S. ONG, M. L. PLATT;
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Abstract: Coordination and cooperation depend on effective social communication. Deciphering the neural basis of social coordination and communication is thus a priority. In humans, brain areas linked to both social behavior and reward are activated by cooperation. The neural processes indexed by these blood flow signals, however, remain unclear. We previously reported neurons in anterior cingulate cortex and amygdala signal vicarious reward in monkeys when they choose to reward another monkey. Such signals may inform computations mediating coordination and cooperation.To test this idea, we developed a new task based on the classic "chicken game." Two monkeys (M1 & M2) face each other across a shared monitor. Two colored annuli framing random dot motion arrays and 4 response targets are presented. Color indicates the annuli and targets belonging to each monkey. On ½ of trials, the larger reward (denoted by visual tokens) lies opposite the controlling monkey behind the opponent's annulus; smaller rewards lie to the left. To obtain the larger reward, M1 goes straight, but if M2 also goes straight the annuli collide and neither monkey gets reward. On some trials, a "cooperation bar" allows both monkeys to obtain larger rewards only if both choose to go left. If only one monkey yields he receives a smaller reward. On some trials, dot motion coherence is randomized to obscure intention signals. When playing a computer opponent, monkeys played the pure Nash equilibrium strategy of maximizing reward by choosing the left target when it offered more reward, regardless of the opponent's choice. When the straight target gave more reward, they played a mixed Nash equilibrium strategy by choosing straight unless the computer opponent signaled it would go straight; then, monkeys chose the left target and obtained less reward.Monkeys' deviated from the Nash equilibrium strategy when playing a live monkey opponent by going straight more often regardless of reward size or the opponent's choice. This behavior depended on both social dominance and information about the opponent's intentions. High-ranking monkeys challenged low-ranking monkeys, who tended to yield. Mid-ranking monkeys switched strategies depending on whether the opponent was higher or lower ranking. When intention signals were obscured, monkeys collided thrice as often as when intentions were

signaled clearly. These findings demonstrate strategic, competitive behavior in monkeys that depends on both social context and communication, thus validating our task as an effective tool to determine the neural basis of social coordination and cooperation.

Disclosures: W.S. Ong: None. **M.L. Platt:** None.

Poster

176. Decision Making: Primates

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Title: Pharmacological manipulation of the serotonergic system modulates the value-based decision making in non-human primate

Authors: *G. DRUI[1], Y. SAGA[1], A. RICHARD[1], P. N. TOBLER[2], V. SGAMBATO-FAURE[1], L. TREMBLAY[1];
[1]CNRS UMR5229 - Ctr. of Cognitive Neurosci., BRON, France; [2]Dept. of Econ. - Zurich Univ., Lab. for Social and Neural Systems Res., Zurich, Switzerland

Abstract: Beyond the behaviors associated with positive outcomes (approach towards appetitive stimuli), a lot of stimuli or events are associated with a negative outcome and induce aversive anticipation to produce avoidance or escape behaviors. Previous studies have linked the serotonin system to anxiety and impulsive behaviors and thus to an inappropriate aversive anticipatory process. To study the neurobiological mechanisms involved in such context, we have developed an instrumental delay task with a condition that allows the expression of aversive anticipations and associated behaviors. Four monkeys were trained to recognize conditioned stimuli (CS) associated with appetitive (juice) or aversive (air puff) outcome in two different conditions. In a choice condition, two opposite CS (i.e. appetitive and aversive) were presented simultaneously. After a delay, if the animals choose to select the target positioned where the appetitive CS was previously located, it performed an active avoidance from the aversive

outcome and an approach toward the reward in the same time. In an imperative condition, only one CS was presented, so monkeys must either approach the appetitive stimulus or actively avoid the aversive one. We showed that monkeys did perform approach for appetitive trials and avoidance for aversive trials. Progressively during the daily session, the strategy of the animals toward the aversive stimuli was modified as they produced premature responses and omissions, which could be both interpreted as escape behaviors. Interestingly, systemic fluoxetine, a selective serotonin reuptake inhibitor (SSRI), delayed these escape behaviors for all monkeys. These results suggest that the modulation of the serotonergic tone modifies the appetitive and/or the aversive encoding and thus the decision-making. The characterization of the fluoxetine's effect in the neural encoding of the appetitive and aversive information in cerebral structures which receive large input of serotonin fibers such as the ventral striatum could help to understand the pathophysiology of obsessive compulsive disorders, depression and anxiety related disorders for which SSRI are effective.

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Poster

176. Decision Making: Primates

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JSPS

Title: Facilitation negative decisions in the non-human primate by stimulating a ventral striato-pallidal pathway linked to the anterior insula

Authors: *Y. SAGA[1,2], G. DRUI[1], V. SGAMBATO-FAURE[1], C. RUFF[3], L. TREMBLAY[1]; [1]Ctr. de Neurosci. Cognitive, Bron Cedex, France; [2]Japan Society for Promotion of Sci., Tokyo, Japan; [3]Dept. of Econ., Lab. for Social and Neural Systems Res., Zurich, Switzerland

Abstract: The limbic territories of the Basal Ganglia (BG) - such as ventral striatum (VS) and ventral Pallidum (VP) - are thought to play important roles in goal-directed behavior by guiding decision to approach rewarding outcomes. However, using neuronal recordings in monkeys

performing a value-based choice task, we have recently identified in these BG structures neurons that selectively respond to negative conditioned stimuli or aversive outcomes (Saga et al. FENS, 2014; Richard et al. SFN, 2014). Recent imaging studies in humans show that negative events or decisions in a negative context lead to active in the anterior insula. Moreover, the anterior insula is known to have a projection to VS (Sgambato-Faure et al. 2014), suggesting that a limbic Cortico-BG circuit may process the negative values of stimuli that can inhibit behavioral approach and facilitate the active avoidance of negative outcomes. The purpose of the present study was to determine the role of the structures in this circuit (VS, VP and Insula) for aversive encoding, decision-making and context-adapted behaviors. For this purpose, we applied micro-stimulation specifically when animals were making value-based decisions. Two monkeys were trained to perform a choice task that involved conditioned stimuli (CS) that were associated with either an appetitive (juice) or aversive (air puff) outcome. When monkeys held the start lever, one or two CSs (single/dual cue conditions) were presented on the screen and were followed after a delay by two targets. Subsequently, monkeys made their choice by touching one of the targets located at the same (approach) or different (avoidance) position from the selected CS. Micro-stimulation (pulse-train of 700 ms with 0,2-0,6 mA as in Worbe et al. 2009) was applied before, during or after CS presentation and induced increases of non-initiated choice (omission) and escape reactions (premature responses) as well as disadvantageous choices (avoidance of positive outcomes or approach to aversive outcomes). Taken together, our micro-stimulation results combined with previous anatomical studies and neural recordings highlight the involvement of a Cortico-BG circuit (passing through the indirect pathway and closely linked to the anterior Insula) in negative value encoding and corresponding choices to escape or avoid disadvantageous outcomes in aversive contexts.

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Poster

176. Decision Making: Primates

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Global COE program A06 (to M.N.)

Title: Conflict between different task rules influences the prefrontal neuronal activities during behavioral choice

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Abstract: While we often use previously acquired rule to choose our behavior, sometimes we need to choose it based on immediate cue ignoring the acquired rule. How is such a flexible behavior realized? Previously, we have examined the neuronal activities of the medial and dorsolateral prefrontal cortex (MPF and DLPF) while the monkeys were performing a delayed matching-to-sample (DMS) task and a transverse patterning (TP) task, which needs long-term memory of relations between the visual cues. In the present study, we examined how the acquired TP rule influences the neuronal activities during performance of the DMS task. The TP task was a set of three visual discrimination problems, in which two out of the three visual stimuli (A, B, C) were presented simultaneously, and the monkey chose the target based on the rule, which was acquired by long-term practice (A+/B−, B+/C−, C+/A−, where + and − indicate the target and the distractor). In the DMS task, the same three stimuli were used, but the correct target was determined by the immediate sample cue that was randomly chosen in each trial. In a half of the trials (congruent trials), the target was the same as in the TP task (e.g., A+/B−), but in the other half (incongruent trials), the target was opposite (e.g., B+/A−). Both monkeys showed lower percent correct in the incongruent trials than the congruent trials ($P < 0.001$), showing interference from the acquired TP rule. We examined activities of 136 DLPF and 56 MPF neurons in the DMS task. The firing rates were analyzed in 2 task periods: cue period shortly after the presentation of the choice cues, and presaccade period immediately before the saccade onset. We examined the influence of the congruency with the TP rule on the neuronal activities by three-way ANOVA (factors: congruency, target shape, and target location). In the cue period, 5 DLPF (4%) and 1 MPF (2%) neurons showed the main effect of congruency. In the presaccade period, the numbers increased to 16 in DLPF (12%, $P = 0.019$) and 5 in MPF (9%, $P = 0.125$). In either area, the large majority of the congruency-dependent neurons showed higher firing rate in the incongruent trials during the presaccade period (13 out of 16 DLPF neurons, $P = 0.009$; 5 out of 5 MPF neurons, $P = 0.031$). These results suggest that the neuronal activities in both the DLPF and MPF, especially in the presaccade period just before the response execution, are affected by the conflict with the currently irrelevant rules in the process of behavioral choice depending on the immediate cue. Such increased activities in presaccade period may contribute to the execution of the correct behavior against the irrelevant rule(s).

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Poster

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Title: Hierarchical hidden Markov modeling of real time changes in strategic behavior in a game theoretic context

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Abstract: In a dynamic environment in which players are continuously interacting in a competitive game, these players must generate flexible strategies that can adaptively respond to their opponent's actions in real time. For example, consider a simplified two-player soccer match in which a kicker's objective is to dribble a ball into a goal, while a goalie's objective is to block the kicker. A strategy a kicker may consider is feinting to mislead the goalie into moving in the opposite direction of the kicker's intended scoring point. However, given the dynamic nature of this game, if the goalie were to be unaffected by this maneuver, then the kicker should adaptively change strategies in real time. Because strategies are adaptively adjusted in real time, it is difficult to determine precisely which strategy is being utilized at any given time. Indeed, a common approach to identify behavioral strategies is to cluster complete behavioral trajectories through k-means clustering or other clustering algorithms to elucidate behaviors. While useful for identifying games which, in their entirety, are played out in similar fashion, these methods are incapable of identifying particular strategies utilized at any given time. To address this issue, we consider a hierarchical hidden Markov model (HHMM) in which high-level states represent current strategies of the subject and low-level states represent current behavior. Sequences of high-level states are used to identify global patterns that summarize entire gameplay sequences, while low-level behavioral states making up a given sequence can be identified as the strategy the player is currently employing. We applied this approach to the aforementioned two-opponent game played by male rhesus macaques. The HHMM automatically discovered four global

patterns of behavior. More importantly, it also identified when the kicker initiated a change in behavior or adaptively transitioned to another. This approach reveals highly nuanced and sophisticated strategies in interactions between non-human primates in a competitive game setting that require advanced theory of mind capabilities and working memory of the other player's intentions and strategies. Here, we demonstrate a novel application of HHMM that identifies current strategies implemented throughout a continuous sequence of a competitive game. Not only does this analysis provide a concise representation of global patterns of behavior, but it may also serve to generate effective predictors of neural activity in higher-level brain areas, providing a novel paradigm for investigating the neural circuitry underlying real-time behavior and strategies in game theoretic contexts.

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Poster

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Support: ZIAMH002886

Title: Dissociating the functions of the macaque ventrolateral and orbitofrontal cortex

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Abstract: Orbitofrontal cortex (OFC) has long been associated with the flexible control of choice behavior in general and efficient performance of the object reversal learning task in particular. In large part, this is based on the repeated finding that aspiration lesions of the OFC in humans, monkeys, and rodents - among other mammals - lead to deficits in stimulus-reward reversal learning. Recently we showed that excitotoxic fiber-sparing lesions of macaque OFC do not affect learning and reversing stimulus-reward associations in a deterministic setting (Rudebeck et al., 2013, Nat Neurosci 16:1140). Evidence suggests that impaired performance on reversal tasks following aspiration of OFC is caused by damage to white matter tracts passing nearby OFC, which disrupts the connections and functions of other frontal cortex areas. The lack of effect on a deterministic task also left open the question of OFC contributions to stimulus-reward learning and reversal in probabilistic settings. Here, we set out to determine the role of

both the OFC (Walker's areas 11,13, and 14) and the ventrolateral prefrontal cortex (VLPFC, Walker's area 12), the area laterally adjacent to the OFC, in learning and reversing probabilistic stimulus-reward associations. Macaques (Macaca mulatta) were trained to perform a stimulus-based three-arm bandit task for food rewards. The probability of receiving a reward from any of the stimuli fluctuated over the course of 300-trial testing sessions. Both controls (n=8) and macaques with excitotoxic lesions of the OFC (n=4) were readily able to learn and track which of the three stimuli was associated with the highest probability of reward. In contrast, macaques with excitotoxic lesions of VLPFC (n=4) were severely impaired. Logistic regression analyses indicate that this was because monkeys with VLPFC lesions were unable to form contingent associations between stimuli and rewards. We are currently assessing whether VLPFC lesions affect all types of flexible choice behavior, including the ability to learn and update specific stimulus-outcome associations, a function dependent on OFC. Irrespective of this, our data are consistent with the idea that the deficits in flexible stimulus-reward learning and reversal previously ascribed to OFC are the result of unintentional damage to projections to or from VLPFC. This work was supported by the intramural research program of the NIMH.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Spatial selectivity of neurons in the primate prefrontal and parietal cortices

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Abstract: Both, the primate prefrontal cortex (Mikami et al., 1982; Suzuki, 1985) and the parietal cortex (Duhamel et al., 1997) are known to be visually and spatially selective. In the prefrontal cortex, neurons have been described as having "memory fields" which circumscribe the area of increased delay period activity of a certain neuron to a preferred object when it is presented in specific part of the visual field (Rainer et al., 1998). Thus, while retaining the representation of an object, they additionally retain its location by having spatial selectivity. Quite remarkably, this spatial selectivity is highly localized and contralateral in many neurons

and not foveal as long believed. Similarly, neurons in the ventral intraparietal area of the posterior parietal cortex have a sophisticated multi-modal representation of objects in space using different reference frames (Avillac et al., 2005; Zhang et al., 2004). These reference frames are sometimes found to shift from eye-centered to head-centered representations, possibly enabling spatial transformations during movement. In short, spatial selectivity in such higher order visual areas appears to subserve the cognitive functions they perform. To address this possibility in another domain, we record single unit responses simultaneously from both areas while monkeys fixate a central spot and a moving bar stimuli is used to scan the visual field for spatial selectivity. Additionally, we record the same units during a delayed match-to-sample task with centrally placed dot array stimuli. We first investigate the spatial selectivity of these cells. We compare the spatially restricted responses during the fixation task to their responses towards numerically rich stimuli during the active matching task. We further test the responses of these cells to varying directions and orientations of the moving bar stimuli.

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Poster

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Title: The neural representation of empty sets in macaque posterior parietal cortex

Authors: *A. RAMIREZ-CARDENAS, A. NIEDER;
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Abstract: A general magnitude system primarily hosted in the posterior parietal cortex is considered responsible for the representation of quantity in primates. However, in the small extreme of the natural numbers the neuronal correlates of zero remain unexplored, even when behavioral studies have shown that non-human primates do possess a pre-symbolic precursor of zero. We trained 2 rhesus monkeys in a delayed match-to-sample task involving as stimuli sets of black dots (0-4) presented against a gray background. We controlled for total dot area, dot density, background shape and area, and total luminance of the stimuli. Importantly, when empty sets were presented as sample, monkeys mistakenly matched them to numerosity 1 more frequently than to numerosity 2. This pattern in behavior, a ‘distance effect’, suggests empty sets

were ordered in the context of other numerosities and so treated in a quantitative way. Single-cell recordings in VIP (ventral intraparietal area) revealed a significant population of numerosity-responsive neurons (16.3% sample, 23% delay), most of which (77.9% sample, 82.8% delay) did not exhibit interactions between number and other factors in their responses. Just as for other numerosities, a distinct subpopulation of responses having zero as preferred numerosity was identified by tuning-curve clustering. Crucially, the averaged tuning curve of zero-preferring responses showed a graded decay in firing rate with increasing numerosities. Error analysis confirmed the behavioral relevance of such responses. Numerosity-preferring responses included empty sets in their response range as if placed in the numerical continuum. A decoding approach corroborated our findings. When a SVM classifier was trained on the responses of numerosity selective neurons, its performance in numerosity discrimination exhibited a distance effect. Altogether, our data show that single neuron responses in the posterior parietal cortex represent empty sets as endowed with null quantity. Unexpectedly, empty sets are actively encoded in this cortical region and find a place in the neural number line.

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Poster

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KAKENHI 23135518

Title: Preference judgment of visual items and contribution of the orbitofrontal cortex in monkeys

Authors: ***S. FUNAHASHI**, W. NAKAMOTO;
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Abstract: Both humans and animals like to watch neutral and biologically insignificant visual stimuli. Behavioral studies have revealed that animals more frequently select stimuli with symmetrical and regular patterns and short movies compared to stimuli with unsymmetrical and irregular patterns and photographs, respectively. Preferred visual stimuli can serve as rewards for

animals performing behavioral tasks. Therefore, preferences for visual stimuli could be related to the magnitude of the pleasant feelings that are experienced when the stimuli are seen. The orbitofrontal cortex is known to participate in the detection and prediction of reward, the estimation of the value of the stimuli as a reward, and positive emotion. Human neuroimaging studies have shown that the magnitude of orbitofrontal responses to the presentation of neutral visual stimuli correlates with the strength of the preference for the stimuli in the behavioral studies. In the present study, to examine whether the magnitude of orbitofrontal single-neuron responses to neutral visual stimuli correlates with the strength of the preference for these stimuli observed in the behavioral study, we first determined two Japanese monkeys' preference for 50 visual stimuli obtained from the FMD database using a simple choice task (select one stimulus from simultaneously presented two stimuli by eye movements) and then examined whether or not the magnitude of orbitofrontal neural responses to these stimuli correlated with the rank order of these stimuli obtained in the behavioral study. The behavioral study showed the rank order of the preference in these 50 stimuli based on the chosen ratio of each stimulus during the simple choice task. Among 188 single-neuron activities recorded from the orbitofrontal cortex, 65 neurons exhibited visual responses to 50 visual stimuli and exhibited stimulus selectivity. One third of these neurons exhibited either positive or negative correlations between the magnitude of visual responses and behaviorally determined preference rank orders of visual stimuli. These results suggest that the orbitofrontal cortex plays an important role in the judgment of the preference for visual stimuli and that the magnitude of orbitofrontal responses to the visual stimuli is related to the strength of the pleasant feelings that are produced by the stimuli.

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Poster

176. Decision Making: Primates

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Title: Predictive coding in macaque area IT

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Abstract: An influential theoretical framework argues that neural systems capitalise on the statistics of stimulus presentation to actively predict forthcoming sensory information, and optimise perceptual decisions. Human functional neuroimaging studies have provided support for predictive theories of perception, but evidence from single-unit neurophysiology has been conspicuously absent. We recorded from 252 single neurons in inferior temporal cortex (IT) while monkeys judged whether a degraded cue image was a face or a fruit, signalling their decision by responding to a matching image after a variable delay. The probability of each object cue (face vs. fruit) was controlled at 5 levels (0%, 25%, 50%, 75% and 100% face probability) that changed unpredictably over the experiment. Monkeys' choices were determined by the physical stimulus (responding "face" when the cue was a face), but they were also strongly biased by the object probability (i.e. responding "face" when faces were more probable). Changes in probability were not signalled overtly, implying that monkeys were learning the most likely cue from the recent trial history. We identified neurons that were selective for both faces and fruit, but critically, averaging responses over the neuronal population revealed embedded signals related to expectation. Starting at about 150 ms following cue onset, responses to expected cues were attenuated relative to responses to unexpected cues ("expectation suppression"), which is consistent with findings from human neuroimaging studies. Control analyses showed that this effect was not due to adaptation to the cue from the previous trial, and the degree of expectation suppression for a given cue type was negatively correlated with the neuron's selectivity for that cue (i.e., expectation suppression for face cues correlated negatively with face selectivity, and expectation suppression for fruit correlating negatively with fruit selectivity). Fitting a reinforcement-learning model to the data, we further observed temporally dissociable neural responses that encoded both stimulus-specific prediction errors (stronger response for unexpected faces > unexpected fruit, starting at 100 ms) and stimulus-general prediction errors (stronger responses for unexpected > expected cues, starting at 150 ms). Together, these findings suggest that the responses of neurons in IT reflect the probability of occurrence of a stimulus over time, derived from the statistics of stimulus presentation. These findings support the view that neural signals in the mammalian visual system actively encode and update predictions about the local sensory environment.

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Poster

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KAKENHI 26119504

KAKENHI 26330266

IRP/NIMH/USA

Title: Representation of reward value by single unit in the monkey orbitofrontal cortex during decision-making

Authors: *T. SETOGAWA[1], T. MIZUHIKI[1,2], F. AKIZAWA[2,3], R. KUBOKI[2], B. J. RICHMOND[4], N. MATSUMOTO[5], M. SHIDARA[1,2];
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Abstract: In our daily life, we often choose one item or action from several alternatives by considering their values and efforts to obtain them. To study the neural mechanism of such decision-making process, we developed a decision-making schedule task to obtain a reward and recorded single neurons from monkey orbitofrontal cortex (OFC). Two monkeys were initially trained to perform a reward schedule task. In this task, the monkey had to complete the schedule composed of 1, 2 or 4 trials of visual discriminations to earn 1, 2 or 4 drops of liquid reward. After the monkey learned this task, the decision-making schedule task was introduced. The decision-making schedule task had a decision-making part and a reward schedule part. In the decision-making part, two choice targets (CT), each a bar of light, were presented sequentially at the center of a computer monitor. Brightness and length of the CT were proportional to the amount of liquid reward (1, 2, or 4 drops) and the required number of the visual-discrimination trials (1, 2, or 4 trials) to be performed, respectively. After both first and second CTs had been presented, the same two CTs simultaneously reappeared along side the fixation point. The monkey was required to choose one of the two CTs by touching the corresponding bar in the chair. Then, the chosen reward schedule task was started. We recorded 253 neurons in the monkey OFC during the decision-making schedule task (137 and 116 neurons from each monkey). The CT values were estimated from the monkey's choice behavior by an exponential

discounting model of reward value. Multiple regression analysis after the second CT period revealed that 172/253 neurons had responses correlated with CT values (either one or the other chosen value, 108/253; both chosen value, 64/253). To investigate whether the recoded neurons could be involved in comparing reward values, the trials were divided into 2 groups: trials when monkeys chose the first CT and the trials when they chose the second CT. Multiple regression analysis for the two groups separately showed that 84/253 (33.2%) of the recorded neurons had different signs for the coefficients of 2 CT terms, and 29/253 (11.5%) neurons had coefficients with the same signs. 76/253 (30.0%) neurons coded currently presented CT values. These results suggest that OFC neurons related to both the predicted outcome values, and to the value comparison between the alternatives.

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Poster

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Title: Dopamine D2 receptor stimulation enhances working memory coding in primate prefrontal cortex neurons

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Abstract: Working memory processing in the prefrontal cortex (PFC) is modulated by dopamine. Manipulation of either dopamine receptor type, the D1 or D2 receptor family, impacts behavioral performance of tasks requiring working memory in humans and animals. However, only D1 receptors modulate the memory fields of single neurons in the PFC during spatial working memory. Despite strong clinical implications of prefrontal D2 receptors, cellular mechanisms by which D2 receptors modulate working memory processing in the PFC remain unknown. Therefore, we trained two macaque monkeys to perform a memory-guided decision-making task. Monkeys had to remember the amount of dots, i.e. the numerosity, of a sample item to decide whether a test item had a larger or smaller numerosity. During the delay between sample and test, no visual information was available. We recorded single units in the PFC while

applying specific dopamine D1 or D2 receptor targeting drugs using micro-iontophoresis. The activity of single units in the PFC was selective for one of the three sample numerosities during the memory period without visual stimulation, i.e. they were tuned to their preferred numerosity. After stimulating D2 receptors with the D2 receptor agonist quinpirole, neuronal responses to the preferred numerosity were increased, thus enhancing their tuning properties. At the population level, D2 receptor stimulation increased the representation of sample numerosities and improved decoding accuracy. In contrast, manipulation of D1 receptors did not strongly affect working memory coding. These results show that prefrontal D2 receptors are involved in working memory processing at the cellular level and serve as a possible basis for cognitive changes observed after D2 receptor manipulation.

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Poster

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Title: Not quite noisy, suboptimal, or wrong: rational process models and strategic decision making

Authors: ***D. L. BARACK**[1], Y. LI[1], M. R. NASSAR[2], J. I. GOLD[1];
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Abstract: Strategies determine the probability of taking an action given a state of the world. Optimal strategies are those likeliest to achieve particular goals, such as maximizing rewards or minimizing costs. However, agents often seem to behave sub-optimally, such as making more errors than expected. Such sub-optimalities might result from mixed strategies, with contributions from both optimal and suboptimal components. Alternatively, these apparent sub-optimalities might result from rational process models that approximate optimal strategies using simpler but psychologically plausible computations. The goal of this study is to develop a framework for distinguishing between these contributing strategies. We focus on the behavior of monkeys performing a ten-choice saccade task. Monkeys chose one target on each trial, receiving a juice reward for correct choices as well as visual feedback informing them of the rewarding target on that trial. On each trial, the rewarding target was drawn probabilistically

from a distribution centered on a single, best target. From time to time, change-points occurred, when the best target was randomly reselected from the remaining nine possible targets. To maximize rewards, monkeys should identify and always pick the best target, even if that target is not rewarded on a given trial. Monkeys' behavior reflected components of an optimal inference process, including a tendency to choose the best target determined from recent history, and apparently simpler heuristics, including a tendency to choose the previously rewarded target. We develop a three-step framework to decompose the behavior into underlying strategies. First, we represent the behavioral data with a matrix that specifies action probabilities given a set of states. Second, we define how a range of strategies would map onto this matrix, using both a priori selection of strategies ranging from simple (e.g., follow the winner) to complex (fully Bayesian) and more data-driven approaches that extract strategy-like features from the behavioral data. Third, we compare these strategies to a rational process reduction of the Bayes optimal model, a mixture of delta rules model with different learning rate time constants. Preliminary results indicate that the rational process model can recapitulate many different heuristics including the optimal strategy by changing the parameter settings governing its computational complexity. Further work will analyze how time-dependent variability in the parameter settings of these kinds of rational process models can generate complex behavioral patterns that appear to reflect mixtures of optimal and sub-optimal components.

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Wellcome Trust

Title: Structural and functional changes in brain circuits associated with learning rules in macaques

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Abstract: In discrimination reversal (DisRev) learning tasks animals learn that one choice leads to reward while another does not. Animals also learn that when the reward assignments are switched, the previously unrewarded choice has become the one followed by reward. Behavioural flexibility in DisRev has often been thought to rely on a brain circuit centered on the orbitofrontal cortex (OFC). To identify the components of this circuit we sought brain regions where structure and activity changes were associated with DisRev experience in a group of nine macaques: 4 animals choosing between target identities, 5 animals choosing between target locations. We obtained structural and functional MRI (fMRI) measures of activity at two time points while animals were at rest. At time 1 the animals already had learned that pressing a target on a touchscreen, either on a left or a right side of the screen, was associated with a juice reward. At time 2 the animals had negotiated 5 sessions with 5 reversals within a session and with performance above 85% correct Compared with control animals, significant grey matter increases were associated with DisRev experience in amygdala, basal forebrain, and medial thalamus, medial orbitofrontal cortex, anterior cingulate cortex (ACC), and the lateral prefrontal cortex (lPFC). No significant changes were observed in the central OFC (cOFC). As well as structural changes functional coupling changes were also found between the same regions. Second, to examine further whether these areas might constitute the circuit affected by OFC aspiration lesions, we examined the impact of OFC lesions in two macaques on grey matter and activity coupling in and between these same areas. Structural and fMRI data revealed that OFC lesions were associated with significant changes in grey matter in many of the same areas including medial thalamus, lPFC, mOFC, medial forebrain, and amygdala. In most cases the changes were decrements in grey matter but increased grey matter was found in the amygdala and hippocampus Although we failed to identify changes in cOFC associated with DisRev we were able to show that adjacent frontal areas, likely to be disconnected by OFC aspiration lesions, are important in DisRev. The results are consistent with the view that fluent DisRev performance is mediated not just by choice-reward association learning mechanisms linked to OFC, but also by acquisition of a cognitive set or task model, dependent on many interacting brain regions, representing the inter-relationships between the different choice-reward associations active in the task at different times

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Poster

176. Decision Making: Primates

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 176.18/Z33

Topic: F.02. Animal Cognition and Behavior

Support: Medical Research Council

Title: Irrational decisions? blame your vmpfc

Authors: *G. K. PAPAGEORGIOU[1], J. SALLET[1], M. J. BUCKLEY[1], M. F. S. RUSHWORTH[1,2];
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Abstract: Aims The medial orbitofrontal cortex (mOFC)/ventromedial prefrontal cortex (vmPFC) has been implicated in decision-making and encoding the value of expected outcomes. Here we investigate its role in valuation and decision-making in the context of multi-faceted outcomes. We report an unusual 'less is more' effect where subjects have the tendency to choose options that are less valuable than others and show the effect is dependent on mOFC/vmPFC. Methods Six adult macaque monkeys (controls: 4, mOFC/vmPFC-lesioned: 2) participated in the experiment. All animals were trained on a 2-choice 6-options behavioural task in which choices of three stimuli led to 3 different types of reward and the other 3 stimuli led to no reward delivery. More specifically, one stimulus led to a highly valued reward ('High option'), a second stimulus led to a low valued reward ('Low option') and a third stimulus could led to the combination of the two ('Compound option'). All possible choice combinations have been used. Once this phase of the experiment was completed, the 4 control animals, executed a modified version of this task inside the fMRI scanner. Results All control animals preferred the high option over the low option and the compound option over the low option. Surprisingly, however, they preferred the high option over the compound option suggesting that the value of the multifaceted compound option was not the sum of the values of its component parts. This unusual choice pattern on high option versus compound was significantly less pronounced in animals with mOFC/vmPFC. Further analyses of the fMRI data at the time of the stimulus presentation showed stronger mOFC/vmPFC activation for the high option in comparison with the compound option. Conclusion Together, lesion and neuroimaging results suggest that mOFC/vmPFC plays an important role in representing values of multi-faceted options.

Disclosures: G.K. Papageorgiou: None. **J. Sallet:** None. **M.J. Buckley:** None. **M.F.S. Rushworth:** None.

Poster

176. Decision Making: Primates

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant EY015260

Title: Neuronal responses of macaque caudate nucleus and frontal eye field during visual perceptual learning

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Abstract: Our ability to detect, discriminate, and identity sensory stimuli can improve with training. This phenomenon, called perceptual learning, is observed widely across sensory modalities and is preserved throughout adulthood. Although psychophysical aspects of perceptual learning have been studied extensively, the underlying neural mechanisms are not clear. Our previous work with monkeys suggested that a learning mechanism based on reward feedback—reinforcement learning—can guide perceptual learning on a visual motion direction-discrimination task. The basal ganglia, a set of neural structures implicated in reinforcement learning, along with interconnected regions of prefrontal cortex may together contribute to the training-based perceptual improvements. Here we recorded single- and multi-unit activity from caudate nucleus, the primary input stage of the basal ganglia, and the frontal eye field (FEF) of prefrontal cortex while a monkey was trained to perform the direction-discrimination task. We presented a patch of noisy, moving dots for a fixed duration at the center of the visual field. After a variable delay, the monkey indicated the perceived direction by making a saccade to one of two choice targets located along the axis of motion. Initially, we used only easy stimuli in which all the dots coherently moved in the same direction. After the monkey learned the correct association between motion and saccadic directions, we introduced more difficult stimuli in which varying portions of dots move in random directions. The percentage of coherently moving dots, termed motion coherence, dictated the strength of motion stimulus. As expected, the monkey's sensitivity to weak motion stimuli improved over the course of training. We found that task-driven responses of caudate neurons also changed during training. Most strikingly, the sensitivity to motion coherence increased, with most neurons responding more to lower coherences but some responding more to higher coherences. These changes were observed for neural responses measured during the delay and post-saccadic periods and occurred before FEF neurons began to show coherence selectivity. The coherence-sensitive responses in caudate tended to be similar for trials that resulted in choice saccades contralateral and ipsilateral to the recorded hemisphere and thus did not appear to be related directly to the monkey's decision.

These responses are consistent with a representation of choice-independent value, which could, in principle, be used to guide reinforcement-driven learning of the perceptual discrimination.

Disclosures: T. Doi: None. **J.I. Gold:** None.

Poster

176. Decision Making: Primates

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH IRP

Title: Observations from a successful DREADD silencing experiment in non-human primate orbitofrontal cortex

Authors: *W. LERCHNER, M. A. G. ELDRIGE, R. C. SAUNDERS, V. DER MINASSIAN, S. BHAYANA, B. J. RICHMOND;
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Abstract: We used the DREADD (Designer Receptors Exclusively Activated by Designer Drugs) technology to reversibly silence virally transduced orbitofrontal cortex (OFC) neurons in two monkeys with contralateral rhinal lesions. On days when hM4Di-DREADD expressing neurons were silenced by systemic injection of the DREADD receptor ligand Clozapine-N-Oxide (CNO), at 10mg/kg, there was a marked reduction in discrimination between expected reward sizes in a simple stimulus-reward association task, and an overall reduction in error rate compared to days without injection of CNO. There was no difference in the total number of trials completed or in the reaction times of the monkeys under these conditions. Therefore the DREADD technology can be used to silence neurons in an extended region of sheet-like structures such as the monkey cortex, to a sufficient degree to induce changes in behavior. In the course of these experiments, we made several observations on the use of virally expressed DREADDs in monkeys: 1) We made 40-70 handheld lentivirus injections into OFC, resulting in dense local expression areas of up to 2000 μm width near the presumed injection tracks, with gaps in between injection sites. The volume coverage of areas with DREADD expressing cells was approximately 7% in Brodmann area 13 and 3.0% in Brodmann region 11. Neuron specific expression, as determined by NeuN overlap, imaged on a confocal microscope, in the 200 x 200 μm center of expression areas, averaged 60% and often reached 100%. Even though it was not possible to cover cortex completely with handheld lentivirus injections, the limited coverage

(<7%) can be enough to change behavior. 2) Dense projections expressing DREADD protein were visible in ventromedial caudate and insular cortex as well as in region TE. Sparser projections were detected in the rhinal cortex. Thus, even with a relatively small percentage of expressing neurons, labeled axons are seen in brain regions previously known to receive projections from the OFC. 3) Time course analysis of CNO levels in cerebrospinal fluid (CSF) showed a peak concentration at 60 minutes after intramuscular injections. Despite concerns of conversion of CNO into clozapine (observed in humans and guinea pigs), only trace amounts of clozapine were detected in the CSF and serum when analyzed in a group of 10 monkeys at the 60-minute time point. 4) Some monkeys showed side effects from 10 mg/kg CNO injections when tested before DREADD virus injections. Side effects included fewer trials completed and lengthened reaction times. Therefore, animals should be screened to know whether they are affected by experimental doses of CNO before virus injection.

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Poster

176. Decision Making: Primates

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Support: NIH Grant 1R01MH104251-01A1

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Title: Adaptive value coding: Temporal influences on choice mediated by divisive normalization in rhesus monkey

Authors: *J. ZIMMERMANN[1,2], T. LOFARO[3], P. W. GLIMCHER[2], K. LOUIE[2];
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Abstract: Any organism has to react and adapt to a constantly changing environment. Its neuronal system is thus confronted with the task of encoding a broad range of sensory information efficiently within the finite constraints of its coding capacity. This problem is widely believed to explain temporal adaptation and spatial normalization in sensory systems. Recent work has demonstrated that temporal adaptation occurs in reward-processing and decision-

related brain areas, but the computational mechanisms and behavioral consequences of this temporal adaptation have remained largely unknown. Here, we present data from a saccadic choice task. Trained monkeys were offered a choice between two options differing in reward magnitude and juice type. Block of trials were presented which were composed of a mixture of "adaptor trials" and "measurement trials". In measurement trials, fixed in structure across all blocks, monkey were asked to choose between an unvarying reference reward (fixed reward magnitude and juice type) and one of 5 variable rewards. These responses allowed us to plot the monkey's probability of choosing the reference reward as a function of the magnitude of the variable reward; exposing a "choice curve". What we systematically varied across blocks was the structure of the adaptor trials. We then examined the effects of the standard deviation of adaptor variability on the slopes of these choice curves. We attempted to account for the effects of adaptor variability on choice behavior using a model of adaptive value coding based on the dynamic normalization models we have previously explored. Our current model implements two cascaded divisive-normalization networks which we refer to euphemistically as OFC and LIP. Adaptation is implemented via divisive normalization, a canonical neural computation widely reported in sensory processing, in both networks. The time constants of the networks, however, differ by several orders of magnitude (with OFC being much slower) and allows the OFC network to effectively adapt the sensitivity of the LIP network. Our behavioral and simulation results demonstrate that the temporal value context significantly influences monkey choice behavior and that the temporal dynamics of our model can account for these changes. The findings suggest that divisive normalization, may underlie adaptive value coding in decision-making areas and shape behavioral choices accordingly. Single unit recordings from real monkey OFC will relate neuronal dynamics to behavioral and model dynamics in a future step.

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Poster

176. Decision Making: Primates

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Title: The dynamics of neural population activity during decision-making

Authors: *P. E. CISEK, J.-F. CABANA, D. THURA, A. FEGHALY;
Univ. of Montreal, Montreal, QC, Canada

Abstract: How does the brain make decisions in a dynamic and constantly changing world? We have recently shown (Thura & Cisek, 2014) that when monkeys perform a task in which relevant sensory information for action selection varies over time and they are free to respond at any moment, many neurons in dorsal premotor (PMd) and primary motor cortex (M1) continuously reflect the evolution of sensory information along with a signal related to the growing urgency to respond. Approximately 280ms prior to movement onset, the activity of these cells reveals the resolution of a competition between the choices, implicating them in the process of volitional commitment. The above conclusions were based on analyses of cells that showed statistically significant directional tuning prior to the moment of commitment and comprise about a third of the total PMd/M1 population. The remaining cells include those that only become tuned during or shortly before movement as well as untuned cells whose relation to the task is difficult to quantify. However, it is well-known that cortical populations are highly heterogeneous and do not neatly partition into clear categories such as “decision” or “movement cells”. Thus, inspired by recently developed methods, here we analyze the activity of the entire population by plotting the dynamical state of the system as a trajectory through a high-dimensional neural space. We use Principal Components Analysis to reduce this high-D space into a lower-dimensional projection and examine 1) how neural trajectories evolve in different conditions, and 2) how individual cells contribute to the dimensions identified by PCA. We found that during deliberation, the neural state evolves on a 2-dimensional “decision manifold” defined by orthogonal directions roughly corresponding to sensory evidence and urgency, and then rapidly falls off this surface at the moment of commitment into trajectories specific to each choice. This is consistent with a dynamical attractor network that implements decisions through a winner-take-all process. During blocks in which monkeys were motivated to make hastier decisions, the neural state evolved on the same decision manifold but was shifted closer to the edge where commitment appears to occur. Finally, we found that the “loading matrix”, which quantifies how each cell contributes to individual PCs is a continuous distribution, without distinct clusters. This does not support the idea of separate functional groups but instead suggests that the entire PMd/M1 population works together to govern the dynamics of decision-making.

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Poster

176. Decision Making: Primates

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Topic: F.02. Animal Cognition and Behavior

Support: ONR Award N000141310561

Title: Rhesus monkeys make probabilistic evaluations of the individual features in compound visual stimuli

Authors: *H. RAO[1], M. RYOO[1], A. TOADER[1], J. BECK[2,3], S. FERRARI[2,4,5], H. OH[2,6], T. EGNER[2,6,7], M. A. SOMMER[1,3,6];
[1]Biomed. Enginnering, [3]Neurobio., [4]Mechanical Engin. and Material Sci., [5]Duke Inst. for Brain Sci., [6]Ctr. for Cognitive Neurosci, [7]Psychology and Neurosci., [2]Duke Univ., Durham, NC

Abstract: Making decisions during real-life analyses of a scene involves the consideration of visual attributes that may be interlinked and probabilistic, such as evaluating apples by virtue of their shape and color. Both feature dimensions provide a probabilistic contribution to the choice of which apple to eat. If time pressures are involved (e.g. someone else may grab the best apple), subjects may use heuristics for expediency rather than a full analysis (e.g. just go for the reddest apple). Prioritization of some sources of information over others in the face of decision pressures is known as satisficing. Here we examine the capacity for rhesus monkeys to evaluate the probabilistic worth of individual features in compound visual objects, to set the stage for neurophysiological investigation of satisficing. Monkeys (and humans for comparison) were presented with two stimuli, each consisting of four "cue dimensions". A stimulus was red or blue (the cue dimension of color), a circle or a square (shape), outlined in white or black (contour), and contained horizontal or vertical stripes (orientation). Within these cue dimensions, each feature carried a differential value, e.g. circle = .8 but square = .2, that contributed to the aggregate conditional probability that selection of the stimulus would result in reward. Subjects selected the "best" stimulus in each trial on that basis. We used Bayesian inference and model comparisons to quantify their decision-making strategies. We found that within a few weeks of training, monkeys learned the differential probabilities associated with each cue dimension. Given ample time to view pairs of stimuli, > 1000ms, the animals based their choices on the 3 most informative cue dimensions. Humans tested in single sessions, in contrast, were adept at using all 4 dimensions. Next steps will impose pressures such as reduced viewing time to determine the satisficing strategies adopted by the monkeys. The overall outcome will be to

establish the parameters under which more naturalistic, information-restricted decision-making may be studied at the single neuron level in the macaque.

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Poster

176. Decision Making: Primates

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Topic: F.02. Animal Cognition and Behavior

Support: NIH RO1-HD059852

Title: The neuronal basis of oxytocin's effect on interactive social behavior

Authors: ***K. HAROUSH**[1], Z. WILLIAMS[2];
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Abstract: Social behaviors such as conspecific bonding, sexual encounters, cooperation and parental care have been shown to be modulated by the neuropeptide oxytocin. However, the mechanisms by which oxytocin affects neuronal activity to mediate these changes in behaving animals remain largely unknown. Building upon our recent findings, that delineate a causal link between neural activity in the dorsal Anterior Cingulate Cortex (dACC) and cooperative behavior in rhesus macaques (Haroush and Williams, 2015), we set to investigate how inhaled oxytocin affects social interactions in behaving individuals and the manifestation of its effect in the dedicated neuronal circuitry underlying this behavior. To this end, we trained pairs of rhesus monkeys on an adapted version of the iterated Prisoner's Dilemma (iPD) game, where the outcome of each monkey depended on the concurrent, joint decision of both animals. On successive trials, each individual could either opt for cooperation which could mutually benefit both individuals, or attempt to maximize personal profit at the other's expense. Each pair of monkeys sat side-by-side facing a screen. The monkeys chose on each trial between two stimuli, representing cooperation and defection, which were operationally defined by the payoff matrix. Neither monkey was aware of the other's choice until both completed their own selection. Since both monkey's decisions influenced their outcome and no one decision could guarantee reward, their choices required an element of trust when making a cooperative decision. While the primates performed the task, we carried out electrophysiological recordings in single cells in the dACC. In the midst of each experimental session we introduced both monkeys with either saline

or a 48IU dose of oxytocin, inhaled via a previously acclimated pediatric nebulizer for a period of 5 minutes, in counterbalanced fashion. Neuronal recordings were continued throughout the session and behavioral paradigm was resumed at the end of inhalation. We found that oxytocin significantly enhanced cooperative social behavior compared to saline inhalation, similar to the previously reported effect of oxytocin in humans. Furthermore, oxytocin effects were dependent on the social context of the task as they were not evident when the monkeys played each other in separate rooms. At the neuronal level, oxytocin significantly affected the computation carried out by the neuronal circuits in the dACC. These results are instrumental in understanding the neural basis by which oxytocin influences interactive social behavior, with implications for the treatment of disorders such as Autism Spectrum Disorder and Schizophrenia.

Disclosures: K. Haroush: None. **Z. Williams:** None.

Poster

176. Decision Making: Primates

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P30EY008126

P30HD015052

Robin and Richard Patton through the E. Bronson Ingram Chair in Neuroscience

Title: Effects of choice errors versus response inhibition on response times

Authors: *P. MIDDLEBROOKS[1], J. D. SCHALL[2];
[1]Psychology, [2]Vanderbilt Univ., Nashville, TN

Abstract: Perceptual choice and response inhibition have each been a major focus of decision-making research for decades. During stop-signal tasks, participants tend to respond slower to a go stimulus following a successfully stopped trial. During speeded choice tasks, participants tend to respond slower following choice error trials. To date no studies have examined the relationship between response inhibition and choice-error slowing. The choice stimulus was a

cyan-magenta checkerboard. Saccade choice was specified by the fraction of cyan or magenta in the checkerboard, varied around discrimination threshold. On 25-40% of trials a visual stop signal replaced the central fixation spot after a variable stop-signal delay. On no-stop signal trials reinforcement was earned for a correct choice. On stop signal trials reinforcement was earned for inhibiting the saccade. Previously we reported that performance measures of choice and response inhibition indicate that choosing and stopping are independent cognitive processes (Middlebrooks & Schall 2014 Atten, Percept & Psychophys). Here we investigated further the extent of this independence by examining whether the effects of stopping inhibition and choice errors on the response time in subsequent trials were additive, interfering or independent. Data were analyzed from two perspectives. First we measured no-stop trial response times following trials of each possible pairwise outcome, comparing them to overall mean no-stop response times. Second, we compared no-stop trial response times following trials of each possible outcome to no-stop trial response times preceding trials of each possible outcome in a triplet trial sequence. No-stop responses were faster after successive no-stop trials. Following canceled stop trials, they were slower relative to the preceding no-stop trial, and they did not change much after non-canceled stop trials. These three findings replicate previous work. However, unlike previous observations, no-stop trials after no-stop choice errors were faster than overall no-stop response times. That is, we found post-choice error speeding instead of slowing. The post-choice error speeding may be due to executive processing dominance of inhibition over choice given the task demands. These behavioral results, in conjunction with ongoing stochastic accumulator modeling and electrophysiological recordings, contribute to weave together two major threads of research on decision-making, perceptual choice and response inhibition.

Disclosures: P. Middlebrooks: None. **J.D. Schall:** None.

Poster

176. Decision Making: Primates

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R21 5R21DA034421-02

Title: Ventral tegmental dopaminergic stimulation causes preference reversals

Authors: *D. FREESTONE[1], L. GRATTAN[2], R. B. RUTLEDGE[3], K. LOUIE[1], P. W. GLIMCHER[1];

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Abstract: In natural environments, animals make foraging decisions based on the values of potential food sources, foraging more at locations associated with high values. But to accomplish this, animals must first learn the values of the available options. The dopaminergic reward prediction error (RPE) hypothesis is the dominant view of how biological reinforcement learning works, proposing that midbrain dopamine neurons encode the difference between actual and predicted rewards. Under this theory, dopaminergic RPEs serve as a teaching signal, with dopamine bursts signaling better-than-expected outcomes that increase the subjective value of associated options. There is an enormous amount of correlational support for this hypothesis, but few studies assess the causal role of dopamine in reinforcement learning. Here, we examine how selective microstimulation of midbrain dopamine neurons impacts reinforcement learning. We trained two rhesus macaques on the matching law foraging task in which two options yielded water rewards with equal probability (25%) but different magnitudes. The reward magnitudes changed abruptly over time, requiring constant updating of subjective value estimates. In select blocks, we stimulated dopaminergic neurons in the ventral tegmental area along with the delivery of the smaller magnitude reward; this stimulation resulted in a preference reversal by which monkeys chose the smaller (but stimulated) option. Consistent with a dopaminergic mechanism, administration of the dopamine D2 receptor antagonist raclopride reduced the size of this effect. Aspects of trial-by-trial choice dynamics following both reward and combined reward-stimulation were captured by standard reinforcement learning models. These results provide causal evidence that midbrain dopamine neurons carry a teaching signal that updates the value estimates that guide value-based decision-making.

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Poster

176. Decision Making: Primates

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Topic: F.02. Animal Cognition and Behavior

Support: Grant from Templeton Foundation to Ben Hayden

Title: Are search strategies a result of adaptation to, or emergence due to, resource distributions?

Authors: *S. SHAH[1], B. HAYDEN[2];
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Abstract: Animal's evolutionary success depends, in part, on the efficiency with which it can search for prey in its environment. Many animals, indeed, can be said to perform efficient searches. How do animals search efficiently? Such efficiency could be a result of the animal understanding the resource distribution and adopting a suitable strategy or heuristic. Alternatively, the animal could be following an innate process that evolved over millennia to optimize foraging in a naturalistic setting. In most natural foraging environments, resources are patchy and sparsely distributed. Theoretical work has shown that the optimal search strategy in such environments would be to perform a Lévy walk. This comprises of many short steps, and a few long ones. A Lévy walk would increase the probability of encountering new patches, reduce the chances of visiting the same site again, and also allows the animal to explore more distant locations. This theoretical result has been supported by empirical work in diverse species like sharks, bony fishes, sea turtles, spider monkeys and penguins. Is this Levy walk search strategy a result of the animal following an innate strategy (adaptation) or does the search behavior merely appear to be a Levy walk due to the nature of the resource distribution (emergent)? We tackle this question in a computerized search task in a lab setting with rhesus macaques. By varying the resource distribution, we are able to dissociate between a resource distribution influenced behavior and an innate behavior.

Disclosures: S. Shah: None. **B. Hayden:** None.

Poster

176. Decision Making: Primates

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Topic: F.02. Animal Cognition and Behavior

Title: Habitual saccades aiming at valuable objects - a role of basal ganglia

Authors: *H. AMITA, O. HIKOSAKA;
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Abstract: Habitual behavior emerges after repetitive learning. After experiencing many fractal objects (n>280), half with a large reward ('good objects') and the other half with a small reward ('bad objects'), for more than 5 days, monkeys freely looked at good objects more frequently, quickly, and intensely than bad objects, even though no reward was given (Hikosaka et al.

Trends Cogn Sci 2013). Is such a habitual behavior useful? To answer this question, we devised a value-biased object saccade task in which the monkey obtained a large or small reward depending on which object (good or bad) was targeted by a saccade, unlike free viewing. On choice trials, two objects (good and bad) were presented at the same eccentric positions (15 deg) as the central fixation point turned off. On forced trials, one object (good or bad) was presented. The objects were seen only in periphery because they changed to white dots as soon as a saccade started. On choice trials, the saccade targeted the good object on most trials (>90%) if the monkey had experienced the objects with a large or small reward for >5days, even when the objects were chosen randomly out of 200 learned objects. On forced trials, the saccade tended to occur earlier to the good than bad object. Interestingly, the good-bad discrimination was absent if the saccade occurred too early. An analysis of cumulative saccade latencies showed that value-biased discrimination started around 150 ms after object presentation. This indicates that neurons responsible for the value-based saccade discrimination must show differential responses to good and bad objects before 150 ms. We hypothesized that neurons in the substantia nigra pars reticulata (SNr) serve this function, since they transmit stable object value signals from the caudate tail (CDt) to the superior colliculus (SC) (Yasuda & Hikosaka, J Neurophysiol 2015). Indeed, the SNr neurons were inhibited by good objects and mostly excited by bad objects, especially when they were presented on the contralateral side, and the discrimination started about 130 ms or less after object presentation. To test the causal role of SNr neurons, we are testing whether the pharmacological manipulations of the CDt-SNr-SC circuit affect the value-biased saccade.

Disclosures: H. Amita: None. **O. Hikosaka:** None.

Poster

177. Learning and Memory: Physiology

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Title: Effects of whole-brain-irradiation (WBI) on primate brain and related cognitive function

Authors: *S. A. DEADWYLER, A. PEIFFER, C. A. SEXTON, J. B. DAUNAIS, D. B. HANBURY, E. L. MITCHELL, K. T. WHEELER, J. D. BOURLAND, J. M. CLINE, R. E. HAMPSON;
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Abstract: A recent publication (Hanbury et al. Radiation Res. 2015), has demonstration effects of fWBI on several vascular regions of the brain, and provided a preliminary estimate of the time course of cognitive function decay associated with progressive brain deterioration in a 12 month window. The study described here assesses the same time course and confirms the degree of change in performance and associated functional brain activity, but using a much more detailed analysis of the cognitive changes that progress temporally from the time of WBI treatment. Two cohorts of nonhuman primates (NHPs) were tested for long-term effects on performance of a complex memory task over a 10-12 month time period following fractionated Whole Brain Irradiation on delayed match to sample (DMS) task. fWBI was administered in the same fashion and with the same target parameters used with human cancer patients. Each subject received eight 5-Gray fractions (40 Gy total) delivered by linear accelerator, with a lead block system to limit the exposure to the cranium and shield the eyes and optic chiasm. Fractions were separated by 3 or 4 days each; total elapsed time for the fWBI regimen was 4 weeks. Cognitive performance was assessed using a visual object and position-oriented delayed-match-to-sample (DMS) task (see Hampson et al. poster, this meeting). A major variable associated with performance decay over months was the number of trials that each animal was capable of performing within a daily session. The decline in this factor over time was directly associated with the decrease in performance of two types of trials, spatial and object, which required differential encoding by hippocampal and prefrontal neurons as shown in past research in this laboratory (Hampson et al. J. Neural Eng. 2012). The decline in performance was directly related to 1) the number of distracter images presented in the nonmatch phase of the task, as well as 2) the duration of the delay interval between Sample and Match phase of the same task. Measures of brain imaged activity in MRI and PET scans across the same time period, showed changes in areas normally activated during the task when performance was at pre-WBI levels. The gradual decline in hippocampal and prefrontal cortex activation was related to increased vascular impairment in regions that normally supply those brain areas. Therefore these results indicate that WBI imposes a major disruption in brain function that progresses relatively slowly over time (months and years), likely related to progressive cell loss in critical brain regions as a consequence of vascular impairment induced by radiation exposure to eliminate other types of pathological cells.

Disclosures: S.A. Deadwyler: None. **A. Peiffer:** None. **C.A. Sexton:** None. **J.B. Daunais:** None. **D.B. Hanbury:** None. **E.L. Mitchell:** None. **K.T. Wheeler:** None. **J.D. Bourland:** None. **J.M. Cline:** None. **R.E. Hampson:** None.

Poster

177. Learning and Memory: Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.02/AA1

Topic: F.02. Animal Cognition and Behavior

Title: The modulating effects of stress, sex and age on early neurogenesis in mouse dentate gyrus

Authors: *H. WU.[1], L. YU[2];
[1]Inst. of Behavioral Med. of NCKU, Tainan, Taiwan, Tainan, Taiwan; [2]Inst. of Behavioral Med. of NCKU, Tainan, Taiwan

Abstract: Unconditioned foot shock followed by restraint in water was used as a stress regimen and the number in cell proliferation and early neurogenesis in mouse dentate gyrus (DG) was counted. This study aimed to assess sex difference and age-related modulating effect on the stressor-altered cell proliferation and early neuronal differentiation. Young (7 to 8-week) of age mice or aged (over 8 months of age) C57BL/6 mice were intraperitoneally injected with a bromodeoxyuridine (BrdU) (100 mg/kg) injection immediately before the stress regimen. Bromodeoxyuridine staining was used to indicate newly mitotic cells and doublecortin co-staining was used to reveal early differentiated neurons. Aged mice had lower baselines in the number of newly proliferated cells and neural progenitors compared to younger mice regardless of sex. Surprisingly, aged female mice exhibit increased numbers in cell differentiation and early neurogenesis in response to our stress regimen. Companions did not seem to prevent the stress-decreased cell proliferation or early neurogenesis in aged male mice.

Disclosures: H. Wu.: None. **L. Yu:** None.

Poster

177. Learning and Memory: Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.03/AA2

Topic: F.02. Animal Cognition and Behavior

Title: Sex difference in conformity behavior

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Abstract: Conformity behavior refers to the act of changing one's behavior to match the response of others. Using an approach dilemma paradigm in mice, we attempted to examine whether female mice are more prone to exhibit behavior of conformity than male mice in this study. First, food-deprived experimental mice were trained to obtain a sucrose pellet at the end of the black arm or white arm in a custom-made double J-shaped maze. In contrast, four corresponding food-deprived mice serving as a group of conformity for each experimental mouse were trained to obtain food pellet at the opposite end of the arm. When the correct arm choice was over 70% for both experimental mice and their respective groups, we, then, started the experiment. The experimental mice alone or along with their respective group were re-introduced into the start box of the maze. The correct arm choice was obtained for each experimental mouse. Approximately 24 hours later, the correct arm choice was obtained immediately after the conclusion of five consecutive extinction trials with pellet omission in the arm end. We found that male and female mice' correct arm choice was not affected by the presence of the group. Nonetheless, female, but not male, mice exhibited a significant decrease in correct arm choice after the extinction trials. More surprisingly, the presence of the group produced an obvious decline in the correct choice in male, not female, mice immediately after the extinction trials. Although the dominance indices at fight was used to determine the social hierarchy of each member of a group, the social hierarchy of the experimental mice did not seem to have impact on their correct arm choice.

Disclosures: C. Wang: None. **L. Yu:** None.

Poster

177. Learning and Memory: Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.04/AA3

Topic: F.02. Animal Cognition and Behavior

Title: Social buffering effects prevent the stressor-decreased dendritic length, branching in dentate granule cell and -decreased memory performance

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Abstract: A robust stressor regimen can decrease early neurogenesis and BDNF level in the dentate gyrus (DG), while the presence of companions reverses the changes. This study was undertaken to assess whether such stressor regimen may render morphological changes in the

existing DG granule cells and whether the presence of companions can prevent the plausible changes. Moreover, we assessed whether the morphological changes in DG granule cells could be associated with the alterations in local extracellular-regulated kinase (ERK) and cAMP-responsive element binding protein (CREB) phosphorylation as well as the performance of the hippocampus-related memory. Single granule cell in slice containing DG was visualized by lucifer yellow labeling in conjunction with the 2-D imaging techniques. Six hours after the conclusion of the stressor procedure, the morphological indices (including the total length of dendrites, the total number of dendritic branches and Scholl analysis results) of the granule cells along the neuraxis (from Bregma -0.94 to -3.5) of the DG (including the suprapyramidal blade, hilus tip, and infrapyramidal blade division) were obtained in three groups of Balb/C mice, the stressor-free control, stressor, and stressor with company groups. The stressor procedure produced significant decreases in the total length of dendrites, number of dendritic branches, and the size of the dendritic field in granule cells, while the social buffering effects prevented all these changes. The stressor-produced decreases and social buffering effects in these morphological indices of the DG granule cells were correlated positively with local ERK phosphorylation immediately after the stressor regimen and the memory performance in the object recognition and location tasks approximately six hours after the conclusion of the stressor procedure.

Disclosures: **W. Tzeng:** None. **L. Yu:** None.

Poster

177. Learning and Memory: Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.05/AA4

Topic: F.02. Animal Cognition and Behavior

Title: Effect of anxiety on learning and memory in mice

Authors: ***T. PARETKAR**[1], E. DIMITROV[2];
[2]Physiol. and Biophysics, [1]Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL

Abstract: Norepinephrine and glucocorticoids play an important role in the consolidation of emotionally arousing memories. The neurons of the amygdala express receptors for both, norepinephrine and glucocorticoids, and the amygdala is critical for integrating affect, learning and memory. We tested the hypothesis that increased anxiety will activate locus ceruleus (LC) and hypothalamic-pituitary-adrenal axis (HPA) and will increase norepinephrine and corticosterone signaling in the amygdala, which in turn will negatively affect both working and

long term memory in mice. The anxiety-like behavior of mice was assessed under conditions of low light intensity (6 lux, LLI) and high light intensity (200 lux, HLI). As expected, the different light conditions affected the anxiety-like behavior of tested mice. The mice spent less time in the open arms of Elevated O-maze and took longer time to exit from the dark compartment of the Light/Dark box when tested under HLI as compared to the group tested under LLI. The animals tested under HLI also showed significantly lower number of correct spontaneous alternations in the Y-maze test and significantly lower discrimination index in the Novel Object Recognition test. Furthermore c-Fos expression in basolateral amygdala (BLA), central amygdala (CeA) and LC was significantly higher in HLI group when compared to the LLI group. Next, we injected cre activated adeno-associated virus expressing inhibitory designer receptor exclusively activated by designer drug (AAV/DREADD/Gi) into the CeA of transgenic VGATcre mice. Clozapine N-oxide (CNO) is the only ligand for DREADDs. The inhibition of the CeA GABAergic neurons by an intraperitoneal injection of CNO abolished the effect of HLI on anxiety-like behavior and memory tasks. Pretreatment of VGATcre mice with the beta adrenergic blocker propranolol reversed the effects of CeA inhibition. Next we evaluated the stress response of the experimental animals to different light conditions. The HPA axis activation was assessed by measuring plasma corticosterone levels 30 minutes and c-Fos expression in the PVN one hour after Y-maze test. Surprisingly, plasma corticosterone levels and c-Fos expression in PVN were similar between the HLI and LLI groups.Our investigation showed that HLI activates LC norepinephrine neurons, increases anxiety-like behavior and negatively affects memory. The activation of LC under HLI occurs before the activation of the HPA axis and depends on GABAergic input from CeA. The increased norepinephrine signaling in the amygdala may modulate initial memory processing independently of the HPA axis.

Disclosures: **T. Paretkar:** None. **E. Dimitrov:** None.

Poster

177. Learning and Memory: Physiology

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Program#/Poster#: 177.06/AA5

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

CIHR

Human Frontiers Science Program

Canadian Foundation for Innovation

Title: Optogenetic manipulation of theta rhythm reveals sleep-dependent cognitive processing

Authors: *R. BOYCE[1], S. GLASGOW[2], S. WILLIAMS[1], A. ADAMANTIDIS[3];
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[3]Univ. of Bern, Bern, Switzerland

Abstract: Rapid-eye-movement (REM) sleep is present in nearly all terrestrial mammals studied to date and has been positively correlated with memory formation. However, identifying a direct causal link between neural activity during REMs and memory has proven difficult due to a sporadic pattern of occurrence in addition to a spectrum of critical caveats associated with REMs deprivation techniques. To overcome these issues we genetically targeted inhibitory archaerhodopsin (ArchT) to GABAergic neurons of the medial septum (MS), a brain region required for generation of *in vivo* theta rhythm, a prominent ~7 Hz oscillation present during active wakefulness and REM sleep that is linked to cognitive processing. We found that green light pulses reliably hyperpolarized ArchT-expressing cells by ~ 40 mV, completely preventing spiking in transfected neurons in MS brain slices *in vitro*. Using a combination of optogenetic and electrophysiological (MS unit recording and dorsal hippocampal CA1 field potential and unit recording) techniques in freely-behaving mice, we further found that green light delivered to the MS could completely and reversibly silence putative MS GABAergic neurons with <1 s temporal resolution. Silencing GABAergic neurons during REMs resulted in a significant (> 60%) and reversible attenuation of theta rhythm power recorded in the dorsal CA1 region of the hippocampus without disturbing the ongoing REMs episode. To determine whether MS GABAergic activity during REMs is involved in memory formation, we tested mice in a novel object place recognition test as well as a fear conditioning task. During the four hour period immediately following the learning phase of each test, MS GABAergic neuronal activity was silenced selectively during REMs. When tested the following day, novel object place recognition and fear-conditioned contextual memory were both found to be significantly impaired relative to controls. Collectively, our results have identified MS GABA neurons as one of the major generators of hippocampal theta rhythm and provide a direct causal link between MS GABAergic neuronal activity during REMs and memory formation.

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Poster

177. Learning and Memory: Physiology

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Program#/Poster#: 177.07/AA6

Topic: F.02. Animal Cognition and Behavior

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

Title: Deep-brain stimulation of central lateral nucleus of thalamus strengthens striatal-thalamic connectivity and enhances cognitive behavior

Authors: *E. T.-H. SHEN[1], Y.-T. TSAI[2], H.-C. PAN[4], J.-A. CHEN[2], H.-C. LIN[3], Y.-Y. CHEN[2], F.-S. JAW[1];
[1]Inst. of Biomed. Engin., Natl. Taiwan Univ., Taipei, Taiwan; [2]Dept. of Biomed. Engin., [3]Dept. and Inst. of Physiol., Natl. Yang-Ming Univ., Taipei, Taiwan; [4]Inst. of Neurosci., Taipei, Taiwan

Abstract: Deep brain stimulation (DBS) is a potent therapeutic approach for several neurological diseases and psychiatry disorders. However, the exact molecular mechanism of DBS remains elusive. Several studies has shown that DBS mediates behavioral improvements and neurological changes, and also indicated that interval stimulation with specific phase and at critical timing of medial temporal lobe, an important region related to memory formation, is dominant for memory processing. Direct hippocampal stimulation may disrupt local neuronal activities and inter-regional connections, thus DBS of central thalamus (CL) may provide more advantages of safety and efficiency for memory enhancement. The CL plays an important role in arousal regulation and awareness. In this study, we demonstrate drastically enhanced learning behavior of water-drinking tasks in rodents, along with reinforced oscillations and connectivity of the central lateral nucleus of thalamus, dorsal striatum, and ventral striatum. Recordings of local field potentials were used to examine memory-related oscillation pattern and behaviors, including theta and alpha oscillation and its spectral density. An increase is shown in the thalamic CL nuclei, along with the ventral and dorsal striatum area following thalamic CL DBS treatment in rodents. The correlation matrices of functional connectivity before and after behavioral training obtained showed a strong bilateral correlation of delta and theta synchronizations in the thalamic CL nuclei, ventral striatum and dorsal striatum, as well as between the thalamic CL nuclei and striatum. The expression of neuronal c-Fos, the proto-oncogene, is a well-known marker of neuronal activity. For comparison of neuronal activation distribution between DBS treated and sham control groups, we investigated c-Fos positive cells in various learning related brain regions and found statistically significant increased expression in DBS treated c-Fos positive neurons relative to the sham controls. The striatal neural circuits are composed of dopaminergic or cholinergic synapses to receive signals from cerebral cortex and propagate to basal ganglia to modulate movement. Thus, we examined the protein level of D2R and nAcha4 of striatum by Western blot analysis, finding statistically significant increases in the level expression for both stratial and hippocampal proteins. We suggest that thalamic CL DBS can strengthen the thalamic-striatal functional connectivity in the brain. The up-regulated

dopaminergic and cholinergic systems by CL DBS-treatment is involved in the water reward learning.

Disclosures: **E.T. Shen:** None. **Y. Tsai:** None. **H. Pan:** None. **J. Chen:** None. **H. Lin:** None. **Y. Chen:** None. **F. Jaw:** None.

Poster

177. Learning and Memory: Physiology

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Program#/Poster#: 177.08/AA7

Topic: F.02. Animal Cognition and Behavior

Title: Reverberating cell assemblies formed by axo-axonic GABA neurons in the rodent basal amygdala

Authors: ***M. BHAGAVATHI PERUMAL**, R. SULLIVAN, P. STRATTON, P. SAH; Queensland Brain Insititute, Brisbane, Australia

Abstract: Donald O Hebb's proposal of reverberating cell assembly remains one of the most elegant hypotheses to describe cognitive processes as discrete synchronized neural networks. Many in-vivo field potential studies have provided evidence for existence of these cell assemblies. But the types of neurons and synapses that form a cell assembly, as well as its temporal dynamics remain elusive. A cell assembly should have strong synaptic interconnectivity among its members to generate temporal precision. Axo-axonic neurons (AAC) are a unique subset of GABA neurons that synapse on to the axon initial segment (AIS) of pyramidal neurons. Depolarized GABA-A reversal at the AIS is presumed to enable GABAergic excitation at the pyramidal AIS in a state dependent manner. AAC in the rodent basal amygdala (BA) and cortex, when stimulated to threshold, receive di-synaptic time-locked feedback excitation. Using *in vitro* whole cell patch clamp recordings, we found that AACs in the rodent BA triggered recurrent time-locked feedback excitation that followed the first di-synaptic feedback with a latency of 4.7 ms ±0.3ms (n=7). The reverberating feedback excitation reached maximum amplitude at 18.1±2.4ms (n=9) and triggered multiple action potentials in the AAC in current clamp. The recurrent feedback excitation lasted up to 48.6±10.2ms (n=9) after the initial AAC spike. These oscillations were abolished with GABAA receptor or AMPA receptor antagonists. Therefore we suggest that the AAC driven reverberating microcircuits may form a novel model for cell assembly with axo-axonic synapses, GABAergic excitation and strong reciprocal glutamatergic connections.

Disclosures: **M. Bhagavathi Perumal:** None. **R. Sullivan:** None. **P. Stratton:** None. **P. Sah:** None.

Poster

177. Learning and Memory: Physiology

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Topic: F.02. Animal Cognition and Behavior

Support: State of California funds (UH & ROM)

University of Texas at Austin startup funds (ROM)

Title: LMO4 regulates the excitability of pyramidal neurons in the basolateral amygdala of mice

Authors: *R. A. MANGIERI[1], R. MAIYA[1], R. O. MESSING[1], U. HEBERLEIN[2], R. A. MORRISETT[1];
[1]Pharmacol., Univ. of Texas at Austin, Austin, TX; [2]HHMI, Janelia Res. Campus, Ashburn, VA

Abstract: The transcriptional regulator LMO4 is highly expressed in pyramidal projection neurons of the basolateral complex of the amygdala (BLA), and we previously demonstrated an important role for BLA LMO4 in fear learning. More recently we have examined the role of LMO4 in cue–reward learning using mice that have a genetrap insertion at the *Lmo4* locus (*Lmo4gt/+*), which results in 50% reduction in *Lmo4* expression. We found that *Lmo4gt/+* mice display a selective deficit in conditioned reinforcement that was recapitulated by RNAi-mediated knockdown of LMO4 in the BLA of wild type mice. Thus, given the BLA as a critical structure in which LMO4 influences fear and reward learning, we sought to investigate whether electrophysiological properties of neurons in this region are altered in *Lmo4gt/+* mice. To that end, we performed whole-cell patch-clamp recordings in current clamp mode to measure membrane properties and the excitability of BLA pyramidal neurons *in vitro*. The resting membrane potential and the threshold potential for firing action potentials in response to depolarizing current injection were similar in BLA neurons from WT and *Lmo4gt/+* mice, as were other action potential characteristics. However, BLA neurons from *Lmo4gt/+* mice exhibited several features of increased excitability such as greater input resistance ($p<0.01$) and lower rheobase current (the amplitude of depolarizing current injection necessary to evoke action potential firing) ($p<0.01$). *Lmo4gt/+* BLA neurons also displayed greater spike firing frequencies in comparison with WT BLA neurons in response to increasing steps of depolarizing current injections ($p<0.01$). In light of this difference in intrinsic excitability, we next evaluated whether

Lmo4gt/+ BLA neurons were equally sensitive to the modulatory effects of dopamine on excitability. As expected, brief bath application of dopamine (10 μM) significantly increased the firing frequency of WT BLA pyramidal neurons in response to depolarizing current step ($p<0.01$). Dopamine application also increased the firing of neurons from *Lmo4gt/+* mice ($p<0.01$), but the magnitude of this effect was markedly attenuated relative to WT ($p<0.05$). Thus, the ability of BLA pyramidal neurons to respond dynamically to an increase in extracellular dopamine concentration appears to be compromised in *Lmo4gt/+* mice. Overall, these findings suggest that reduction of LMO4 in the BLA causes changes in pyramidal neuron physiology, which may contribute to the behavioral differences observed between WT and *Lmo4gt/+* mice.

Disclosures: R.A. Mangieri: None. **R. Maiya:** None. **R.O. Messing:** None. **U. Heberlein:** None. **R.A. Morrisett:** None.

Poster

177. Learning and Memory: Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.10/AA9

Topic: F.02. Animal Cognition and Behavior

Title: Effect of chronic stress and streptozotocin treatment on choline acetyltransferase and neuronal plasticity in adolescent rats

Authors: K. HERNÁNDEZ-MERCADO[1], L. REYES-CASTRO[2], E. ORTA-SALAZAR[3], *C. PEREZ-CRUZ[1];
[1]CINVESTAV, Mexico City, Mexico; [2]Natl. Inst. of Nutr. Salvador Zubirán, Mexico City, Mexico; [3]Neurbiology Inst., Queretaro, Mexico

Abstract: Chronic stress triggers maladaptive changes altering learning and memory functions. During adolescence some parts of the brain are still under development and exposure to stress events may cause plastic changes in the central nervous system leading to behavioral alterations. Furthermore, metabolic diseases such as diabetes type two, also triggers cognitive decline. Importantly, animal models have shown that chronic stress dampers the development of diabetes after streptozotocin (STZ) administration. Despite the importance of those two factors involved in cognitive functions, there are no reports regarding the effect of chronic stress in development of diabetes in adolescent rats, and its impact in cognitive functions and neuroanatomical alterations. In this study, we hypothesized that chronic restraint stress in adolescent rats will cause neuroplastic changes at structures involved in learning and memory functions and modify

the further effect of STZ injection. Our results showed that stress and STZ causes behavioral alterations, and its effects are related to modifcations in choline acetyltransferase and postsynaptic density protein 95 immunoreativity and number of dendritic spines in CA3 region of hippocampus and prefrontal cortex. These data may offer important information to develop strategies to manage the incidence of stress-related pathologies during the adolescence.

Disclosures: K. Hernández-Mercado: None. **L. Reyes-Castro:** None. **E. Orta-Salazar:** None. **C. Perez-Cruz:** None.

Poster

177. Learning and Memory: Physiology

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Topic: F.02. Animal Cognition and Behavior

Support: NINDS Grant NS45260

NINDS Grant NS085709

Title: Non-RGD ß1 integrins regulate cytoskeletal reorganization underlying long-term potentiation in the dentate gyrus

Authors: *W. WANG[1], S. KANTOROVICH[1], C. M. GALL[1,2], G. LYNCH[1,3];
[1]Dept. of Anat. and Neurobio., [2]Dept. of Neurobio. and Behavior, [3]Dept. of Psychiatry and Human Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: A now substantial body of evidence indicates that membrane adhesion receptors belonging to the integrin family play a critical role in the consolidation of long-term potentiation (LTP) and memory. Moreover, particular subunits of the integrin alpha/beta dimers have been linked to these physiological and behavioral effects. Studies to date have focused on hippocampal field CA1 or forms of learning dependent upon that field; very little is known about integrins and plasticity at other sites. Here we report that i) integrins are critically involved in LTP in the lateral perforant path (LPP) projections to the dentate gyrus, and ii) the pertinent integrins differ from those involved in plasticity in field CA1. Peptides or toxins (disintegrins) that block the consensus RGD binding site for a large family of integrins block LTP in field CA1 but these reagents had no effect in the LPP. However, neutralizing antisera against the ß1 integrin subunit, which prevents LTP consolidation in CA1, proved to be very effective in the LPP. Moreover, mice with a conditional knockout of the ß1 integrin subunit also had a severe

LTP impairment in the dentate. Two homologous, integrin associated tyrosine kinases (FAK, Pyk2) provide essential links between the adhesion receptors and cytoskeletal reorganization. Studies using Fluorescence Deconvolution Tomography showed that LTP induction is accompanied by FAK activation at LPP synapses, a result that is highly suggestive of integrin activation. Collectively, the results indicate that engagement of non-RGD, ß1 integrins are required for memory-related changes to LPP contacts. The list of such integrins is relatively short, particularly given what is known about integrin subunit expression in the entorhinal-dentate system. Hypotheses about the functional significance of regional differences in integrin driven LTP will be described.

Disclosures: W. Wang: None. **S. Kantorovich:** None. **C.M. Gall:** None. **G. Lynch:** None.

Poster

177. Learning and Memory: Physiology

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.12/AA11

Topic: F.02. Animal Cognition and Behavior

Support: NIA grant AG035379

Title: Excitatory and inhibitory disabled-1 knockdown reveals cell-specific role of reelin signaling in adult synaptic plasticity

Authors: *A. L. LUSSIER[1], H. L. MAHONEY[2], J. H. TROTTER[3], A. DODGE[2], B. CAPRARO[2], B. CAPRARO[2], H. SOUEIDAN[2], G. D'ARCANGELO[4], E. J. WEEBER[2]; [1]Mol. Pharmacol. and Physiol., [2]Mol. pharmacology and physiology, Univ. of South Florida, Tampa, FL; [3]Mol. and cellular physiology, Stanford Univ., Stanford, CA; [4]Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ

Abstract: Disabled-1 (Dab1) is an intracellular adaptor protein that acts downstream of the Reelin signaling pathway to promote neuronal migration in the developing brain. In the adult brain the Reelin signaling pathway is important in dendritic and spine growth, learning and memory, and synaptic plasticity. Using novel Dab1 knockout mice in excitatory (eKO; Camk2 promoter) and inhibitory (iKO; GAD2 promotor) cells, we examined the role of Reelin on cell-specific activation of Dab1 in adult synaptic plasticity. In support of the well characterized role of Reelin signaling in excitatory neurotransmission, our eKO mice revealed a deficit in induction and maintenance of long-term potentiation. Surprisingly, the inhibitory Dab1 knockout mice (iKO) also show a deficit in long-term potentiation induction and maintenance. To examine the

role of Reelin in GABAergic cell activation we injected Reelin into the ventricles of eKO mice which revealed an activation of GABAergic interneurons. These data support a role of the Reelin signaling pathway in GABAergic interneurons and open new avenues for understanding the role of the Reelin signaling pathway in synaptic plasticity and learning and memory. Collectively, these data demonstrate an important new method for examining Reelin-Dab1 signaling in the adult brain, and underscore the importance of this pathway in synaptic plasticity.

Disclosures: A.L. Lussier: None. **H.L. Mahoney:** None. **J.H. Trotter:** None. **A. Dodge:** None. **B. Capraro:** None. **B. Capraro:** None. **H. Soueidan:** None. **G. D'Arcangelo:** None. **E.J. Weeber:** None.

Poster

177. Learning and Memory: Physiology

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Program#/Poster#: 177.13/AA12

Topic: F.02. Animal Cognition and Behavior

Support: Israel Science Foundation

Title: Simultaneous and persistent CaMKII, PKC and PKA activation is required for maintaining learning-induced enhancement of AMPAR-mediated synaptic excitation

Authors: *S. GHOSH, E. BARKAI, R. LAMPRECHT;
Univ. of Haifa, Haifa, Israel

Abstract: Learning leads to changes in AMPA receptor (AMPAR) synaptic expression level and conductance. The mechanisms for maintaining such alterations needed for memory formation remain to be clarified. Here we report a novel molecular mechanism needed for maintaining high-skill learning-induced AMPAR-mediated enhancement of synaptic excitation. We show that training rats in a complex olfactory discrimination task, such that requires rule learning, leads to the enhancement of averaged amplitude of AMPAR-mediated miniature excitatory post-synaptic currents (mEPSCs) in piriform cortex pyramidal neurons for days after learning. Inhibiting calcium/calmodulin-dependent kinase II (CaMKII) or protein kinase C (PKC), days after learning, reduced the averaged mEPSC amplitude in neurons from the trained rats to the level where they are not significantly different from mEPSC of control animals. Inhibition of protein kinase A (PKA), days after learning, reduced the averaged mEPSC amplitude in trained rats significantly but not to the level of the control rats. Concomitant inhibition of CaMKII, PKC and PKA activities abolished the learning-induced enhancement of mEPSC in a non-additive

manner. We conclude that the maintenance of learning-induced enhancement of AMPAR-mediated synaptic excitation requires the persistent and simultaneous activation of CaMKII, PKC and PKA.

Disclosures: **S. Ghosh:** None. **E. Barkai:** None. **R. Lamprecht:** None.

Poster

177. Learning and Memory: Physiology

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR Grant

Title: Changes in brain structure volume following training depend on long term memory formation and CREB

Authors: ***D. A. VOUSDEN**[1,3], M. VAN EEDE[2], A. YIU[2], L. SPENCER NOAKES[2], B. J. NIEMAN[2,3], M. HENKELMAN[2,3], S. A. JOSSELYN[2,4], P. W. FRANKLAND[2,4], J. P. LERCH[2]; [1]Neurosci. & Mental Hlth., [2]Hosp. For Sick Children, Toronto, ON, Canada; [3]Dept. of Med. Biophysics, [4]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Recent human imaging studies show that learning can change the brain at a scale detectable with MRI. For instance, learning to juggle or navigate a complex environment alters the volume of task-specific brain areas [1]. These findings have been recapitulated in rodent studies, which show spatial training increases the volume of the hippocampus, a critical area for spatial memory [2]. However, the cellular mechanisms and signaling pathways underlying these volume changes remain unknown. . It is well established that learning and memory formation require cellular changes in the brain. Initially, learning depends on changes in synaptic strength; long-term memory (LTM) formation, however, requires protein synthesis, which enables structural alterations to neurons and surrounding glia. We hypothesize that the training-associated neuroanatomical changes seen with MRI are due to neuronal and glia remodelling associated with LTM formation. Accordingly, we hypothesize that volume changes can be blocked by targeting the signaling pathways implicated in LTM. . CREB is a transcription factor that is critical for LTM. It regulates the transcription of many genes whose products are involved in LTM, and also modulates dendritic growth. Here, we used CREB mutant mice to ask whether changes in brain structure volume following training depend on CREB-dependent signaling and LTM formation. . Methods: CREB mutant mice (CREB -/-, CREB +/- & CREB +/+) were

trained on either the spatial or non-spatial Morris water maze for 6 days (N=20/group). A probe trial was performed 24 hours after training. Eight days after training mice were sacrificed and brains were imaged with high-field MRI. Automated image processing algorithms were used to detect volumetric brain changes associated with training. . Results: All mice learned the platform location, but CREB -/- mice had significant deficits in LTM on the probe. Preliminary results suggest that consistent with prior studies, hippocampal volume is larger in spatially trained wildtype mice than in untrained controls. Conversely, there was little effect of spatial training on hippocampal volume in the CREB -/- mice. This suggests that volume changes following maze training depend on LTM formation and CREB-dependent signaling, and that CREB-dependent cellular remodelling may underlie the neuroanatomical changes seen with MRI. Intriguingly, CREB +/- mice trained on the spatial water maze had normal LTM, but there was no effect of training on hippocampal volume. This suggests both LTM formation and CREB dosage underlie the volume changes seen with maze training. . **References** 1. Zatorre et al Nat Neurosci 2012 2. Lerch et al NeuroImage 2011

Disclosures: D.A. Vousden: None. **M. van Eede:** None. **A. Yiu:** None. **L. Spencer Noakes:** None. **B.J. Nieman:** None. **M. Henkelman:** None. **S.A. Josselyn:** None. **P.W. Frankland:** None. **J.P. Lerch:** None.

Poster

177. Learning and Memory: Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.15/AA14

Topic: F.02. Animal Cognition and Behavior

Title: Role of voltage-gated potassium channel Kv1.5 in CNS-based behavior

Authors: *S. BHATTA[1], C. PATEL[2], H. MCGEE[2], W. CHILIAN[3], G. CASADESUS SMITH[2,1];
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Abstract: Voltage-gated potassium channels, such as the Kv1 family, are responsible for maintaining membrane potential and are capable of modulating electrical excitability in excitable tissues. Previous studies have shown that loss of the Kv1.5 channel in the heart causes atrial fibrillation. Accumulating evidence suggests Kv1.5 is present in the brain, however its role in CNS function is unknown. As maintenance of neuronal excitability is essential for synaptic transmission, we proposed that genotypic loss of Kv1.5 channel would induce changes in CNS

associated behaviors. To address this, we carried out a full phenotyping battery to determine whether CNS functions including locomotor, emotion, and learning and memory would be altered in the Kv1.5 knockout mouse. Our results demonstrate that Kv 1.5 knockout shows impairments in learning and memory function in addition to habituation and anxiety processes when compared to wild-type littermate controls. Taken together, our work suggests Kv1.5 channel is crucial for functionality of the brain in addition to its known role in the heart and provides potential mechanisms of through which changes have occurred.

Disclosures: S. Bhatta: None. **C. Patel:** None. **H. Mcgee:** None. **W. Chilian:** None. **G. Casadesus Smith:** None.

Poster

177. Learning and Memory: Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.16/AA15

Topic: F.02. Animal Cognition and Behavior

Title: Circadian levels of serotonin in plasma following a mild traumatic brain injury in rats

Authors: *C. C. TENN, N. CADDY, M. GARRETT;
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Abstract: Introduction There has been a lot of interest especially among the military population, in the role blast exposure plays in traumatic brain injuries (TBI) and how this may link to the clinical features of the injury. The mechanism by which exposure to a shockwave can affect memory, concentration, balance, sleep and other neurobehavioral functions is still unknown. Previous studies have shown that various neurotransmitter systems including serotonin (5-HT) are affected following brain injury. Moreover, 5-HT has been suggested as one of several biomarkers associated with the cognitive dysfunction following TBI. Understanding the serotonin responses following brain injury could aid in predicting cognitive outcomes of TBI patients. In this study, we assessed the circadian levels of 5-HT in plasma using a brain injury model previously shown to induce cognitive impairment. **Methods** Rats were housed under controlled environmental conditions of light (12h light/dark cycles) and temperature. A mild brain injury was induced in anaesthetized animals by exposure to a single shockwave intensity of 140 kPa for 7.1ms duration. Control animals underwent the same treatment except for the shockwave exposure. Blood samples were taken at various times during the light and dark periods within a 24h period. All handling during the dark period was done under a dim red light. Sampling was done several days prior to the shockwave exposure (baseline), 24h and 7d

following exposure. Serotonin levels were determined by means of a commercially available enzyme immunoassay kit. **Results** Baseline data revealed a circadian variability in plasma levels of 5-HT during the 24h test period. The highest level of 5-HT was measured at 2h after lights-off with the lowest levels occurring late in the dark period. Twenty-four hours after shockwave exposure, 5-HT levels were significantly elevated in brain injured animals and the circadian pattern was altered when compared to controls. One week following the exposure, the levels of 5-HT were similar between the two groups of animals during the day. Although the neurotransmitter levels remained higher during the dark period for the head-injured animals, the circadian pattern returned to that of controls. **Conclusion** The findings from this study demonstrate that exposure to mild blast resulted in transient changes in the circadian levels of serotonin in blood. These changes in 5-HT levels in the acute phase following a mild brain injury may play an important role in the pathophysiology of blast-induced neurotrauma.

Disclosures: **C.C. Tenn:** None. **N. Caddy:** None. **M. Garrett:** None.

Poster

177. Learning and Memory: Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.17/AA16

Topic: F.02. Animal Cognition and Behavior

Support: ADA Basic Science Grant 7-12-BS-126

Title: The cognitive and neural impact of recurrent hypoglycemia is mediated by glucocorticoids in the hippocampus

Authors: *E. C. MCNAY, D. M. OSBORNE;
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Abstract: Recurrent hypoglycemia (RH) is primarily caused by repeated over-administration of insulin. This occurs most commonly among patients with Type I Diabetes; however, it is increasingly common in Type 2 diabetes also. Although acute hypoglycemia impairs cognitive and neural function, the long-term effects of RH are more complex: during subsequent euglycemia, both humans and animals show enhanced hippocampal memory following RH along with altered hippocampal metabolism and activity. Hypoglycemia causes systemic elevation of counter-regulatory hormones including glucocorticoids (GCs), and RH alters this secretion profile. Because GCs modulate cognitive performance and modulate the hippocampus, we investigated whether the impact of RH on the hippocampus might be mediated, at least in part,

by GCs. Using our previously-validated rat model of RH, hypoglycemia was induced once a day for three consecutive days, with or without an infusion of GC antagonists to the hippocampus. Following the final RH bout, rats were trained and 24 hours later tested in contextual fear. Hippocampal tissue was immediately extracted following tested for analysis by western blot. RH increased fear memory, and this effect was completely attenuated by hippocampal GC blockade during the RH episodes. Additionally, the improved cognitive performance in RH rats was accompanied by increased hippocampal expression of glucocorticoid receptor, SGK1, pCREB, as well as plasma membrane levels of AMPA and NMDA receptors; ALL of these effects were regulated by GC activity. These results indicate that RH produces several physiological changes in the dorsal hippocampus that are conducive to enhanced memory, and support the hypothesis that GCs are responsible for mediating the impact of RH on hippocampal function. Moreover, changes induced by RH are adaptive, indicating a response that would reduce physiological damage which could otherwise arise following repeated exposure to hypoglycemia.

Disclosures: **E.C. McNay:** None. **D.M. Osborne:** None.

Poster

177. Learning and Memory: Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 177.18/AA17

Topic: F.02. Animal Cognition and Behavior

Support: CNPq

FAPERJ

CAPES

Title: Defective brain insulin signaling and long-term cognitive impairment in a mouse model of sepsis

Authors: *F. CAMPOS RIBEIRO[1], F. S. NEVES[2], P. T. MARQUES[3], R. L. FROZZA[4], C. BENJAMIM[5], J. DE OLIVEIRA[6], D. F. ENGEL[6], A. F. DE BEM[6], F. G. DE FELICE[4], S. T. FERREIRA[4], J. R. CLARKE[3], C. P. FIGUEIREDO[3];
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Abstract: Substantial improvements have been achieved in recent years in the treatment of sepsis and its sequelae. However, a large portion of sepsis patients develops long-term cognitive impairment, and the underlying causes of such a devastating symptom remain to be established. Here, we subjected mice to sepsis induced by cecal ligation and puncture (CLP) to examine the possibility that brain insulin signaling might be dysregulated and associated with cognitive impairment in post-septic mice, similarly to what has been described in Alzheimer's disease (AD) brains. Mice underwent CLP or sham-operation and were treated with antibiotic therapy. Thirty days thereafter, mice were tested in the novel object recognition (OR) paradigm, followed by molecular analysis to evaluate possible impairment in hippocampal insulin signaling. Sepsis-surviving mice (30 days after surgery) failed to acquire the OR memory, but exhibited normal performance when re-evaluated 45 days after surgery. Increased tumor necrosis factor (TNF- α) and decreased cAMP response element-binding protein (CREB) mRNA levels were found in hippocampal extracts from post-septic animals (30 days after CLP). Significantly, CLP impaired brain IRS-1 signaling, indicated by increased hippocampal IRS-1 phosphorylation at serine residue 636 (IRS- 1pSer636) and decreased phosphorylation at tyrosine residue 465 (IRS-1pTyr465). We also found that phosphorylation of Akt at serine residue 473 (AktpSer473) and phosphorylation of GSK3 5; at serine residue 9 (GSK3βpSer9) were decreased in hippocampi of post-septic animals, supporting the notion that, as in AD, brain IRS-1 inhibition and increased GSK3β activity are linked to long-term memory impairment in sepsis. Conclusions: By establishing that disrupted insulin signaling is a common denominator between post-septic and AD brains, results improve our understanding of the pathophysiology of sepsis-associated encephalopathy and cognitive impairment, and open new avenues for therapeutic approaches targeting this condition.

Disclosures: **F. Campos Ribeiro:** None. **F.S. Neves:** None. **P.T. Marques:** None. **R.L. Frozza:** None. **C. Benjamim:** None. **J. de Oliveira:** None. **D.F. Engel:** None. **A.F. de Bem:** None. **F.G. De Felice:** None. **S.T. Ferreira:** None. **J.R. Clarke:** None. **C.P. Figueiredo:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.01/AA18

Topic: F.02. Animal Cognition and Behavior

Title: Paracetamol and object recognition memory in mice

Authors: *R. A. ANTONAWICH[1], R. S. GORE[2], I. L. KENT[2], J. T. CANNON[2,3], P. T. ORR[2,3];
[1]Neurosci. Program, Univ. of Scranton, Scranton, PA; [2]Neurosci. Program, [3]Psychology Dept., Univ. of Scranton, Scranton, PA

Abstract: Paracetamol, commonly known as tylenol, is the most commonly used over-the-counter pain and fever medication. However, there has been little investigation into its cognitive effects. Recent evidence suggests that the analgesic effects of paracetamol may be due, in part, to its actions as a cannabinoid reuptake inhibitor. Cannabinoids can interfere with memory consolidation through CB1 receptors in the hippocampus. Thus, paracetamol may interfere with memory consolidation through its interactions with cannabinoids. In this study, 24 male C57Bl/6 mice were tested on the object recognition (OR) task, which is dependent on the dorsal hippocampus. Following training, mice were assigned to receive regular drinking water or one of two doses of paracetamol (200 mg/ml or 400 mg/ml) suspended in drinking water for 12 hours. No group demonstrated an object or side preference during training. Mice receiving regular drinking water showed a preference for the novel object during testing, indicating intact memory for the familiar object. Mice in the 200 mg/ml and 400 mg/ml groups did not show a preference for the novel object during testing, suggesting no memory for the familiar object. Based on these results, it appears that 12 hours of paracetamol exposure interferes with OR memory in mice.

Disclosures: R.A. Antonawich: None. **R.S. Gore:** None. **I.L. Kent:** None. **J.T. Cannon:** None. **P.T. Orr:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.02/AA19

Topic: F.02. Animal Cognition and Behavior

Title: Chronic nicotine treatment normalizes hypothyriodism induced suppression of camkii pathway during learning and memory processes

Authors: *K. H. ALZOUBI[1], K. ALKADHI[2];
[1]Jordan Univ. of Sci. & Technol., Irbid, Jordan; [2]Univ. of Houston, Houston, TX

Abstract: Calcium/calmodulin protein kinase II (CaMKII) is a crucial molecule for hippocampus-dependent learning and memory. Activation of CaMKII is triggered by an increase in intracellular calcium concentration, which will activate PKCγ. This will release calmodulin

that will activate CaMKII forming phosphorylated (P-) CaMKII. Recently, we have shown that hypothyroidism impairs hippocampus-dependent learning and memory in adult rats. Nicotine, on the other hand, alleviates cognitive deficits during hypothyroidism. In this study, we investigated the interactive effect of nicotine and hypothyroidism on the CaMKII pathway during learning and memory processes. To locate a hidden (2cm under water) platform, each rat was trained in the radial arm water maze (RAWM) for 8 consecutive trials. Thirty minutes later, a memory test was done, and immediately after that hippocampi were dissected out, and CA1 areas were removed for determination of P-CaMKII, total CaMKII, PKCγ and calmodulin protein levels. RAWM trained rats were compared with age-matched controls, nicotine-treated, hypothyroid and nicotine treated/hypothyroid animals that underwent the same number and duration of swimming trials, but to locate a clearly visible (2cm above water) platform in an open (no radial arms) swim field. Western blot analysis revealed a significant increase in P-CaMKII, total CaMKII and PKCγ in the CA1 region of the hippocampus in RAWM-trained control, nicotine-treated, and nicotine treated/hypothyroid groups, but not in the hypothyroid group. However, no significant increase was found in the level of calmodulin in CA1 area of the hippocampus of all RAWM trained rats. Therefore, our study shows that chronic nicotine treatment normalizes hypothyroidism induced suppression of P-CaMKII, total CaMKII and PKCγ levels during hippocampus dependent learning and memory.

Disclosures: K.H. Alzoubi: None. **K. Alkadhi:** None.

Poster

178. Learning and memory: Pharmacology

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Program#/Poster#: 178.03/AA20

Topic: F.02. Animal Cognition and Behavior

Support: NRF Grant 2012R1A2A2A01046132

Title: Protective effects of choline alfoscerate (L-alpha-glycerylphosphorylcholine, α-GPC) on seizure-induced neuron death and cognitive impairment

Authors: *S. LEE[1], J. KIM[2], M. SOHN[3], H. SONG[4], H. CHOI[1], S. SUH[2];
[1]Dept. of Neurol., Hallym Univ., Chuncheon, Korea, Republic of; [2]Hallym University, Col. of Medicine, Dept. of Physiol., Chuncheon, Korea, Republic of; [3]Inha University, Dept. of Nursing, Incheon, Korea, Republic of; [4]Hallym Univ. Dept. of Neurology, Col. of Med., Chuncheon, Korea, Republic of

Abstract: An epileptic seizure is a brief episode of abnormal electrical activity in the brain that manifests as behavioral or sensory changes. While the episodes are transient, patients with epilepsy are at an increased risk for developing lasting deficits to cognitive function and/ or behavioral abnormalities. Choline alfoscerate (α-GPC) is a natural choline compound found in the brain. It is also a parasympathomimetic acetylcholine precursor that has been shown to be effective in the treatment of Alzheimer's disease and dementia. α-GPC is used to enhance memory and cognition for stroke and Alzheimer's patients but currently remains untested in patients suffering from epilepsy. This study aimed to evaluate whether α-GPC treatment after seizure can ameliorate seizure-induced cognitive impairment and neural injury. The potential therapeutic effects of α-GPC on seizure-induced cognitive impairment were tested in an animal model of pilocarpine-induced epilepsy. Seizure was induced by intraperitoneal (i.p) injection of pilocarpine (25 mg/kg) in adult male rats. α-GPC (250 mg/kg) was injected into the intramuscular (i.m) space three weeks after seizure onset for three weeks once-daily administration. To evaluate if treatment with α-GPC provides protection to hippocampal-dependent cognitive abilities following seizure we analyzed subject performance using a standard water maze test protocol and brain NeuN immunohistochemistry to determine hippocampal neuronal survival. All groups were sacrificed at 6 weeks post-seizure. In the present study, we observed enhanced survival of hippocampal neurons and improved cognitive function in animals receiving α-GPC injection after pilocarpine-induced seizure. Therefore, choline alfoscerate (α-GPC) injection may serve as a beneficial treatment for improvement of cognitive function in epilepsy patients.

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Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.04/AA21

Topic: F.02. Animal Cognition and Behavior

Support: Smoking Research Foundation in Japan

Title: Doxorubicin and cyclophosphamide treatment causes anxiety-like behavior and spatial cognition impairment in rats

Authors: ***Y. KITAMURA**[1,2], S. WATANABE[2], S. YONEDA[2], M. SUGIMOTO[2], E. KANEMOTO[2], H. KANZAKI[1], A. MACHIDA[2], I. MIYAZAKI[3], M. ASANUMA[3], T.

SENDO[1,2];
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Abstract: Recently, the importance of the quality of life and mental condition of patients being treated for cancer has become recognized. Anxiety and depression are the most common mental disorders among all cancer patients. In addition, it was reported that cancer patients receiving chemotherapy experienced some degree of cognitive impairment. Previously, we examined that doxorubicin and cyclophosphamide led to anhedonia-like, anxiety-like, and spatial cognitive impairment in rats. The chronic administration of doxorubicin and cyclophosphamide decreased serum brain-derived neurotrophic factor (BDNF) protein levels and BrdU-positive cells in the dentate gyrus of the hippocampus. These behavioral changes may be attributed, in part, to decreased hippocampal neurogenesis, and chemotherapy may lead to these impairments by altering neurogenesis. In this study, we determined the effect of doxorubicin and cyclophosphamide treatment on neurochemical mechanisms in rats. [METHODS] Male Wistar rats were administered doxorubicin (2 mg/kg, i.p.) and cyclophosphamide (50 mg/kg, i.p.) once per week for 4 weeks. [RESULTS] Hippocampal BDNF and Bdnf mRNA levels were not decreased by treatment with doxorubicin and cyclophosphamide. However, hippocampal Cyclin D1 levels were significantly decreased by chemotherapy. [DISCUSSION and CONCLUSION] These results suggest that doxorubicin and cyclophosphamide induce anhedonia, anxiety, and spatial cognitive impairment, in addition to negatively affecting hippocampal neurogenesis, which may be related to hippocampal cyclin D1 levels but not hippocampal BDNF levels.

Disclosures: Y. Kitamura: None. **S. Watanabe:** None. **S. Yoneda:** None. **M. Sugimoto:** None. **E. Kanemoto:** None. **H. Kanzaki:** None. **A. Machida:** None. **I. Miyazaki:** None. **M. Asanuma:** None. **T. Sendo:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.05/AA22

Topic: F.02. Animal Cognition and Behavior

Support: Smoking Research Foundation in Japan

Title: Effects of nicotine on doxorubicin and cyclophosphamide-induced spatial cognition and anxiety in rats

Authors: *M. SUGIMOTO[1], E. KANEMOTO[1], S. WATANABE[1], I. MIYAZAKI[2], M. ASANUMA[2], Y. KITAMURA[1,3], T. SENDO[1,3];
[1]Dept. of Clin. Pharm., [2]Dept. of Brain Sci., Okayama Univ., Okayama, Japan; [3]Dept. of Pharm., Okayama Univ. Hosp., Okayama, Japan

Abstract: Many patients who receive chemotherapy to treat cancer experience depressive- and anxiety-like symptoms or cognitive impairment. However, irrespective of the evidence for anxiety, depressive impairment, and cognitive impairment due to chemotherapy, the underlying mechanisms are still not understood. Previously, we examined that doxorubicin and cyclophosphamide led to anhedonia-like, anxiety-like, and spatial cognitive impairment in rats. On the other hand, nicotine, an agonist for nicotinic acetylcholine receptors, is well-known to have cognitive and anxiolytic effects in both animals as well as humans. This study was undertaken to determine the effect of nicotine on doxorubicin and cyclophosphamide-induced anhedonia-like, anxiety-like, and spatial cognitive impairment in rats. [METHODS] Doxorubicin (2 mg/kg) and cyclophosphamide (50 mg/kg) were injected intraperitoneally once per week for 4 weeks. Nicotine (2 mg/kg, s.c.) was administered daily. We performed sucrose preference and novel location recognition tests. [RESULTS] Doxorubicin and cyclophosphamide led to anhedonia-like and spatial cognitive impairment in rats. The spatial cognitive impairment was reversed by nicotine treatment. [DISCUSSION and CONCLUSION] These results suggest that nicotine can reverse anticancer drug-induced spatial cognitive impairment. Studies are underway to clarify the subtypes of nicotine receptor mediating nicotine's improvement of spatial cognition impairment and mechanism of neurogenesis in rats repeatedly treated with doxorubicin and cyclophosphamide.

Disclosures: M. Sugimoto: None. **E. Kanemoto:** None. **S. Watanabe:** None. **I. Miyazaki:** None. **M. Asanuma:** None. **Y. Kitamura:** None. **T. Sendo:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.06/AA23

Topic: F.02. Animal Cognition and Behavior

Title: Evaluation of chronic treatment with quetiapine on executive function and prefrontal cortex volume, perfusion and ERK/MAPK signaling in rats

Authors: *I. PODDAR[1], C. M. HERNANDEZ[1], P. M. CALLAHAN[1], L. VANDENHUERK[2], M. G. BARTLETT[3], X. YANG[3], A. V. TERRY, Jr.[1];

[1]Pharmacol. and Toxicology, [2]Small Animal Behavior Core, Georgia Regents Univ., Augusta, GA; [3]Dept. of Pharmaceut. and Biomed. Sci., Col. of Pharmacy, The Univ. of Georgia, Athens, GA

Abstract: Cognitive dysfunction is considered one of the most debilitating symptoms of schizophrenia, however, our current understanding of how the primary treatments of this disease, antipsychotics (APs) affect cognition remains inadequate. Our previous work in animals has established that some APs can negatively affect several domains of cognition including attention, spatial learning, and memory as well as some neurobiological substrates of cognition (e.g., growth factors, cholinergic receptors). Here, we were interested in extending these studies to the commonly prescribed AP, quetiapine. The first objective was to determine a clinically-relevant dosing approach for quetiapine, in rats, by measuring plasma and brain levels over several chronic time points. Rats were thus treated with Quetiapine (25 mg/kg/day) orally in drinking water and plasma and brain quetiapine levels were measured after 15, 30, 60 and 120 days of exposure. In parallel groups, the effects of chronic quetiapine treatment on executive function was assessed using a set-shifting task and as an additional measure of cognition (also known to rely in part on the prefrontal cortex-PFC) extinction of fear memory was assessed. No changes were associated with shorter delays (i.e., 24 hours) and analyses are in progress to assess longer delays: 4, 7, 14, and 21 days following an unconditioned stimulus. Test subjects were also evaluated for extrapyramidal side effects using standard catalepsy tests: bar, grid and paw tests. Quetiapine treatment was not associated with any signs of catalepsy after any period of exposure that might have influenced performance in set-shifting or fear memory tasks. To correlate any structural changes in the PFC with quetiapine treatment, we utilized magnetic resonance imaging (MRI) to measure both perfusion and volume between 30 and 120 days exposure. No notable changes in PFC volume were associated with 30 days of quetiapine treatment. Analyses are underway to determine if quetiapine treatment is associated with time-dependent changes in PFC volumetric and/or intraflow rate. To supplement the MRI studies, multiple signaling molecules are being assessed after 30 and 120 days of treatment (ERK/MAPK, Akt/PKB, CREB and histone H3 motifs) to gain insight into molecular mechanisms that may underlie altered memory function. To date, we have observed significant alterations in Akt phospho-signaling after 30 days of treatment. In future experiments we will continue evaluating the chronic effects of quetiapine on additional domains of cognition, the integrity of the PFC, relevant signaling proteins, and we will make comparisons to other commonly prescribed APs.

Disclosures: I. Poddar: None. **C.M. Hernandez:** None. **P.M. Callahan:** None. **L. Vandenhuerk:** None. **M.G. Bartlett:** None. **X. Yang:** None. **A.V. Terry:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.07/AA24

Topic: F.02. Animal Cognition and Behavior

Title: Comparison of chronic treatment with quetiapine to risperidone on hippocampal-dependent memory function, volume and ERK/MAPK signaling in rats

Authors: *C. M. HERNANDEZ[1], I. PODDAR[1], P. CALLAHAN[1], S. SINHA[1], X. YANG[2], M. BARTLETT[2], A. V. TERRY, Jr[1];
[1]Pharmacol. and Toxicology, Georgia Regents Univ., Augusta, GA; [2]Univ. of Georgia, Athens, GA

Abstract: Cognitive dysfunction is considered one of the most debilitating symptoms of schizophrenia. However, our current understanding of how antipsychotics (AP), the primary treatment of this disease, affect cognitive function remains inadequate. Not only does this lack of knowledge extend to how APs affect the neurobiological processes that support cognitive function but also the development of therapeutic interventions to improve cognition in schizophrenia. Our previous work in animals has established that both representative typical and atypical APs can negatively affect attention, spatial learning, and memory if administered chronically, however, the mechanism for these adverse effects have not been clearly elucidated. In this study, male Wistar rats were dosed daily in drinking water up to 120 days, in mg/kg: Quetiapine (QUE, 10 or 25) or Risperidone (RISP, 2.5). Hippocampal (HIP)-dependent memory function was assessed after 30 days treatment using both an object recognition task (delay intervals: 4-, 6- and 48-hours) and water maze (spatial acquisition test). In a parallel cohort, brain AP levels were measured after 30, 60 and 120 days of exposure periods. After 30 days treatment, QUE treatment was associated with impaired recognition memory after an intermediate 6-hour delay compared to vehicle-treated controls. After 60 days treatment, no spatial learning deficits were associated with either AP. Locomotor activity was assessed in all rats and no AP-associated motor deficits were observed. To identify potential HIP structural changes associated with memory deficits, we utilized magnetic resonance imaging (MRI) to measure HIP volume and perfusion rate after 30 and 120 days treatment. To date, no notable volumetric changes were measured after 30 days treatment and analyses are in progress in the same cohort following an additional 90 days treatment. To supplement these structural and functional analyses, multiple signaling molecules (ERK/MAPK, Akt/PKB, CREB and histone H3 motifs) are being assessed in hippocampal extracts harvested after 30 and 120 days exposure to gain insight into potential molecular mechanisms that may underlie altered memory functions but also differ between QUE and RISP. In the future it is our objective to compare QUE and RISP (and other APs) and measure the timeline for these APs on cognition and HIP structural integrity.

Disclosures: **C.M. Hernandez:** None. **I. Poddar:** None. **P. Callahan:** None. **S. Sinha:** None. **X. Yang:** None. **M. Bartlett:** None. **A.V. Terry:** None.

Poster

178. Learning and memory: Pharmacology

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Program#/Poster#: 178.08/AA25

Topic: F.02. Animal Cognition and Behavior

Support: NIH NINDS R21 NS085471

Title: Regulation of a cerebellar voltage-gated potassium channels and cerebellum-dependent learning and memory

Authors: *J. R. FUCHS[1], A. D. MORIELLI[2], J. T. GREEN[3];
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Abstract: Eyeblink conditioning (EBC) is governed by sites of plasticity in the cerebellar cortex and the interpositus nucleus. However, the cellular mechanisms supporting EBC are poorly understood. The voltage-gated potassium channel alpha-subunit Kv1.2 is densely expressed at basket cell (BC) axon terminals (Koch et al., 1997) where they form inhibitory synapses with Purkinje cells (PCs). Kv1.2 is also expressed on PC dendrites (Koch et al., 1997). Our previous work showed that intra-cerebellar blockade of Kv1.2 facilitated EBC (Williams et al., 2012) and manipulations to trafficking mechanisms associated with Kv1.2 endocytosis lead to impairments or enhancement during delay EBC (Fuchs et al., 2014; Williams et al., 2012). In the current work, we addressed the question of whether EBC regulates surface expression of Kv1.2 in cerebellar cortex. Rats underwent three days of training, and training consisted of either delay EBC, explicitly unpaired stimulus presentations, or no stimuli. Following the last trial, cerebellar tissue surrounding the primary fissure and ipsilateral to the conditioned eye was fixed, harvested, and analyzed via biotinylation/western blot (WB) and multiphoton microscopy (MP) techniques. These two techniques offer a global and quantitative measure of Kv1.2 surface expression, and a region-specific level of analysis, respectively. After three days of training, the Unpaired group showed significantly less surface Kv1.2 at BC axon terminals as measured by MP and a trend towards greater surface Kv1.2 at BC axon terminals and PC dendrites as measured by WB. We hypothesized that inhibition of the inferior olive by the interpositus nucleus resulting from the generation of conditioned eyeblink responses contributed to differences observed between the Paired and Unpaired groups following three days of training. Thus, if we measure surface Kv1.2 expression earlier in training, we hypothesized that the Paired group and Unpaired group would appear similar, showing reduced surface Kv1.2 expression at BC axon terminals (MP) and increased surface Kv1.2 expression across the entire section (WB). This hypothesis is currently

being tested by repeating the experiment with only a half-day of EBC training. Overall, these data suggest that regulation of surface Kv1.2 in cerebellar cortex may be involved in acquisition but not expression or maintenance of the learned response during cerebellar-dependent EBC.

Disclosures: J.R. Fuchs: None. **A.D. Morielli:** None. **J.T. Green:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.09/AA26

Topic: F.02. Animal Cognition and Behavior

Support: NIH-NINDS R21 NS085471

Title: Cerebellar mGluR1 modulates cerebellar-dependent learning

Authors: *M. SHIPMAN[1], S. C. MADASU[2], A. D. MORIELLI[3], J. T. GREEN[4];
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Abstract: Eyeblink conditioning (EBC), a type of cerebellar-dependent learning, is an ideal model for studying learning because its neural circuitry is well characterized and behavior is easily quantifiable. In EBC paradigms, rats receive training sessions that consist of many trials of conditioned stimulus (CS) and unconditioned stimulus (US) pairings. In these trials, a tone (CS) predicts a periorbital shock (US) and rats learn to blink to the tone (CS) in anticipation of shock (US). This learned, anticipatory eye blink is the CR. Input from the CS and US are relayed separately through the brainstem onto both Purkinje cells in cerebellar cortex and to one of the deep cerebellar nuclei, the interpositus nucleus. CRs occur following disinhibition of the tonically inhibited interpositus nucleus. Mice lacking metabotropic glutamate 1 receptors (mGluR1) show impairments in EBC as well as parallel fiber (PF)-Purkinje cell (PC) long-term depression (LTD) (Kishimoto et al., 2002; Aiba et al., 1994; Ohtani et al., 2014); restoration of the mGluR1a splice variant in PCs restores both EBC and PF-PC LTD (Kishimoto et al., 2002; Ohtani et al., 2014). To examine the consequences of mGluR1 stimulation in cerebellar cortex on cerebellar-dependent learning, we infused 0.5 uL of the mGluR1 agonist DHPG (1 uM) into the lobulus simplex prior to training sessions 1 and 2 of delay EBC. Rats received 6 training sessions across 6 days, with 80 paired trials intermixed with 10 CS-alone and 10 US- alone trials per session. We found that mGluR1 stimulation enhances delay EBC. We then utilized the same experimental design to determine the effects of an mGluR1 antagonist in cerebellar cortex on

EBC using the mGluR1 antagonist YM-298198 (50 uM). Data collection for this second experiment is ongoing. Our results might be explained by drug effects on PF-PC LTD, although one study found that mice with mutations that prevent internalization of GluR2 AMPA receptors, a process necessary for expression of LTD, showed normal EBC (Schonewille et al., 2011). Other work from our lab has shown that blockade of the voltage gated potassium channel alpha-subunit Kv1.2, which is densely expressed at basket cell (BC)-PC synapses, enhances EBC and that DHPG reduces surface expression of Kv1.2. Reduction in surface Kv1.2 at BC-PC synapses could also explain how mGluR1 stimulation enhances EBC.

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

Poster

178. Learning and memory: Pharmacology

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Topic: F.02. Animal Cognition and Behavior

Support: NIH-NINDS R21 NS085471

NIH 5 P30 RR032135

NIGMS 9 P30 GM103498

Title: PKM-ζ is involved in cerebellar-dependent learning and memory

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Abstract: PKM-ζ has long been implicated in a hippocampal cellular correlate of learning, long-term potentiation (LTP), through its regulation of hippocampal AMPA receptors (Ling et al., 2002; Yao et al., 2008). Disruption of PKM-ζ with Zeta-inhibitory peptide (ZIP) can irreversibly disrupt hippocampal memory that has been maintained for many weeks (Hernandez et al., 2003). Despite being highly expressed in the cerebellum (Oster et al., 2004), no studies have examined how regulation of cerebellar PKM-ζ may affect cerebellar-dependent learning and memory. We have shown for the first time that infusion of ZIP in the lobulus simplex of the cerebellum can significantly disrupt delay eye-blink conditioning (EBC) in rats, a form of cerebellar-dependent learning. Infusion of 0.50 µl 20 mM ZIP or 0.50 µl PBS vehicle occurred 2 hours prior to the first acquisition session of EBC, ipsilateral to the conditioned eye; rats underwent a total of 6

daily sessions of 350-ms delay EBC. We hypothesized that PKM-ζ may have regulatory effects on voltage-gated potassium channel alpha-subunit 1.2 (Kv1.2). Several studies have shown that PKC-ζ can co-immunoprecipitate with and phosphorylate a β subunit that associates with cerebellar Kv1.2 (Gong et al., 1999; Croci et al., 2003). Kv1.2 is highly expressed in cerebellar basket cell axon terminals and Purkinje cell dendrites and our lab has shown that Kv1.2 is important for cerebellar EBC in rats (Williams et al., 2012). Here we demonstrate that PKM-ζ can significantly reduce Kv1.2 surface expression in HEK 293 cells.

Disclosures: K. Chihabi: None. **J.T. Green:** None. **A.D. Morielli:** None.

Poster

178. Learning and memory: Pharmacology

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Topic: F.02. Animal Cognition and Behavior

Support: Internal Grant: JMU

Title: Sleep deprivation and voluntary alcohol consumption: the neuroplasticity of chronic behaviors

Authors: *J. DYCHE[1], K. PONDER[2];
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Abstract: Alcohol is one of the most common psychoactive drugs, and has been used by humans for thousands of years. Research has focused on the effects of alcohol on sleep, however recent trends in the literature have taken a more bidirectional approach to the relationship between alcohol and sleep. Research on transcription factors involved in the drugs of abuse, including alcohol, suggests a maladaptive alteration of these proteins in the presence of chronic use. The research on transcription factors and chronic sleep deprivation has been sparse. The present study extends the results of previous research on sleep and alcohol to voluntary alcohol consumption and the subsequent buildup of addiction related transcription factors. This research investigates the effects of chronic, partial sleep deprivation on alcohol consumption. Twelve Sprague Dawley rats had free access to two bottles at all times, one containing water and one containing a 7% alcohol and water solution. Five rats had no access to alcohol. Sleep deprivation was achieved by using a forced exercise wheel. All rats were sleep restricted every day for 7 days in one week. There were three total weeks of chronic partial sleep deprivation that were interspersed with a week for recovery. Three rats experienced an alcohol only condition, and three rats were purely

control rats with no exposure to sleep deprivation or alcohol. There was a significant effect of sleep condition on voluntary alcohol consumption, $F(4, 44) = 9.191$, $p < .001$, partial $\eta 2 = .455$. The partial eta squared showed a large effect, 45.5% of the variance in error associated with alcohol consumption can be explained by sleep condition. It was found that sleep deprivation increased alcohol consumption. Histology was performed on all rats to stain for differences in Delta Fos B levels in areas involved with reward and sleep.

Disclosures: J. Dyche: None. **K. Ponder:** None.

Poster

178. Learning and memory: Pharmacology

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NIDA 5 R25 DA035161-03

Title: Sex specific developmental effects of caffeine and taurine on cognitive function

Authors: *S. L. PEREZ[1], K. CHAUHAN[2], K. URIBE[4], D. WOO[2], M. EVELYN[2], M. GUZMAN[2], U. AKPARA[2], F. JACQUES[2], S. SINGH[2], M.-R. MURITALA[2], S. AYO[2], S. SOYEMI[2], A. COLE[2], P. DUVALSAINT[2], A. ELZANIE[2], D. HARRIS[2], S. MARACHERIL[2], D. PETERS[4], A. ALEXANDER-STREET[5], K. Y. SALAS-RAMIREZ[3];
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Abstract: Energy drinks contain caffeine and taurine, among other compounds, which have been associated with improved cognitive performance. In the last 8 years, increased sales of drinks like Red Bull and Monster reflect an increase in energy drink consumption among the general

population. Few studies have directly compared the effects of these compounds on cognitive function during different developmental stages. Adolescence is a critical period of development when significant brain maturation occurs with varying rates of growth between sexes, in comparison to the adult brain. The objective of this study was to determine whether caffeine and taurine differentially effect cognition in adolescent and adult male and female rodents. Sixty-four Sprague Dawley rats of each sex were randomly divided into four groups (1) caffeine (n=8; 20mg/kg; ip), (2) taurine (n=8; 100mg/kg; ip), (3) caffeine and taurine (n=8; cocktail) or (4) saline. Adolescent rats began treatment on postnatal (P) day 33 and adults began treatment on P68. Animals were pre-treated for one week before beginning consecutive behavioral assessment for working, visual and spatial memory. Treatment was continued throughout the days of testing. Females were the least affected by compounds found in energy drinks, showing no effects on visual or working memory. However, a repeated measures three way ANOVA revealed a main effect of treatment ($p = 0.0075$) and age ($p=0.0016$) on the spatial memory task. Specifically, adolescent females showed impaired spatial memory, while taurine improved memory. Interestingly, adolescent males treated with taurine displayed improved working memory ($p = 0.05$) when compared to the saline controls. Nevertheless, they showed impairments in the visual and spatial memory tasks when exposed to caffeine, taurine and the cocktail. Adult males exhibit impairments only on the spatial memory task when exposed to caffeine and the cocktail. Three way repeated measures ANOVA revealed a significant interaction of age by treatment ($p = 0.0071$) in the spatial memory tasks, highlighting how compounds found in energy drinks can differentially effect adolescent and adult animals. Our findings suggest that males are more vulnerable to the separate and combined effects of caffeine and taurine when compared their female counterparts both adolescence and adulthood. In addition, these studies also underscore that the adolescent brain is more susceptible to the effects of exogenous compounds and their impact on behavior. Further studies will investigate the mechanisms by which these compounds differentially affect the cognitive behaviors.

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Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.13/AA30

Topic: F.02. Animal Cognition and Behavior

Title: Self-administration of caffeine in rats

Authors: *C. A. BRADLEY, A. M. BROWN, E. A. WILLIAMS, C. N. SWYMER, M. I. PALMATIER;
East Tennessee State Univ., Johnson City, TN

Abstract: Caffeine is the most consumed psychoactive drug in the world and functions as a reinforcer in participants who are low to moderate consumers of caffeine. However, no study has effectively established caffeine as a reinforcer in pre-clinical paradigms. In humans, the relationship between caffeine and operant behavior includes salient orosensory stimuli that could moderate the reinforcing effects of the drug (e.g., coffee, energy drinks, etc.). Therefore, we hypothesized that adding a salient gustatory reinforcer (saccharin) to a rodent caffeine self-administration paradigm would result in higher levels of operant responding. In Experiment 1, thirty rats were instrumented for intravenous self-administration and assigned to one of three groups: saccharin only (SACC, 0.2% w/v), caffeine only(CAFF, 0.5 mg/kg/inf.), and caffeine+saccharin (CAFF+SACC). The oral reinforcer (saccharin) was delivered in a liquid dipper cup, caffeine was delivered intravenously. Rats were tested in 1-hr sessions and their assigned reinforcer was available under a Progressive Ratio (PR) reinforcement schedule. This schedule measures motivation by increasing the number of responses required to earn each reinforcer (saccharin and/or caffeine). Lever pressing in the CAFF group declined across sessions, confirming previous studies showing that caffeine is not a reliable reinforcer. Saccharin maintained moderate levels of responding for rats in the SACC group. However, responding increased in the CAFF+SACC group and was significantly higher than both control groups by the end of testing, confirming our hypothesis that caffeine would increase operant behavior if it was delivered with a salient orosensory stimulus. In Experiment 2 we investigated whether this same approach would result in oral caffeine self-administration. Rats from CAFF+SACC and CAFF groups were combined into a control group that could earn dipper presentations of a vehicle solution (0.5% decaffeinated coffee and 0.2% saccharin). For the SACC group, the same vehicle was available but was spiked with different caffeine concentrations (0.5, 1.8, 2.5, 3.5, 5.0 mg/ml) in a pseudo-random order. The oral reinforcers were presented under the same PR schedule as Experiment 1. Oral caffeine dose-dependently increased responding for the vehicle solution, with the peak concentration between 1.8 and 2.5 mg/ml. These findings indicate that caffeine interacts with non-drug gustatory reinforcers to increase a well-defined operant response and are the first demonstration of reliable and repeatable caffeine self-administration in non-human subjects.

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Poster

178. Learning and memory: Pharmacology

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Program#/Poster#: 178.14/AA31

Topic: F.02. Animal Cognition and Behavior

Title: Aspartame consumption affects hippocampus, memory and anxiety in mice

Authors: *P. U. NWOHA[1], A. Y. ONAOLAPO[1,2];
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Abstract: Aspartame is a widely used sweetener and food additive. Yet nothing is known about its effect on the hippocampus and memory. In the present study, 30 adult male albino mice weighing, 20-22g were used. The mice were divided into five groups of six mice each. Group A is control and received distilled water. Groups B, C, D, and E were experimental and received 20, 40, 80, and 160mg/kg body weight respectively of aspartame dissolved in distilled water. Administration of distilled water or aspartame was daily by oral intubation for 28 days. Spatial memory was measured by recording sponataneous alternation behaviour in Y-maze, and anxiety was measured by noting open arm entries in elevated plus maze. Behavioural tests were conducted before and at end of the treatment period. After 28 days, the mice were anaesthetised by ketamin, brains removed processed for routine histology, sectioned at 5μ, and stained with H & E, Cresyl violet, and Bielschowsky's silver. Under the microscope the morphology and stereology of the hippocampi were studied. Values obtained were analysed statistically by ANOVA and Tukey's test. Results of the H & E, and cresyl violet methods showed extensive degeneration of pyramidal cells of CA1, CA2, CA3 and CA4 layers of the hippocampus, and granule cells of dentate gyrus. On Y-maze there was significant increase in % alternation at 20mg/kg, but significant decrease at dose 160mg/kg aspartame compared to control ($p<0.05$). Elevated plus maze showed significant increase in % time spent in the open arms ($F=26.6$) for all doses of aspartame compared to control ($p<0.05$). These suggest that aspartame consumption for such long period and for the doses affect the morphology of the hippocampus, reducing spatial memory and increasing anxiety in mice.

Disclosures: P.U. Nwoha: None. **A.Y. Onaolapo:** None.

Poster

178. Learning and memory: Pharmacology

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: JUST deanship of research

Title: Involvement of endogenous opioid system in sucrose-induced analgesia during infancy and long-term effects on learning and memory during adulthood in rats

Authors: *K. NUSEIR, K. ALZOUBI, A. BAWAANE;
Jordan Univ. of Sci. and Technol., Irbid, Jordan

Abstract: Premature human infants and newborns are often exposed to painful procedures. Several studies show that early pain experiences may have long-term consequences such as altered neurobehavioral development, and long-term changes in responsiveness of the neuroendocrine and immune systems to stress at maturity. Impairment in memory and learning process could be a long-term consequence of pain at early life. Pain is difficult to assess especially in neonates and young infants. Studies show that fear of adverse reactions and long-term effects often lead to inadequate use of analgesics in neonates. This leads to inadequate pain management in this group of patients. We have shown previously that orally administered sucrose solution given to rat pups exposed to painful stimuli during early life protected against short-term memory impairment. Sucrose induced analgesia is thought to be mediated, at least in part, via endogenous opioid system. Opioid antagonists inhibit sweet substances induces antinociception, and sweet solutions given orally augment morphine analgesia. In this study we will examine the involvement of the endogenous opioid system in sucrose-induced analgesia during infancy by using the orally effective pure opioid receptor antagonist naltrexone. We also will study the long-term effect of this treatment on learning and memory formation during adulthood in the rat. As well as examine the ability of sucrose solution for analgesia to reverse pain-induced learning and memory impairment. And weather this is mediated via endogenous opioid system.

Disclosures: **K. Nuseir:** None. **K. Alzoubi:** None. **A. Bawaane:** None.

Poster

178. Learning and memory: Pharmacology

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Topic: F.02. Animal Cognition and Behavior

Title: Effect of Local Kappa Opioid modulation on forebrain dependent trace associative learning: An eyeblink conditioning analysis

Authors: *R. LOH, S. SHAH, R. GALVEZ;
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Abstract: There have been several findings indicating a primary role for the opioid receptor system in the modulation of learning and memory. Spatial tasks, fear conditioning, and y-maze spontaneous alternation have all demonstrated that opioid modulation can impair task acquisition. Much of the current literature investigating opioid effects in learning and memory have focused on the mu opioid receptor; however, several reports have indicated that there is a likely role for the kappa opioid receptor in various behavioral learning paradigms. We have recently demonstrated that systemic administration of the kappa specific opioid antagonist, NorBNI, prior to training also delays acquisition for whisker-trace eyeblink (WTEB) associations in mice. In investigating the specific brain region mediating this effect, it is well known that primary somatosensory neocortex (S1) is required for the acquisition as well as consolidation of the trace association. The following study utilized intra-S1 injections of NorBNI with the behavioral paradigm WTEB to determine if the kappa opioid receptor is mediating its learning induced effect through primary somatosensory cortex. In WTEB, animals are trained to associate whisker stimulation (CS) with a salient unconditioned stimulus (US) that causes an unconditioned response. After several trials in which the CS is paired with the US, the animal begins to exhibit a conditioned response in anticipation of the US. Separating the CS and US with a stimulus free trace interval makes this paradigm forebrain dependent by recruiting higher brain structures such as the hippocampus and most importantly, the neocortex. The current study utilized localized primary somatosensory injections of 10μg of NorBNI paired with WTEB behavioral training. We found that local injections of NorBNI significantly retarded learning relative to saline controls, suggesting that the kappa opioid receptor is neocortically involved in the acquisition of forebrain-dependent trace associative learning. Further research will focus on determining the specific downstream effects of the kappa opioid receptor that is mediating this effect in learning.

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Poster

178. Learning and memory: Pharmacology

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R15MH093918-01A1

Title: Grape powder supplementation prevents cognitive, behavioral and biochemical impairments in rat model of social defeat

Authors: ***N. SOLANKI**[1], G. PATKI[2], F. ATROOZ[2], S. SALIM[2];
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Abstract: Psychological stress has been known to cause behavioral and cognitive impairments. In our previous study we used the modified version of the resident-intruder rat model of social defeat (SD) to induce stress. In the same study we have reported that SD-induced psychological stress is associated with increased oxidative stress, anxiety and depression-like behavior and cognitive deficits. Furthermore, we have reported protective effects of grape powder (GP) on behavioral impairments in pharmacologically-induced oxidative stress. However, the protective effect of GP treatment in SD-induced behavioral and cognitive deficits is unknown. Therefore, in the present study using the rat model of SD, we examined the protective effect of GP on SD-induced cognitive, behavioral and biochemical impairments. Male Sprague Dawley rats were randomly assigned into five groups: Naïve control, Naïve control treated with GP, Social defeat, control exposure, social defeat treated with GP. Anxiety-like behavior tests (open-field, light-dark and elevated plus maze) and forced swim test data suggested that GP treatment prevented SD-induced anxiety and depression-like behavior of rats. Moreover, GP also improved SD-induced impairment of memory function of rats, when examined using radial arm water maze test. Results suggest that GP ameliorates SD-induced behavioral and cognitive deficits possibly via reducing oxidative stress in rats.

Disclosures: **N. Solanki:** None. **G. Patki:** None. **F. Atrooz:** None. **S. Salim:** None.

Poster

178. Learning and memory: Pharmacology

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CONACYT Grant 130524

CONACYT Scholarship 371741 to C.S.P

Title: Glucocorticoids enhance emotional memory consolidation via recruitment of striatal endocannabinoids

Authors: C. SILLER PÉREZ[1], E. SOTELO BARRERA[1], N. SERAFÍN[1], R. PRADO-ALCALÁ[1], P. CAMPOLONGO[2], B. ROOZENDAAL[3], *G. L. QUIRARTE[1];
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Abstract: After a stressful experience, glucocorticoid hormones target brain structures that regulate the stress response as well as memory processes. One such structure is the dorsal striatum, which integrates different types of information associated to context, procedures, and arousal. Administration of corticosterone into the dorsal striatum enhances memory consolidation of an inhibitory avoidance task. Additionally, glucocorticoids in association with acetylcholine enhance memory consolidation. However, it is unknown if different neuromodulators interact with glucocorticoids to store a memory. Our objective was to asses if glucocorticoids interact with striatal endocannabinoids to consolidate a memory trace. Male Wistar rats were implanted bilaterally with cannulae into the anterodorsal striatum and were trained on an inhibitory avoidance task. In Experiment 1, the CB1 cannabinoid receptor antagonist AM251 (0.28 or 0.56 ng/µl) or its vehicle was administered intra-striatally immediately after training with a higher foot-shock intensity (0.6 mA, 1 s), and memory retention was evaluated 48 h later. In Experiment 2, rats were trained with a lower foot-shock intensity (0.45 mA, 1 s) and immediately after training they received 0.56 ng/µl of AM251 or its vehicle, followed by a systemic administration of corticosterone (3 mg/kg, i. p.) or its vehicle; a retention test was performed 48 h after training. We found in Experiment 1 that AM251 administration (0.56 ng/µl) caused a significant impairment in retention. In Experiment 2, systemic administration of corticosterone enhanced memory consolidation; importantly this effect was blocked by the prior intra-striatal administration of AM251 (0.56 ng/µl). Our findings provide evidence of the interaction of endocannabinoids and glucocorticoids in the modulation of emotional memory, suggesting that the endocannabinoid system needs to be active up-stream for glucocorticoids to enhance memory consolidation of an emotional training experience. We thank the excellent technical support from Cristina Medina, Ángel Méndez, Leonor Casanova, Lourdes Lara. This work was supported by PAPIIT-DGAPA, UNAM IN202414, and CONACYT (Grant 130524, and scholarship 371741 to C.S.P).

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Poster

178. Learning and memory: Pharmacology

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PAPIIT-UNAM IN214111

PAPIIT-UNAM IN202414

Title: Basolateral amygdala-striatal interactions regulating glucocorticoid effects on memory retrieval of a cued water-maze task

Authors: *J. PARGA-MARTÍNEZ[1], S. N[1], A. P[2], P.-A. RA[1], R. B[2], Q. GL[1];
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Abstract: Evidence indicates that glucocorticoid hormones, released after a stressful experience, impair the retrieval of hippocampus-dependent spatial/contextual memory in rats and declarative memory in humans. Recent findings suggest that glucocorticoids also act in the dorsal striatum to impair the retrieval of procedural memory. The present experiments investigated whether these effects depend on noradrenergic activity within the basolateral amygdala (BLA). Adult male Wistar rats were trained on a session of eight trials in a water maze to find a platform that was marked with a visual cue. The platform position and rat´s starting position were repositioned randomly on each trial. Retention of the training was tested 48 h later. In the first experiment, rats received infusions of either vehicle or corticosterone (5, 10 or 20 ng) into the dorsal striatum 30 min before the retention test. Corticosterone (10 ng) significantly impaired escape latencies to find the platform. In the second experiment, rats received corticosterone (10 ng) or vehicle into the dorsal striatum and the β1-adrenoceptor antagonist atenolol (0.5 μg) or β2−adrenoceptor antagonist zinterol (0.5 μg) or saline into the BLA 30 min before the retention trial. A blockade of noradrenergic activity (β1 and β2 receptors) in the BLA prevented the impairing effect of corticosterone on memory retrieval. These findings indicate that the BLA interacts with the striatum in mediating the impairing effects of glucocorticoids on retrieval of procedural memory.
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Poster

178. Learning and memory: Pharmacology

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Program#/Poster#: 178.20/AA37

Topic: F.02. Animal Cognition and Behavior

Support: ADA Basic Science Grant 7-12-BS-126

Title: Chronic resveratrol treatment ameliorates cognitive deficits associated with a high fat diet

Authors: *K. O'LEARY, R. ANKAWA, E. C. MCNAY;
Behavioral Neurosci., Univ. At Albany, Albany, NY

Abstract: Resveratrol is a polyphenol antioxidant, found in red wine and known to have anti-aging, anti-cancer, and anti-obesity effects in both humans and other animals. Resveratrol's primary biological action is as an antioxidant, causing a reduction in reactive oxygen species (ROS). ROS become abnormally elevated in individuals who consume a high fat diet (HFD) or who have metabolic conditions like Type II Diabetes Mellitus (T2DM); in these circumstances, elevated ROS lead to cellular damage and cell death. Another common effect of a diet high in fat and calories is cognitive impairment, and specifically impaired memory. Here, we investigated whether resveratrol administration would attenuate or reverse cognitive impairment caused by the ingestion of a high fat diet. Rats on either a control (chow) diet or high fat diet (60% kcal fat) were treated with resveratrol (or vehicle) once weekly for 20 weeks. Unexpectedly, no metabolic effect of resveratrol were seen: treatment did not affect body mass or fat level. However, resveratrol treatment reversed cognitive deficits seen in high-fat-fed rats, indicating a possible role of resveratrol as a therapeutic intervention to improve memory in individuals with metabolic dysregulation. Intriguingly, resveratrol treatment of control animals impaired, rather than enhancing, cognition, suggesting a possible inverted-U dose-response curve. Post-mortem measurement of molecular markers linked to memory formation, insulin signaling, and oxidative stress, and results suggested that resveratrol's impact may be mediated at least in part through regulation of AMPK activity. Our data suggest a possible role for resveratrol in preventative care for individuals prone to metabolic disease and related cognitive dysfunction.

Disclosures: **K. O'Leary:** None. **R. Ankawa:** None. **E.C. McNay:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.21/AA38

Topic: F.02. Animal Cognition and Behavior

Support: CPUB

SRG

BSI

Neimeyer-Hodgson

FRG

Title: Do estrogen and progesterone act synergistically to accentuate the cognitive effects of a serotonergic antagonist in female rats?

Authors: ***S. S. MASWOOD**, J. HASSELL;
Biol., Millersville Univ., Millersville, PA

Abstract: The neurotransmitter serotonin (5-HT) is involved in the modulation of cognitive functions. Although the precise mechanism of action of the 5-HT system in the facilitation of cognitive functions has not been clearly identified, compounds such as tropisetron that act as 5-HT3 receptor antagonist enhance cognition. Similar to the cognitive enhancing effects of tropisetron, female gonadal hormones such as estrogen and or progesterone also improve cognitive behavior in rodents. Interestingly, both estrogen and progesterone inhibit the function of the 5-HT3 receptors. The objective of our study is to evaluate the cognitive effects of tropisetron in rats primed with both estrogen + progesterone. Since tropisetron, estrogen and progesterone all act as antagonists at the 5-HT3 receptors we are expecting to see an accentuation of tropisetron's effect on cognition in rats primed with both estrogen + progesterone. We are evaluating the effects of these compounds in ovariectomized Sprague-Dawley female rats using the object recognition task. The object recognition task is a model of cognition in rodents in which the natural tendency of rats to explore novel aspects of the environment is utilized. Rats spend more time exploring the novel object, suggesting that rats recognize previously explored objects. Our preliminary data are in agreement with earlier studies

and show that in comparison to rats receiving no estrogen, rats primed with 20 µg of estrogen (subcutaneous) show an increase in cognition by spending greater percentage of time with the novel object. Furthermore, tropisetron's (2.5 mg/Kg, intraperitoneal) effect on cognition is further accentuated in rats primed with 20 µg of estrogen. Ongoing studies evaluating the combined effects of both estrogen + progesterone (250 µg) priming in rats are expected to show the greatest increase in cognition in response to tropisetron.

Disclosures: S.S. Maswood: None. **J. Hassell:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.22/AA39

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS-0923301

Title: Oxytocin (OT) and arginine-vasopressin (AVP) act on OT receptors (OTRs) to influence social but not non-social recognition in Syrian hamsters (mesocricetus auratus)

Authors: *Z. E. SONG, T. E. LARKIN, M. O'MALLEY, H. ALBERS;
Neurosci. Institute, Ctr. for Behavioral Neurosci., Georgia State Univ., Atlanta, GA

Abstract: OT and AVP play essential roles in a variety of social behaviors through their actions in the central nervous system. Here we report effects of intracerebroventricular (ICV) injection of OT and AVP on social versus non-social recognition. Because OT and AVP can activate each other's receptors due to structural similarities in both the peptides and their receptors, we determined whether OT and AVP influence social recognition by acting on OT or AVP V1a receptors. A two-trial discrimination test was used to measure social recognition. During the social recognition test, male hamsters were first exposed to a flank gland odor of another male adult conspecific for 3min and subsequently exposed to a flank gland odor of that same individual together with a flank gland odor from a novel individual for another 3min. Hamsters discriminated between the pre-exposed and novel odors by spending more time on the latter for 20min and 24h, but not 48h or 7 days. Twenty minutes, 24h, 48h, and 7d after the pre-exposure, hamsters spent 71.6% ± 6.7%, 65.5% ± 5.0%, 58.0% ± 10.6%l, and 46.5% ± 11.9% of total sniffing time, respectively, on the novel odor. ICV injections of OT and AVP, but not saline, prolonged the recognition of the previously encountered odor up to 48h. OT and AVP injected hamsters spent 63.0% ± 3.1% and 67.0% ± 3.5% of total sniffing time, respectively, on the novel

odor tested 48h after the pre-exposure, while saline hamsters spent 51.3% ± 4.3% of sniffing time on the novel. This enhancement of recognition after OT or AVP injections was mimicked by injection of highly selective OTR but not V1aR agonists. Furthermore, the selective OTR antagonist but not the V1aR antagonist blocked recognition of the odor 20min after the pre-exposure. In the non-social recognition test, hamsters were pre-exposed to a lemon scent and subsequently exposed to a cocktail of lemon and vanilla. Control hamsters were not pre-exposed to either lemon or the cocktail and were directly tested with the two scents. Hamsters discriminated the lemon scent from the cocktail scent by spending more time on the novel scent for 20min, 60min, but not 24h. They spent 64.5% ± 11.4%, 67.8% ± 7.5%, and 42.0% ± 11.7% of total sniffing time on the cocktail with ITIs of 20min, 60min, and 24h, respectively. Neither the selective OTR nor V1aR antagonist blocked the recognition of the scents, they spent 71.1% ± 3.8% and 67.0% ± 2.8% of total sniffing on the cocktail. Our results suggest both OT and AVP enhance social recognition by acting on OTRs and the recognition enhancing effects of OT and AVP may be limited to social stimuli.

Disclosures: **Z.E. Song:** None. **T.E. Larkin:** None. **M. O'Malley:** None. **H. Albers:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.23/AA40

Topic: F.02. Animal Cognition and Behavior

Support: Jordan University of Science and Technology 224/2013

Title: Melatonin prevents memory impairment induced by chronic sleep deprivation

Authors: ***O. F. KHABOUR**, K. ALZOUBI, F. MAYYAS, F. BANI SALAMEH, N. MHAIDAT;
Jordan Univ. of Sci. and Technol., Irbid, Jordan

Abstract: Sleep deprivation (SD) has been associated with memory impairment through induction of oxidative stress. Melatonin, which promotes the metabolism of many reactive oxygen species (ROS), has antioxidant and neuroprotective effects that attenuate oxidative stress related risks. In this study, the effect of melatonin on memory impairment induced by 4 weeks of sleep deprivation was investigated using rat animal model. Animals were sleep deprived using modified multiple platform model. Melatonin was administered via oral gavage (100mg/kg/day). Spatial learning and memory were assessed using the radial arm water maze (RAWM). Changes

in oxidative stress biomarkers in the hippocampus following treatments were measured using ELISA procedure The result revealed that SD impaired both short and long term memory ($P<0.05$). Use of melatonin prevented memory impairment induced by SD. Furthermore, melatonin normalized SD induced reduction in the hippocampus activity of catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD). In addition, melatonin enhanced the ratio of glutathione GSH/GSSG ratio in sleep deprived rats ($P<0.05$) without affecting TBARS levels ($P>0.05$). In conclusion, SD induced memory impairment, whereas melatonin prevented this impairment probably through normalizing antioxidant mechanisms in the hippocampus.

Disclosures: O.F. Khabour: None. **K. Alzoubi:** None. **F. Mayyas:** None. **F. Bani Salameh:** None. **N. Mhaidat:** None.

Poster

178. Learning and memory: Pharmacology

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Program#/Poster#: 178.24/AA41

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH098985

NIH Grant R21AG044530

NIH Grant R21DA037673

Title: Validation of PSD95-nNOS inhibitors as pharmacotherapies with limited cognitive side effects

Authors: *A. E. SMITH[1], Y. Y. LAI[1], P. M. KULKARNI[2], G. A. THAKUR[2], A. G. HOHMANN[1,3], J. D. CRYSTAL[1];
[1]Indiana Univ., Bloomington, IN; [2]Ctr. for Drug Discovery, and Dept. of Pharmaceut. Sci., Northeastern Univ., Boston, MA; [3]Gill Ctr. for Biomolecular Science, Indiana Univ., Bloomington, IN

Abstract: A major barrier to deployment of NMDAR receptor antagonists as pharmacotherapies is the fact that they exhibit poor therapeutic ratios and produce debilitating cognitive impairments. Thus, NMDARs are largely considered therapeutically untargetable because blockade of these receptors produces memory as well as motor impairment. Consequently, drug discovery efforts have been directed toward the development of small molecules that interrupt the signaling cascades downstream of NMDARs by targeting protein-protein interactions. The

scaffolding protein postsynaptic density 95kDA (PSD95) tethers the enzyme neuronal nitric oxide synthase (nNOS) to NMDAR. Thus, the PSD95-nNOS protein-protein interface represents a therapeutic target due to its role linking excessive NMDAR activity to nitric oxide (NO) production. One of us (Lai) developed IC87201, the first in class small molecule inhibitor of PSD95-nNOS protein-protein interactions (Florio et al. (2009) British Journal of Pharmacology 158: 494-506). Because PSD95-nNOS inhibitors such as IC87201 and ZL006 act downstream of NMDARs, they may lack cognitive impairment associated with NMDAR antagonists. We used a source-memory preparation (Crystal et al. (2013) Current Biology 23:1-5) we developed for use in rats to evaluate possible impairments of both spatial memory and higher order memory functions in the same task. Source memory is a representation of the origin (i.e. source) of information. We compared the cognitive profiles of an NMDAR antagonist (i.e. MK801) with that induced by PSD95-nNOS inhibitors (i.e. IC87201 and ZL006) in this source memory task. MK801, at doses that did not impair motor function, preferentially impaired source memory under conditions in which spatial memory was spared. These observations suggest that source memory is more vulnerable than spatial memory to impairment. By contrast, IC87201 and ZL006, administered at doses that are effective in suppressing pain behavior in rats, spared source memory, spatial memory, and motor function. Thus, PSD95-nNOS inhibitors are likely to exhibit favorable therapeutic ratios compared to NMDAR antagonists. These results lend support to the translational value of animal models of episodic memory for validation of pharmacotherapies with limited cognitive side effects.

Disclosures: **A.E. Smith:** None. **Y.Y. Lai:** None. **P.M. Kulkarni:** None. **G.A. Thakur:** None. **A.G. Hohmann:** None. **J.D. Crystal:** None.

Poster

178. Learning and memory: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 178.25/AA42

Topic: F.02. Animal Cognition and Behavior

Support: West Virginia University Internal Funding

Title: Enhanced object recognition memory following inhibition of cyclic nucleotide phosphodiesterase 2 (PDE2): role of NOS/cGMP pathway

Authors: *L. M. LUEPTOW[1], Y. XU[2], J. M. O'DONNELL[2];
[1]Neurobio. and Anat., West Virginia Univ., Morgantown, WV; [2]The State Univ. of New York at Buffalo, Buffalo, NY

Abstract: Cyclic nucleotide phosphodiesterases (PDEs) are critical regulators of second messenger cAMP and cGMP signaling. PDE2 is of special interest due to its significant level of expression in forebrain regions, which are highly implicated in learning and memory. In order to examine the role of PDE2 in the early memory consolidation, we trained mice in the object recognition test (ORT). Immediately following exposure to two identical objects, mice were injected with a PDE2 inhibitor, Bay60-7550 (3 mg/kg) or ND7001 (3 mg/kg). Twenty-four hours later, mice were exposed to the familiar object and a novel object. Mice injected with either Bay60-7550 or ND7001 spent significantly more time exploring the novel object, indicating PDE2 inhibition enhanced ORT memory. Next, we pre-treated mice with inhibitors of various molecules in the cGMP pathway, to better understand the underlying signaling that mediates the memory enhancing effects of PDE2 inhibition. When given 30 minutes prior to either Bay60-7550 or ND7001, the nitric oxide synthase (NOS) inhibitor L-NAME (20 mg/kg) and the soluble guanylyl cyclase inhibitor ODQ (20 mg/kg) prevent both Bay60-7550 and ND7001 mediated enhanced ORT memory. This indicates that the memory enhancing properties of PDE2 inhibition during early consolidation are likely mediated via the NOS/cGMP pathway. Ongoing research will continue to investigate other potential molecules in this pathway and the cAMP pathway, to further elucidate the role of PDE2 in ORT memory. Developing a greater understanding of the role of PDE2 in these memory processes will allow for potential drug development for the intervention of a variety of human diseases related to cognitive decline and memory impairment, which plague millions of individuals each year.

Disclosures: **L.M. Lueptow:** None. **Y. Xu:** None. **J.M. O'Donnell:** None.

Poster

178. Learning and memory: Pharmacology

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Program#/Poster#: 178.26/AA43

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AT007222

WSU College of Arts and Sciences

WSU Honors College

Title: A mechanistic study of the effects of nitrous oxide on spatial working memory in mice

Authors: *E. KLEIN[1], D. EMMANOUIL[3], Y. ZHANG[2], D. Y. SHIRACHI[4], R. M. QUOCK[1]; [1]Psychology, [2]Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA; [3]Pediatric Dent., Univ. of Athens, Athens, Greece; [4]Physiol. and Pharmacol., Univ. of the Pacific, Stockton, CA

Abstract: Nitrous oxide (N_2O) is a safe, fast-acting psychoactive drug, and it is the most popular sedative in the field of pediatric dentistry. A recent clinical study demonstrated that in combination with propofol, N_2O potentiated amnesia in patients during dental procedures [Yokoe *et al., J Oral Maxillofac Surg* 73:P402-409, 2015]. Other studies have indicated that prolonged exposure to high concentrations of N_2O (70%) in oxygen affected spatial learning memory in rodents [Culley *et al., Anesth Analg* 105:83-88, 2007]. Therefore, the aim of the study was to explore potential mechanisms of N_2O in reducing spatial working memory in mice. Studies have shown that flumazenil reversed N_2O-induced anxiolysis in mice [Emmanouil *et al., Psychopharmacol* 115:167-172, 1994]. Furthermore, there has been evidence indicating that hyperbaric oxygen (HBO_2) improved cognitive functions in both humans and mice that have suffered brain injury [Boussi-Gross *et al., Neuropsychology,* Nov 10, 2014, doi.org/10.1037/neu0000149; Liu *et al., Neural Regen Res* 8:3334-3343, 2013]. Therefore, flumazenil and hyperbaric oxygen (HBO_2) were used to study potential mechanisms of N_2O-induced spatial working memory dysfunction. The T-Maze Spontaneous Alternation Task (T-SAT) has been utilized for decades to test spontaneous alternation behavior (SAB) and, therefore, spatial working memory in mice in this study. It was found that mice exposed to 70% N_2O (in O_2) exhibited severely reduced alternation behavior in the T-SAT. Mice in this environment alternated their route only 33.04% of the time, in comparison to the control (room air) rate of alternation at 62.81%. Three groups of mice received intraperitoneal injections of flumazenil (0.1, 1.0 or 10 mg/kg) and demonstrated a dose-dependent restoration of spatial working memory under 70% N_2O in the T-SAT. Lastly, mice pretreated for 60 min with HBO_2 at 3.5 atmospheres absolute demonstrated 47.32% alternation under 70% N_2O in the T-SAT. This study verified that 70% N_2O reduced spatial working memory in mice, which can be improved independently by flumazenil and HBO_2. Further, this study implied that N_2O possibly affect the $GABA_A$ receptor complex in inhibiting spatial working memory. The mechanism of HBO_2 has not been elucidated, though this study introduced that HBO_2 might share a similar mechanism with flumazenil in restoring spatial working memory.

Disclosures: E. Klein: None. **D. Emmanouil:** None. **Y. Zhang:** None. **D.Y. Shirachi:** None. **R.M. Quock:** None.

Poster

179. Learning and Memory: Aging II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 179.01/AA44

Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

Office of Research and Discovery, University of Arizona

BIO5 Institute, University of Arizona

State of Arizona and ADHS

Title: Understanding behavioral networks: A novel, scalable microscope designed to enable whole brain imaging of behavior-driven circuits with subcellular resolution

Authors: R. LIANG[1], C. WANG[1], S. PACHECO[1], B. K. BAGGETT[2], M. K. CHAWLA[3,4], D. T. GRAY[3,4], U. UTZINGER[2], *C. A. BARNES[3,4,5];
[1]Col. of Optical Sci., [2]Dept. of Biomed. Engin., [3]Evelyn F. McKnight Brain Inst., [4]ARL Div. of Neural Systems, Memory and Aging, [5]Departments of Psychology, Neurol. and Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: One impediment to progress in understanding the critical differences between brains of normal versus diseased individuals is the difficulty of reconstructing, from sectioned tissue, wiring diagrams that reveal structural or molecular circuit differences. This will be a fundamental requirement for a deeper understanding of how electrical and chemical signals code information in neural circuits and give rise to sensations, thoughts, emotions and actions. With recent advances in whole brain clarification methods (i.e., CLARITY [Chung et al., Nature, 2013, 497:332] and CUBIC [Susaki et al., 2014, Cell, 157:726]), the ability to identify cells, their long-range projections as well as protein and RNA composition of neural networks is a reasonable goal. The physical sectioning required for standard confocal microscopes, however, is not optimal for whole brain imaging because of low imaging speed, layer misalignment and surface mismatching. A number of approaches have been developed (such as the light-sheet microscope) to address fundamental requirements of resolution and speed; however no approach has addressed speed, resolution, working distance and field-of-view simultaneously. We are developing a novel, scalable **h**igh speed, **h**igh resolution, **l**ong working distance, **l**arge field of view **c**onfocal **f**luorescence **m**icroscope ("**H^2L^2-CFM**") that overcomes key limitations of standard single scanning point microscopes, to meet all of these four needs for whole-brain imaging. The novelty of our H^2L^2-CFM approach is that it divides the field of view of the objective (that can scale to the size of an entire mouse or rat brain) into subregions (3x3, 5x5, etc.) with each region having its own multifocal scanning channel. This multi-region, multifocal scanning configuration compensates the remaining aberrations of the objective in each subregion with a collimating lens for each channel. The outcome, once scaled, will be a more than 200x

increase in imaging speed compared to the conventional fluorescence microscope. This novel confocal fluorescence microscope design should overcome the major impediments in current technologies to realize single-cell imaging of large-scale brain networks, and allow for large volumes of brain tissue to be studied on a timescale of weeks to months instead of years. Such an increase in the speed of anatomical data acquisition will enable highly significant experimental questions to be answered including how brain networks change during development, aging or in models of disease.

Disclosures: **R. Liang:** None. **C. Wang:** None. **S. Pacheco:** None. **B.K. Baggett:** None. **M.K. Chawla:** None. **D.T. Gray:** None. **U. Utzinger:** None. **C.A. Barnes:** None.

Poster

179. Learning and Memory: Aging II

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH grant AG048907

Title: Is Arc mRNA expression regulated by the threshold for dendritic Ca++ plateau potentials generated from integration of entorhinal cortical inputs to granule cells?

Authors: *M. K. CHAWLA[1,2], D. T. GRAY[1,2], M. J. HUENTELMAN[1,4], C. A. BARNES[1,2,3];
[1]Evelyn F. McKnight Brain Inst., [2]ARL Div. of Neural Systems, Memory and Aging,
[3]Departments of Psychology, Neurol. and Neurosci., Univ. of Arizona, Tucson, AZ;
[4]Translational Genomics Res. Inst., Phoenix, AZ

Abstract: Immediate-early genes (IEGs) are rapidly and transiently induced following excitatory neuronal activity including maximal electroconvulsive shock treatment. The rapid RNA response can be blocked by the sodium channel antagonist tetrodotoxin, without blocking seizures, indicating a role for electrical excitation in induced mRNA responses (Cole et al., 1990). Using the IEG *Arc* (Lyford et al., 1995) that is selectively synthesized at active synapses, we examined whether lowering the current used to elicit seizures reduces the numbers of *Arc*-positive granule cells in rat hippocampus. F344 rats (5-6 mo old) were divided into 4 groups. Three groups received electroconvulsive shock treatment (20, 40 and 85 mA), and a fourth group did not receive shock. The electrically-induced seizures were produced following attachment of saline-

soaked earclips, using a UGO Basile ECT unit (1sec, 100Hz, 0.5ms square wave pulse) at the three stimulus intensity levels. Five minutes following treatment, brains were rapidly extracted and quick frozen. Twenty micron thick sections were cut and thaw-mounted on frosted slides. Fluorescence *in situ* hybridization was performed as described previously (Chawla et al., 2005) using the full length *Arc* cDNA. Confocal images were acquired using a Leica SP5 microscope equipped with 405 nm and 543 nm lasers, with a 20x dry lens. Overlapping images were obtained of entire dentate gyrus, and *Arc* mRNA-positive granule cells were counted. An estimate of total number of granule cells was used to obtain the percentage of total *Arc*-positive cells in the 0, 20 and 40 mA stimulus conditions. For the 85 mA condition, negative cells were counted to derive the percentage of *Arc* expression. Although seizures were induced by the 20 and 40 mA conditions, these currents resulted in very low *Arc* mRNA expression (20 mA = ~4% +/- 0.4; 40 mA = ~6.0 +/- 0.6 *Arc*, 0 mA ~1% +/- 0.3 mRNA positive cells). Excitation at 85 mA resulted in *Arc* mRNA expression in ~ 93% of granule cells. The minimal expression observed in the lower current conditions may reflect a threshold effect for granule cell burst spiking activity. Indeed, recent observations in CA1 pyramidal neurons suggest that a supralinear summation of electrical activity occurs when entorhinal layer III input to distal CA1 dendrites crosses a threshold to produce a Ca^{++} plateau potential. These plateau potentials appear to drive burst firing, characteristic of CA1 place cell discharge (Milstein and Magee, SfN Abstr, 2014). Our data suggest the possibility that similar plateau potential processes may regulate both the *in vivo* patterned firing that occurs when granule cells express place fields and when granule cells express *Arc* mRNA.

Disclosures: **M.K. Chawla:** None. **D.T. Gray:** None. **M.J. Huentelman:** None. **C.A. Barnes:** None.

Poster

179. Learning and Memory: Aging II

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Program#/Poster#: 179.03/AA46

Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

CIHR SIB171357

NIH grant AG012609

Title: Enhanced single unit firing to unexpected large rewards in aged amygdala neurons

Authors: *R. D. SAMSON[1,2], L. DUARTE[1,2], C. A. BARNES[1,2,3];
[1]Evelyn F. McKnight Brain Inst., [2]ARL Div. of Neural Systems, Memory and Aging,
[3]Departments of Psychology, Neurol. and Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: With aging, changes in emotional regulation can lead to biases in decision making towards certain or safe options. In fact, we found that aged rats are more risk averse and will more readily select a small certain reward over a larger probabilistic one. The activity of neurons in the basolateral complex of the amygdala (BLA) was examined in young and aged rats during rest and while acquiring and performing a probabilistic decision making task. The greatest modulation in firing rate occurred following reward, with the largest change occurring after uncertain large rewards, a modest change in firing rate following small/certain rewards and no change when rewards were not delivered. When all neurons that fired at or above 0.1 Hz were analyzed, the variability and amplitude of change following rewards was much larger in old than in young rats. When neurons with a firing rate below 1Hz were excluded from analysis in both age groups, the young and aged rats did not differ in the amplitude of change of their firing rates in response to uncertain/large rewards. To better characterize how BLA neurons change their firing rate to uncertain rewards, BLA cells were separated into four categories: regular, irregular, irregular/bursty and bursty, based on their local variance, which is a measure of the variability between adjacent inter-spike intervals (ISI). Regular firing neurons accounted for only 1-2% of the 10,000 BLA neurons recorded, and had high firing rates at rest, which were reduced following reward delivery in aged rats. In contrast, irregular neurons accounted 20% of the BLA neural population, and showed the greatest increase in firing rate following large rewards, in both age groups. The vast majority of neurons (60%) had a local variance around 1.5 and an ISI distribution indicating that these cells alternated between irregular and burst firing modes. Following rewards, some of these cells displayed increases and others decreases in firing rates. This effect was consistent across age groups. Finally, bursty neurons accounted for the remaining 20% of the BLA population and only in aged rats did these cells show an increase in firing rate following uncertain/large reward delivery. Thus the change in firing rates of BLA neurons to unexpected rewards appears to be mediated by neurons from all categories, but aging appears to selectively impact low firing rate BLA neurons in a way that allows the aged network to be more responsive to rewards. This effect may contribute to the age-related increases in risk aversion found during probabilistic decision making tasks.

Disclosures: R.D. Samson: None. **L. Duarte:** None. **C.A. Barnes:** None.

Poster

179. Learning and Memory: Aging II

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Program#/Poster#: 179.04/AA47

Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH grant AG012609

Title: Enhanced beta band activity in the aged amygdala during probabilistic decision making

Authors: *L. DUARTE[1,2], R. D. SAMSON[1,2], C. A. BARNES[1,2,3];
[1]Evelyn F. McKnight Brain Inst., [2]ARL Div. of Neural Systems, Memory and Aging,
[3]Departments of Psychology, Neurol. and Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: With aging, older adults tend to use strategies that differ from those used by young adults to solve decision making tasks. When compared to young adults, older adults perform better in tasks that are best solved with a win-stay-lose-shift strategy. The amygdala is known to be involved in both the acquisition and maintenance of optimal probabilistic decision making strategies. We recorded single units and local field potentials from this structure in young and aged rats for up to fifty days as they learned three versions of a decision making task. Two versions were discrimination tasks in which either the reward magnitude (reward magnitude discrimination) or the probability of receiving a reward (probability discrimination) was manipulated. The third version was a probability discounting task in which rats had a choice between a small/certain reward and a large/uncertain reward (probability discounting). In aged rats, we found increased oscillatory power in the beta range (20-40Hz, peak ~24 Hz) in the basolateral complex of the amygdala (BLA). These increases occurred in the delay between the chosen lever presses and resulting outcomes, and lasted for ~1 second. Beta band oscillations were not observed in the younger rats. This effect was not present immediately, but developed after an acquisition period of ~ 6 days in aged rats. Furthermore, the effect was only present in the reward magnitude discrimination and probability discounting tasks. It was of higher amplitude during free choice trials, for both tasks. While beta band activity increased in all aged rats, some showed a stronger increase after choosing the uncertain/large reward, while others showed greater increases in beta power after choosing the certain/small reward. This effect was present in spite of the consistent finding that the old animals all became more risk averse over training compared to the younger animals. Beta power did not increase during the probability discrimination task. One possible explanation for this observation is that this task was only administered for 6 days. This may have been too brief a period for the increased beta band power to emerge. Since behavioral performance on the reward magnitude discrimination version of the task was similar between age groups, this suggests the possibility that aging, rather than differences in behavior, impacts BLA networks in a way that promotes the emergence of beta band activity. Because beta oscillation increases occur in associative learning and motor function, it is possible that our findings reflect a compensatory mechanism in the amygdala of

aged rats, which supports the instrumental associations formed during probabilistic decision making.

Disclosures: **L. Duarte:** None. **R.D. Samson:** None. **C.A. Barnes:** None.

Poster

179. Learning and Memory: Aging II

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH grant AG003376

Title: Age-related changes in external cue-based navigation in the medial entorhinal-hippocampal network

Authors: *A. W. LESTER[1,2], A. J. KOUTIA[1,2], C. A. BARNES[1,2,3];
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Abstract: The hippocampus and entorhinal cortex are critical for spatial navigation and are highly susceptible to age-associated brain changes. As with older adults, aged rats are impaired in many spatial navigation tasks. These impairments are accompanied by changes in the degree to which the spatial tuning properties of hippocampal place fields are influenced by external visual cues in an environment. Such alterations may arise from circuit disruptions caused by known age-related functional and anatomical changes in the hippocampal processing pathway, which could have the effect of either slowing external cue processing or weakening the ability of these cues to influence firing field alignment. To address these possibilities, a novel behavioral apparatus has been developed that allows for complete and immediate control of all visual cues in the environment. The apparatus is composed of a 1.4 m diameter circular track. Projectors arranged around the outside of the apparatus project a 360 degree panorama of visual cues on 68 cm tall cylindrical walls that enclose the track. There are 36 identical feeders evenly spaced along the perimeter of the track and animals learn to run to only one of them for food reward. The projected cues around the rewarded feeder are identical for 50 degrees to either side which eliminates any local visual cue information, forcing the rat to use the full panorama of cues to navigate to the rewarded feeder. By instantaneously rotating the cues we can precisely

characterize when and to what degree animals update their internal representation of space to realign to the rotated external cues. In this context, we can: 1) investigate how spatial representations are updated both at very short time-scales of tens of milliseconds and over longer time intervals of seconds to minutes; 2) identify how different processing stages within the hippocampal formation are affected by age. Behavioral pilot data collected from three young (9 – 13 months old) and two aged (24 month old) animals show that immediately following a 40 degree rotation of the projected cues, animals typically visited feeders that were offset from the learned feeder by a comparable distance (i.e., 20 – 40 degrees). These findings suggest that rats rapidly update their behavior to maintain alignment with the orienting cues in an environment. The next step of the study will be to perform simultaneous high density recordings from both medial entorhinal cortex and the CA1 region of the hippocampus as rats perform the task. With these measurements we hope to more precisely characterize the loci, timing and effect of age-related functional changes within the medial entorhinal-hippocampal network.

Disclosures: **A.W. Lester:** None. **A.J. Koutia:** None. **C.A. Barnes:** None.

Poster

179. Learning and Memory: Aging II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH grant AG012609

Title: Time-dependent decrease in the peak frequency and power of hippocampal sharp-wave ripples and high-gamma events during post-behavior sleep in aged and young rats

Authors: ***J.-P. WIEGAND**[1,2], D. T. GRAY[1,2], L. A. SCHIMANSKI[1,2], P. LIPA[1,2], C. A. BARNES[1,2,3,4], S. L. COWEN[1,2,3];
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Abstract: Sharp-wave ripples (SPW-Rs) are brief (20-150 ms), high-frequency (130-180 Hz) oscillations in the hippocampus (Buzsaki et al., 1992) linked to the process of memory consolidation. Previous work has demonstrated that neural activity associated with recent experience is reactivated during SPW-Rs (e.g., Wilson and McNaughton, 1994) and that the

strength of this reactivation decreases to approximately 20% of original levels within 30 minutes after behavior (Kudrimoti et al., 1998). We investigated the general hypothesis that the rapid decrease in reactivation strength is correlated with a time-dependent reduction in frequency and power of the ripple oscillation. Given the association between age and memory decline, we also investigated whether the time course of such change differs between aged and young animals. CA1 local field potentials (LFPs) were recorded in aged (n = 5) and young (n = 6) male F344 rats during rest periods following a place-dependent eyeblink-conditioning task. To examine the time course of the probability of occurrence of high frequency LFP events during the 20 minutes following the behavior experience, we bandpass filtered between 80-300Hz, rectified the data, set a power threshold, and then found the onset and offset of these events throughout this time period. Peak frequency and power at higher frequencies were reduced in aged rats compared to young, and both frequency and power decreased gradually during the first 20 minutes to ~90% of initial levels in both age groups (unpaired t-test, $p < 0.05$). In the older animals, the entire probability distribution of peak frequency oscillatory events was significantly shifted downward. This reduction could reflect an increase in the proportion of fast gamma to ripple events, or age-associated changes in the CA1 network that limit the maintenance of high-frequency oscillatory activity. Given that high gamma activity is associated with increased entorhinal input to CA1 (Colgin et al., 2009), these results suggest that CA1 may receive increasing input from entorhinal cortex throughout the course of sleep, and that this effect may be particularly strong in aged animals.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH grant AG012609

Title: Behavioral evidence for enhanced interference during working memory and associative learning tasks in aged macaques

Authors: *D. T. GRAY[1,2], S. L. ASHFORD[1,2], W. PYON[1,2], S. N. BURKE[4], A. C. SMITH[1], C. A. BARNES[1,2,3];
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Abstract: The ability to protect ongoing cognitive processes from distracting stimuli is known as interference control. Human studies investigating this phenomenon have revealed that despite impressive flexibility in most cognitive domains, there is a severe capacity limitation in the ability to perform multiple tasks simultaneously. Efforts to develop behavioral paradigms for animal models to study interference control at the single-neuron level have recently led to insights into the neuronal mechanisms behind these limitations. For example, Wantanabe and Funahashi (2014) demonstrated that during a spatial attention task, neurons in the lateral prefrontal cortex show a decreased ability to represent task-relevant information proportional to the cognitive demand of a competing task. During normal aging this capacity limit is further reduced, but our understanding of the neural basis underlying these age-related declines is minimal. To this end, a computer-controlled associative learning task with varying levels of interference is described. Learning of this task is characterized using a state-space modeling algorithm (Smith et al., 2004). The ability of monkeys to form associations with reward between images in novel object pairs was significantly reduced as the number of object pairs to simultaneously learn increased. This interference effect was proportionally greater in aged than in young monkeys. Additionally, behavioral data demonstrating age-related increases in the susceptibility to interference during a manually-presented working memory interference task (adapted from Clapp et al., 2009) are described here (and in Plange et al., 2011). In this task, distractors are presented in the delay period of a delayed nonmatching-to-sample task, which decreases the performance of aged monkeys significantly more so than young monkeys. Together these data support the observation that older macaque monkeys exhibit age-related deficits in interference control. As with the first associative learning paradigm described above, this second task is currently being implemented under computer control to facilitate the temporal precision required to monitor behavior in relation to electrophysiological recordings, which will provide a novel opportunity to study these aging deficits at the single-neuron level.

Disclosures: D.T. Gray: None. **S.L. Ashford:** None. **W. Pyon:** None. **S.N. Burke:** None. **A.C. Smith:** None. **C.A. Barnes:** None.

Poster

179. Learning and Memory: Aging II

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Support: University of Arizona Neuroscience and Cognitive Science Summer Research Program

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RR000169

Title: Species- and age-related differences in learning and performance on working memory tasks in two species of macaque monkeys

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Abstract: Deficits in executive function, such as working memory, are characteristic of human aging. Because of similar aging phenotypes and homology in the organization of the lateral prefrontal cortex, nonhuman primate research continues to be especially informative for understanding the underlying mechanisms of normal cognitive brain aging. Two nonhuman primate models that have been used for studying such age-related changes include the rhesus macaque (*Macaca mulatta*) and the bonnet macaque (*Macaca radiata*). It is unknown how these two macaque species compare in their abilities to learn and perform working memory tasks, and how these skills change throughout healthy aging. We employed state-space modeling algorithms (Smith et al., 2004) to analyze behavioral data from young and aged rhesus and bonnet macaques from two separate colonies in order to characterize any possible species or age-related differences in task acquisition and levels of performance. The macaques were trained on two behavioral tasks that engage working memory systems for optimal performance. These tasks include the delayed response (DR) and delayed nonmatching-to-sample (DNMS) tests, which have been shown to be dependent on different association areas of the brain, including the hippocampus and prefrontal cortex. The data suggest that, although performance on the tasks after reaching criterion is comparable across age and species, bonnet macaques appear to learn working memory tasks faster than do rhesus macaques and also show smaller age-related differences in performance than do rhesus. This finding occurred even though the ages at which the animals were tested were comparable between the two species (i.e., mean rhesus old = 24 years, bonnet old = 25 years; mean rhesus young = 11 years, bonnet young = 11 years). To obtain human equivalent ages from macaque ages, Tigges et al. (1988) have suggested that the

age conversion of 3 human years for every one macaque year is a good approximation. Thus these data reflect animals that range in age from 21 to 90 human equivalent years - a significant portion of the lifespan. An understanding of the differences in cognitive aging between these species may inform future choices in selecting models of normal aging for experiments, and should help to connect respective bodies of primate literature using each species.

Disclosures: A. Comrie: None. **D.T. Gray:** None. **S.N. Burke:** None. **A.C. Smith:** None. **C.A. Barnes:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH grant AG003376

RR000169

Title: Behavioral impact of long-term chronic implantation of neural recording devices in the rhesus macaque

Authors: *C. KYLE[1], M. R. PERMENTER[4], J. A. VOGT[4], C. A. BARNES[1,2,3];
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Abstract: Although ensemble recording methods are pervasive throughout neuroscience, little is known about how chronic electrode implants affect behavioral performance. Here we investigate the effect of chronic hippocampal tetrode array implants on behavioral performance in a delayed nonmatching-to-sample (DNMS) task. Five female rhesus macaques were tested (mean age: 16 yrs (+/-) 7.7, ranging 7 – 26 yrs) prior to implant, and re-tested (mean age: 21 yrs (+/-) 6.9, ranging 15-31 yrs) after implant (mean elapsed time between tests: 5.1 yrs (+/-) 1.5). DNMS testing was conducted at delay intervals of 10, 15, 60, and 120 seconds using the Wisconsin general testing apparatus. A 4x2 (delay interval by test session) repeated measures ANOVA revealed a main effect of retention interval ($F(3,12) = 9.02$, $p=.002$), and a trending delay by test session (pre vs post implant) interaction ($F(3,12) = 2.8$, $p=.08$) on accuracy. While the main

effect of delay interval confirms typical DNMS findings that performance decreases as delay interval increases, the lack of a significant main effect of test session (pre- vs post-implant) suggests that the cumulative impact of aging between tests and the effect of the implant did not significantly affect performance for all delay periods. Rather, the trending interaction may reflect that the cumulative effects of the implant and aging affect different delay periods differentially. Future analysis will seek to compare our results to typical age related declines in DNMS to better assess the effects of aging versus the effects of the implant.

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Poster

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Support: McKnight Brain Research Foundation

State of Arizona, and ADHS

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Title: Age-associated regional network pattern of MRI gray matter in the bonnet macaque

Authors: *P. K. BHARADWAJ[1], S. N. BURKE[7], T. P. TROUARD[2,3], K. CHEN[8], J. R. MOELLER[9], C. A. BARNES[3,4,5], G. E. ALEXANDER[1,3,6];
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Abstract: Studies of healthy aging in humans have consistently observed brain atrophy, often preferentially involving frontal and selective temporal brain regions, which is associated with cognitive aging. We have previously reported regionally distributed patterns of magnetic resonance imaging (MRI) gray matter in healthy aging in humans and a nonhuman primate (rhesus macaque) model of aging (Alexander et al., 2006; 2008) using multivariate network

analysis with the scaled subprofile model (SSM; Alexander and Moeller, 1994) and voxel-based morphometry (VBM). In this study we investigated the effects of aging on the regional pattern of MRI gray matter in a group of 13 healthy adult female bonnet macaques (BM) with ages ranging from 10.2 to 30.8 years (human equivalent age range of 31-92 years). Volumetric T1 MRI scans were acquired on a GE 3T Signa scanner with 600 micron isotropic voxel resolution. Image processing for VBM included brain extraction, inhomogeneity correction, and tissue segmentation with high dimensional warping and smoothing using Statistical Parametric Mapping (SPM12; Wellcome Department of Imaging Neuroscience, London, UK) to produce smoothed gray matter maps. Regional network analysis was performed on the gray matter maps with the SSM using Akaike information criterion with small sample correction, which identified a linear combination of three component patterns that was associated with age in the BM (R^2 = 0.66, $p \leq 0.017$). This pattern was characterized by reductions in prefrontal and bilateral posterior temporal/visual association regions with increasing age in the group, as well as with areas of relative increase in the bilateral primary motor/somatosensory regions. These findings indicate that aging in BM is associated with a regionally distributed pattern of MRI gray matter reduction involving selective frontal and temporal brain regions that are generally consistent with previous findings of structural covariance in human and other non-human primate models of aging. This work provides further support for the application of multivariate network analysis methods on a voxel basis, to investigate gray matter integrity in the context of healthy aging and its potential relation to the effects of cognitive aging.

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Poster

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Intramural Research Program of the National Institute on Aging

Title: Synaptic GPER1 distribution in the monkey prefrontal cortex is altered with aging, modulated by estrogen, and correlated with working memory

Authors: *J. L. CRIMINS[1], A. C. WANG[2], F. YUK[1], R. PURI[1], W. G. JANSSEN[1], Y. HARA[1], P. R. RAPP[3], J. H. MORRISON[1];
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Abstract: Humans and nonhuman primates are vulnerable to age- and menopause-related deficits in working memory, an executive function mediated by layer III neurons of the dorsolateral prefrontal cortex (dlPFC). Long-term cyclic estrogen replacement therapy rescues cognitive deficits in aged rhesus monkeys that have undergone surgical menopause by ovariectomy, in part through the restoration of thin dendritic spines on dlPFC neurons. However, relatively little is known about how the distribution of an estrogen receptor(s) within synapses might contribute to the procognitive effects of estrogen treatment. Here, young and aged vehicle- and estrogen-treated ovariectomized (OVX) rhesus monkeys were first tested on a delayed response (DR) test of spatial working memory. Then, quantitative immunoelectron microscopy was used to evaluate the effects of age and estrogen treatment on the distribution of G protein-coupled estrogen receptor 1 (GPER1) in layer III dlPFC synapses, and its potential relationship to working memory performance. Our data indicated that aged OVX monkeys had a lower density of GPER1-containing synapses than did young OVX monkeys due to the specific loss of synapses with non-perforated postsynaptic densities (PSDs); importantly, this synaptic subclass was partially restored with estrogen treatment. The majority of axon terminals (93%) and dendritic spines (81%) expressed GPER1, but the abundance of GPER1 did not differ across age or treatment groups for either synaptic specialization. GPER1 was predominately localized to mitochondria in axon terminals, and was also highly expressed in the plasmalemmal and cytoplasmic domains of both terminals and spines, as well as at the PSD. There was an age-dependent reduction in the percentage of GPER1 gold particles subjacent to the PSD, which was a strong positive correlate of synaptic density. Intriguingly, working memory performance (average DR accuracy) inversely correlated with the density of GPER1 in the presynaptic active zone across all monkeys. Taken together, these data suggest that GPER1 is strategically positioned to perform diverse estrogen-dependent functions key to the modulation of synaptic plasticity, and ultimately to the functional integrity of dlPFC.

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Poster

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NIA Grant P01 AG16765

Intramural Research Program of the NIA

Title: Differential expression of phosphorylated LIM kinase in non-human primate prefrontal cortex correlates with working memory

Authors: F. J. YUK[1], E. BLOSS[1], R. PURI[1], J. L. CRIMINS[1], *W. G. JANSSEN[1], Y. HARA[1], P. R. RAPP[2], J. H. MORRISON[1];
[1]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Lab. of Behavioral Neurosci., Natl. Inst. on Aging, Baltimore, MD

Abstract: Pyramidal neurons in layer III of the dorsolateral prefrontal cortex (dlPFC) play a critically important role in working memory and goal-directed behaviors in non-human primates (NHP). These executive functions are highly vulnerable to aging, and age-related cognitive impairments have been associated with a loss of thin, highly plastic spines. The spine actin cytoskeleton is directly linked to its stability and phosphorylated LIM kinase (pLIMK) is thought to contribute to this stability by phosphorylating cofilin and inhibiting its actin severing activity within the spine. In the current study, young and aged NHP were first tested on a delayed response task, a well-characterized measure of working memory. Using post-embed immunogold electron microscopy and subsequent three-dimensional reconstruction of axospinous synapses in layer III of dlPFC, we asked if pLIMK expression is related to promoting spine stability with aging in NHP dlPFC. Consistent with previous studies, we found an age related 36% decrease in synaptic density, specifically due to a loss of synapses on thin spines. pLIMK immunogold count per synapse was significantly increased with aging in the postsynaptic density (PSD) as previously reported by our group in rats. However, when immunogold density measures were derived by normalizing for area and volume, this significant difference in PSD label was diminished and instead, significant age-related losses in the densities of spine cytoplasmic and subsynaptic pLIMK gold particles emerged. A cluster analysis by spine size and synaptic type revealed that this cytoplasmic decrease selectively occurred in perforated synapse spines, while the subsynaptic decrease was localized exclusively to small non-perforated synapse spines. Furthermore, the decrease in subsynaptic label was inversely correlated with performance on the delayed response task (average accuracy) across all age groups. These data support our hypothesis that age-related alterations in proteins mediating spine dynamics associated with plasticity are associated with age-related cognitive deficits.

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Poster

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Title: Effect of androgen supplementation on cognitive performance in old male rhesus macaques

Authors: *H. F. URBANSKI[1], K. G. SORWELL[1], A. MARQUEZ LOZA[1], D. I. BROWN[2], L. RENNER[1], M. NEURINGER[1], S. G. KOHAMA[1];
[1]Div. Neurosci., Oregon Natl. Prim Res. Ctr., Beaverton, OR; [2]Facultad de Ciencias, Univ. de Valparaíso, Valparaíso, Chile

Abstract: Cognitive decline in the elderly appears to be causally related to the marked age-related decline in circulating levels of several hormones, including estradiol (E2), testosterone (T), and the adrenal androgen dehydroepiandrosterone (DHEA). We have previously demonstrated beneficial effects of E2 replacement on cognitive function in aged female rhesus macaques, as determined by performance in a delayed response (DR) task, and wondered if androgen supplementation in aged male rhesus macaques would result in similar cognitive benefits. We developed a physiological replacement paradigm for old males, involving daily T and DHEA supplementation, which resulted in circulating E2 and 5α-dihydrotestosterone levels similar to those of young adults - both in terms of magnitude and in terms of circadian profile. As expected, ~6 months of combined androgen supplementation resulted in a slight decrease in the size of the testes, but they were approximately the same size as those of young adults, with no obvious pathology. In the DR memory test, both the young and old androgen-supplemented animals showed improvement with practice. In contrast, the old untreated controls showed no improvement. Consequently, after ~6 months their performance in moderately difficult (15-s delay) DR tasks was significantly worse than in the young animals, whereas the performance in

old androgen-supplemented animals was similar. In a delayed matching-to-sample (DMS) memory test, all of the animals showed improvement in performance with time, but no obvious beneficial effect of androgen supplementation was observed even after ~6 months. Taken together, the data suggest that physiological hormone supplementation paradigms have the potential to alleviate some of the cognitive deficits associated with aging in elderly men, without negatively impacting the neuroendocrine reproductive axis, but significant benefits on cognitive function may require longer periods of treatment.

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Poster

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Title: Intrahippocampal blockade of L-type calcium channels increases unit activity in dorsal CA1 of aged rats

Authors: *D. M. CURLIK, II, X.-W. YU, M. M. OH, J. F. DISTERHOFT;
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Abstract: The calcium (Ca2+) hypothesis of aging predicts that age-related cognitive impairments result from disruption of Ca2+ homeostasis. In aged animals Ca2+ influx is increased in CA1 pyramidal neurons, resulting in a decrease in the intrinsic excitability of those cells. A common measure of intrinsic excitability, the post-burst afterhyperpolarization (AHP), is increased in CA1 pyramidal neurons from aged-cognitively impaired animals, resulting in impaired neuronal function and plasticity in those animals. Increased intracellular Ca2+ is believed to underlie these age-related biophysical deficits, as bath application of L-type voltage-gated Ca2+ channel (LVGCC) antagonists reduces Ca2+ influx and the amplitude of AHP *in vitro*. Additionally, systemic intravenous administration of these antagonists increases hippocampal activity *in vivo*, and ameliorates age-related learning impairments. Moreover, we have recently reported strong correlations between expression of Cav1.2 protein in dorsal CA1 and age-related cognitive impairments. That is to say, aged rats with the greatest expression of Cav1.2 protein in area CA1 were the most cognitively impaired. Together, these results suggest

that an age-related increase in the number, and/or function, of LVGCCs in CA1 pyramidal neurons mediates age-related cognitive deficits. However, to date no experiments have directly demonstrated whether intrahippocampal blockade of LVGCCs is sufficient to ameliorate age-related biophysical and behavioral deficits *in vivo*. To determine whether dorsal CA1 is a therapeutic site for ameliorating age-related deficits through intrahippocampal blockade of LVGCCs we measured activity of dorsal CA1 neurons before and after direct intrahippocampal infusion of varying concentrations of the LVGCC antagonist nimodipine (100nM through 10uM). Results revealed that 100nM nimodipine was sufficient to increase activity in dorsal CA1 of aged rats. However, the maximal increase in unit activity was observed following infusion of 10uM nimodipine. These results suggest that intrahippocampal infusion of nimodipine will ameliorate age-related cognitive deficits. To test this hypothesis we will infuse nimodipine or vehicle into dorsal CA1 of aged rats every day before training with trace and delay eyeblink conditioning.

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Poster

179. Learning and Memory: Aging II

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Program#/Poster#: 179.15/BB10

Topic: F.02. Animal Cognition and Behavior

Title: Assessment of neuronal function during normal aging in male sprague dawley rats

Authors: *A. J. IDOWU[1], I. I. OLATUNJI-BELLO[2];
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Abstract: During normal aging, there are physiological changes especially in high energy demanding tissues including the brain and skeletal muscles. Aging may disrupt homeostasis and allow tissue vulnerability to disease. To establish an appropriate animal model which is readily available and will be useful to test therapeutic strategies during normal aging, we applied behavioral approaches to study age-related changes in memory and motor function as a basis for neuronal function in ageing in male Sprague Dawley rats. 3 months, n=5; 6 months, n=5 and 18 months, n=5 male Sprague Dawley Rats were tested using the Novel Object Recognition Task (NORT) and the Elevated plus Maze (EPM) Test. The results showed a gradual decline in exploratory behavior, locomotor activity and memory function. Importantly, we established a novel finding that the minimum distance from the novel object was statistically significant and may be an index for age-related memory impairment in the NORT. Altogether, we conclude that

the male Sprague Dawley rat show age-related changes in neuronal function and is a useful model for carrying out investigations into the mechanisms involved in normal ageing or possible therapeutic strategies.

Disclosures: A.J. Idowu: None. **I.I. Olatunji-Bello:** None.

Poster

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Title: Increased basal levels of NFκBp65 in the nucleus of hippocampal cells is related to maintenance of hippocampus-dependent spatial memory in aged rats

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Abstract: While the association between aging and inflammation is well known, the cellular mechanisms by which inflammation could influence age-related memory impairment are not well understood. One point of convergence between aging and inflammation is the 65 kDa subunit of the transcription factor Nuclear Factor-κB (NFκBp65) which, unlike other subunits, contains a transactivation domain (Schmitz & Baeuerle, 1991). It is sequestered in the cytoplasm as a hetero- or homodimer until its inhibitor, IκB, is phosphorylated, releasing IκB from NFκBp65 and allowing it to translocate to the nucleus (Brown et al., 1993). Once in the nucleus, NFκBp65 activates transcription of genes related to pro-inflammatory cytokines, growth factors, cell adhesion molecules, and apoptosis (Salminen & Kaarniranta, 2009). Aged rats demonstrated increased nuclear NFκBp65 (Toliver-Kinsky et al., 1996), increased levels and activity of pro-inflammatory cytokines (Coppe et al., 2010) and enhanced reactivity to inflammatory signaling (Krabbe et al., 2001). Additionally, experiments that induce inflammation via endotoxins demonstrated impaired spatial memory in young animals which was exacerbated in aged animals (Chen et al., 2008). However, research into the role of basal levels of NFκBp65 and spatial memory during aging is lacking. To test the hypothesis that increased basal levels of NFκBp65 in the nucleus of hippocampal cells during aging is related to impaired spatial memory, 3- and 20-month-old male Brown Norway rats were tested on a spatial water maze task, and basal levels of NFκBp65 were measured in hippocampal nuclear and cytoplasmic fractions using quantitative

western blotting. Aged rats demonstrated increased levels of nuclear NFκBp65 and worse spatial memory in comparisons to young rats. Elevated levels of NFκBp65 in the nucleus were measured in aged rats that had spatial memory within the range of the young, but not in those with spatial memory impairment. Given that 20 months is approximately middle-aged in the Brown Norway strain, the results suggest that increased NFκBp65 is a compensatory mechanism which benefits spatial memory. In a future experiment, we will test this hypothesis by determining if nuclear NFκBp65 is still related to maintenance of spatial memory in a group of rats aged 30 months, which is the approximate average lifespan for the Brown Norway strain.

Disclosures: **A.F. Jones:** None. **N.R. Pilgeram:** None. **P.J. Colombo:** None.

Poster

179. Learning and Memory: Aging II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 179.17/BB12

Topic: F.02. Animal Cognition and Behavior

Support: WM-EVMS Collaborative Research Grant

Title: Effects of prior distracter exposure on learning in aged rats

Authors: C. T. KOZIKOWSKI, A. TAPP, C. LEONG, *J. A. BURK;
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Abstract: Previous research in our lab showed that the addition of a visual distracter during a two choice sustained attention task enhances young adult rats' acquisition of a novel discrimination task when compared to animals that were not exposed to the distracter. The present study was designed to investigate if similar results were observed in aged animals. FBNF1 hybriderats (N = 20) were trained on two choice sustained attention task, that required discrimination of a variable duration visual signal (illumination of a panel light for 500, 100 or 25 ms) from trials when the panel light was not illuminated, until 20 months of age. Rats then continued in a non-distracter condition in which they performed the same attention task with a stable house light (n = 10) or began a distracter condition where a flashing house light was introduced while performing the attention task for 20 sessions (n = 10). After the addition of the distracter there was a significant session block x signal duration x condition interaction. Subsequent analyses revealed that the basis for this interaction was a significantly lower hit rate by the distracter animals compared to rats that were not exposed to the distracter for the 100ms signal duration trials during block 1 (sessions 1-5) and for the 500ms trials and 100ms trials

during block 2 (sessions 6-10). There were no significant differences in hits between the two groups during block 3 (sessions 11-15) or block 4 (sessions 15-20). After these testing sessions, novel visual discrimination task trials were intersperse with trials of the sustained attention task for both groups for 20 sessions. The new visual discrimination task required animals to press a lever under either an illuminated left or right light. Initially, 40% of the trials contained the novel signal discrimination task. For the next 20 sessions 70% of the trials contained the novel visual discrimination task. During the 70% novel signal discrimination sessions, there was a significant signal duration x condition interaction when assessing accuracy of signal detection on the sustained attention task. Follow up analyses revealed a significant difference in performance of the between the two groups for the 25ms trials with the distracter-exposed animals showing higher accuracy levels compared to rats that were not exposed to the distracter. There were no differences in performance between the groups when evaluating correct rejections, omissions or performance on the novel signal discrimination task for the 40% or 70% sessions. The present results suggest that aged animals can still benefit from distracter exposure, but the extent of the beneficial effects is weaker compared with young adult animals.

Disclosures: **C.T. Kozikowski:** None. **A. Tapp:** None. **C. Leong:** None. **J.A. Burk:** None.

Poster

179. Learning and Memory: Aging II

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Program#/Poster#: 179.18/BB13

Topic: F.02. Animal Cognition and Behavior

Support: R01AG043972

P30NS47466

P30DK056336

Title: Ghrelin agonist as an aging modifying peptide

Authors: *I. KADISH[1], M. SHARPE[1], T. VAN GROEN[1], K. L. GAMBLE[2], M. E. YOUNG[3], D. B. ALLISON[4];
[1]Dept Cell, Developmental and Integrative Bio, [2]Dept. of Psychiatry, [3]Dept. of Med., [4]Sch. of Publ. Health, Office of Energetics, Nutr. Obesity Res. Ctr., Univ. Alabama Birmingham, Birmingham, AL

Abstract: Caloric restriction (CR) is a long established paradigm which extends longevity and slows signs of aging through mechanisms which have yet to be clearly elucidated. Ghrelin is a hunger-inducing gut peptide, and the interoceptive cues caused by ghrelin are likely similar to those produced by CR. In this long term treatment study we tested the novel hypothesis that a ghrelin agonist attenuates behavioral and cognitive decline, and also changes energy metabolism and glucose tolerance in aged C57BL/6J mice and that these changes involve interoceptive cues, rather than reduced energy intake per se. Two groups of C57BL/6J male 2 month old mice were used in this study. One group (control) had ad libitum access to food, while the second group (ghrelin) received the mean amount of diet consumed by the control group and a ghrelin agonist (LY444711; 30 mg/kg of LY in a 45 mg sucrose pellet) daily for 10 or 22 months. At the end of the experimental feeding protocol all mice were analyzed for body composition using quantitative magnetic resonance (QMR). A battery of behavioral and cognitive tests was carried out to analyze behavioral/cognitive differences. All animals were also assessed in CLAMS cages for energy expenditure. Glucose tolerance test was performed for both time points. Biochemistry and immunohistochemistry analysis were carried out to assess the changes in brain tissue and plasma. Ghrelin agonist treatment did not alter the body composition of either group. Cognition was significantly improved in ghrelin group of 24 month old mice compared to control group, but did not significantly influenced the cognitive outcome of 12 month old mice. In a 12:12 light-dark cycle, both control and ghrelin agonist treated mice exhibited typical 24-hour rhythmicity in general cage activity, respiratory exchange ratio (RER), and energy expenditure. Ghrelin agonist treatment improved glucose tolerance in 24 month old mice. In conclusion, “hunger” without caloric restriction has similar cognitive and glucose handling benefits to caloric restriction, without the potential problem of weight loss in aging individuals.

Disclosures: I. Kadish: None. **M. Sharpe:** None. **T. van Groen:** None. **K.L. Gamble:** None. **M.E. Young:** None. **D.B. Allison:** None.

Poster

179. Learning and Memory: Aging II

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Topic: F.02. Animal Cognition and Behavior

Support: CONACyT Grant 221092

CONACyT Scholarship Grant 575857 to Yermein Benitez

Title: Morris Water Maze execution on senile rats exposed to stress at early stages of life

Authors: *Y. BENITEZ[1], G. YAÑEZ-DELGADILLO[2], P. HERNANDEZ-CARRILLO[2], J. GARCÍA-ESTRADA[3], F. JAUREGUI-HUERTA[4], S. LUQUIN[4];
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Abstract: Aging is a complex biological process characterized by a marked decrease on the cognitive skills of all organisms. Stress is also an important regulator of cognition. Effects of stress on learning and memory have been demonstrated on developing, mature, and aged subjects. In this experiment, a group of aged rats were exposed to chronic stress and their MWM execution profiles were compared as a function of whether or not they experienced stress at early stages of live. To this end, we exposed male Wistar rats to chronic noise stress when juveniles, and then, they were re-exposed to chronic variable stress when reached the age of 18 months. We found an improving effect of stress in senile rats that also experienced stress when juveniles. So, early stressful experiences may become beneficial for aged subjects when new aversive conditions demand cognitive adaptation.

Disclosures: Y. Benitez: None. **G. Yañez-Delgadillo:** None. **P. Hernandez-Carrillo:** None. **J. García-Estrada:** None. **F. Jauregui-Huerta:** None. **S. Luquin:** None.

Poster

179. Learning and Memory: Aging II

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 179.20/BB15

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R15AG042155

Title: Effects of testosterone and age on spatial memory and BDNF in male rats

Authors: *M. D. SPRITZER, C. E. SUPER;
Dept. of Biol. and Program in Neurosci., Middlebury Col., Middlebury, VT

Abstract: Spatial memory, the ability to remember spatial aspects of one's environment or one's own position in space, has been shown to have a positive correlation with circulating testosterone levels. In men, testosterone levels decline due to a number of reasons, including treatments for prostate cancer and the natural process of aging. Interestingly, some studies have demonstrated

that low testosterone levels lead to an increased risk of age-related memory loss. Furthermore, brain-derived neurotrophic factor (BDNF) may be the mechanism by which testosterone influences memory. To investigate this connection, we tested young (2 month old) and old (20-21 month old), Fisher 344 castrated and sham-castrated male rats over a period of 25 days for working and reference memory on an eight-arm radial maze. All rats showed a significant reduction in working memory errors over the course of testing, demonstrating learning. Importantly, old rats showed significant impairments in spatial working memory compared to young rats. Contrary to previous results, the young rats showed no differences in working memory between castrated and intact individuals. Among aged rats, however, the castrated group performed significantly more working memory errors than did the intact group during the later days of testing. This suggests that castration exacerbated an age-related impairment in working memory. There was no significant change in the number of reference memory errors overall across the 25 days of testing, suggesting minimal learning on this component of the task. Interestingly, young rats performed significantly more reference memory errors than did the aged rats. This may be due to more use of a response strategy by the young rats compared to the old rats. There were no effects of castration on number of reference memory errors. Testosterone ELISA revealed that old, intact rats had significantly lower testosterone levels than young, intact rats, which indicates that age-related memory loss is associated with decreased testosterone levels. Preliminary ELISA's indicate that old rats had higher BDNF levels in the hippocampus while young rats had comparatively higher BDNF levels in the cortex and striatum. Castrated rats had higher BDNF levels in the hippocampus and cortex while intact rats had comparatively higher BDNF levels in the striatum. Thus, both age and castration seem to impact BDNF levels in different brain regions. Overall, our results suggest that age-related deficits in spatial working memory may be due to decreased testosterone levels, and testosterone may have this effect by influencing BDNF levels in specific brain regions.

Disclosures: **M.D. Spritzer:** None. **C.E. Super:** None.

Poster

179. Learning and Memory: Aging II

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS 13-18490

NIH P30 AG034464 Center for Aging and Policy Studies, Syracuse University

Title: Aging in rats leads to task-dependent impairments and improvements in learning that are accompanied by changes in markers of brain energetics

Authors: *L. A. NEWMAN, R. S. GARDNER, B. V. HAMLING, D. L. KOROL, P. E. GOLD; Biol., Syracuse Univ., Syracuse, NY

Abstract: We found that while aged rats are impaired on hippocampus-sensitive tasks compared to young rats, aged rats are superior to young rats when trained on striatum-sensitive tasks. Thus, cognitive decline is not a universal age-related change in brain function. In the current study we examined whether changes in markers of brain energetics might accompany changes in learning. Young adult (3-mo-old) and aged (24-mo-old) male Fischer-344 rats were trained on two versions of the same maze: a place version that is hippocampus-sensitive and a response version that is striatum-sensitive. These mazes have the same motivational (food) and locomotor (arm entry) requirements yet differ in the rule used to solve the maze: after leaving the start arm a) go to a location in the room for place learning (hippocampus) or b) turn in a specific direction for response learning (striatum). In comparison to young rats, aged rats were impaired in learning the spatial version of the task but better at learning the response version. During training, extracellular lactate levels increased in both the hippocampus and striatum at both ages and for both tasks. However, when trained on either task, aged rats had lower lactate release in the hippocampus than did young rats; the lowest training-related increases were seen during place learning in aged rats. The results were somewhat different for striatal measures of lactate release. Surprisingly, increases in striatal lactate levels were substantially higher in young rats during place learning than during response training. Moreover, striatal lactate levels increased less in old than in young rats during place training. In contrast, the striatal lactate increases were similar in old and young rats trained on the response task. To obtain clarification about metabolic processes that may produce this pattern of results, we are currently examining age-related differences in other metabolic markers such as extracellular glucose levels during and tissue glycogen levels before and after place and response training. The data collected thus far suggest that during place learning, lactate production is decreased in both the hippocampus and striatum of aged rats. However, lactate production during response learning is not depressed in the striatum of aged rats, though it is in the hippocampus. These changes in extracellular lactate may reflect altered metabolic responses to training in aged vs. young rats, perhaps contributing to an age-related shift from hippocampus- toward striatum-based strategies in learning.

Disclosures: L.A. Newman: None. **R.S. Gardner:** None. **B.V. Hamling:** None. **D.L. Korol:** None. **P.E. Gold:** None.

Poster

179. Learning and Memory: Aging II

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 AG 037868

Title: Influence of chronic restraint (psychosocial stress) on young and aged rats

Authors: *K. STAGGS, J. POPOVIC, S. QUTUBUDDIN, E. M. BLALOCK;
Pharmacol., Univ. of Kentucky, Lexington, KY

Abstract: Psychosocial stress is a non-painful stimulus associated in humans with major life changes, such as loss of a job or spouse or social isolation, and strongly influences multiple systems (e.g., corticosterone level, body temperature regulation, sleep and cognition). There is an increased likelihood of experiencing chronic psychosocial stress with age, and the negative consequences to that exposure are more severe. Despite this, little work has investigated mechanistic changes with age in the chronic psychosocial stress response. We hypothesize that, compared to young, aged subjects' initially blunted psychosocial stress response will be followed by chronically worsened outcomes. To test this, young (3 mos) and aged (19 mos) male Fischer 344 rats were assigned to control or psychosocial stress groups and implanted with wireless telemetry from DSI (Data Sciences International) to monitor sleep architecture and body temperature. Chronic psychosocial stress (restraint, 3 h/ day, 4 days/ week, 4 weeks) effects on Morris water maze and body temperature were collected (sleep architecture, blood corticosterone, and transcriptome-level data are being reserved for future analysis). Aged animals showed significant deficits in water maze while hyperthermic responses were conditional on stress exposure.

Disclosures: K. Staggs: None. **J. Popovic:** None. **S. Qutubuddin:** None. **E.M. Blalock:** None.

Poster

179. Learning and Memory: Aging II

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Program#/Poster#: 179.23/BB18

Topic: F.02. Animal Cognition and Behavior

Support: CalciGenix

Title: Effects of the calcium-binding protein apoaequorin on acquisition of trace fear conditioning in adult and aging rats

Authors: *V. L. EHLERS[1], K. L. FELDMANN[1], J. R. MOYER, Jr.[1,2];
[1]Psychology, [2]Biol. Sci., Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Normal aging is associated with an increased susceptibility to cognitive impairment. Of great importance is the ability to develop neurotherapeutic tools that can mitigate cognitive decline and ultimately enhance the quality of life in old age. The hippocampus is widely implicated in learning and memory, and many forms of learning that are hippocampus-dependent are impaired in aging animals, including trace fear conditioning (Moyer & Brown, 2006). A proposed contributor to aging-related cognitive impairment is aging-related calcium (Ca2+) dysregulation. Normally involved in the regulation of Ca2+ transients, Ca2+-binding proteins (CaBPs) are reduced with advancing age, and evidence suggests that this reduction is associated with aging-related cognitive impairments (Soontornniyomkij et al., 2012). Previous data from our lab indicate that a single hippocampal infusion of the CaBP apoaequorin (AQ) is neuroprotective in the event of an ischemic insult (Detert et al., 2013), however, the effect of AQ on cognitive function in aging rats has yet to be investigated. Briefly, adult (3 mo.) and aged (22 mo.) F344 rats received bilateral dorsal hippocampal infusions of either AQ or vehicle. Twenty-four hours following infusion, rats underwent trace fear conditioning, and were tested the following day. Consistent with prior reports, aged vehicle-treated rats demonstrated a significant impairment in trace fear conditioning as evidenced by a reduction in freezing following CS presentation during the test relative to adult vehicle-treated rats ($p < .05$). In adult rats, both vehicle- and AQ-treated animals demonstrated similar freezing during the test (79% and 75%, respectively), suggesting that a single infusion of AQ into the dorsal hippocampus had no adverse effects on trace fear learning. Similarly, a single infusion of AQ did not significantly improve trace fear conditioning in aged rats ($p = .12$). These data demonstrate that although a single intra-hippocampal infusion of AQ is neuroprotective, this treatment did not improve memory in adult or aged rats. However, it is possible that a chronic treatment regimen may be required to observe improvements in aging-related behavioral deficits. Thus, to further determine the effectiveness of AQ in mitigating aging related-cognitive decline, the effect of multiple infusions of AQ on trace fear memory will also be investigated. These studies may shed new light on the role of AQ in trace fear conditioning in adult and aging rats, and will address its value not only in neuroprotection, but also as a potential neurotherapeutic tool for mitigating aging-related cognitive decline.

Disclosures: V.L. Ehlers: None. **K.L. Feldmann:** None. **J.R. Moyer:** None.

Poster

180. Reward: Dopamine

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 180.01/BB19

Topic: F.03. Motivation and Emotion

Support: T32 DA024635-07

NIH Grant DA035443

Title: Nucleus accumbens core muscarinic and nicotinic acetylcholine receptors differentially modulate phasic dopamine release and mediate cue-induced incentive motivation

Authors: *A. L. COLLINS[1], V. GREENFIELD[1], I. XU[1], S. OSTLUND[2], K. WASSUM[1]; [1]UCLA, Los Angeles, CA; [2]Univ. of California, Irvine, Irvine, CA

Abstract: Recent primarily *in vitro* data has demonstrated that within the striatum muscarinic and nicotinic acetylcholine receptors differentially modulate dopamine signaling. The working hypothesis is that striatal acetylcholine levels acting at nicotinic receptors on dopamine terminals act as a low-pass filter on phasic dopamine signaling, attenuating release when acetylcholine levels are high, and facilitating release when levels are low. Muscarinic receptors located on striatal cholinergic interneurons regulate acetylcholine release and therefore regulate this terminal modulation of dopamine release. Whether this occurs in awake, behaving animals and, vitally, whether such modulation has functional consequences related to motivated behaviors is currently unknown. Phasic dopamine is robustly released in the nucleus accumbens core (NAc) in response to reward-paired cues and such release tracks the incentive motivational impact of these cues- their ability to elicit excitation/arousal and to enhance a non-selective range of reward-seeking actions. Therefore, here we used the Pavlovian-instrumental transfer (PIT) task to evaluate the hypothesis that activity at NAc acetylcholine receptors terminally modulates the phasic release of dopamine to mediate the ability of reward-paired cues to invigorate reward-seeking actions. First, we evaluated the role of NAc muscarinic and nicotinic acetylcholine receptors in PIT and found opposite contributions. Blockade of NAc nicotinic receptors (with intra-NAc mecamylamine) enhanced the invigorating influence of a reward-paired cue over reward-seeking actions, while blockade of muscarinic receptors (with intra-NAc scopolamine) selectively attenuated this effect. To determine if these effects were mediated through modulation of dopamine release, in a second experiment we monitored phasic NAc dopamine concentration changes with fast-scan cyclic voltammetry in rats behaving in the PIT task following unilateral infusion of either mecamylamine, scopolamine or ACSF vehicle directly into the recording zone. Early results suggest that local blockade of NAc nicotinic receptors accentuates and blockade of NAc muscarinic receptors attenuates cue-induced phasic dopamine release during PIT. These preliminary data support the hypotheses from *in vitro* data and provide

a potential functional role for acetylcholine modulation of dopamine release in mediating the excitatory influence of reward-paired cues over reward-seeking actions.

Disclosures: **A.L. Collins:** None. **V. Greenfield:** None. **I. Xu:** None. **S. Ostlund:** None. **K. Wassum:** None.

Poster

180. Reward: Dopamine

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Title: Arithmetic and local circuitry underlying dopamine prediction errors

Authors: ***N. ESHEL**[1,2], M. BUKWICH[2], V. RAO[2,3], V. HEMMELDER[2], J. TIAN[2], N. UCHIDA[2];
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Abstract: As we encounter stimuli in the environment, we make predictions about their value. When outcomes match our predictions, learning is not required. When outcomes are unexpected, however, we update our predictions to reflect our experience. This learning process is thought to involve midbrain dopamine neurons, which encode prediction errors, or the difference between actual and expected value. Although dopamine prediction errors have been extensively characterized, little is known about how dopamine neurons calculate these responses. In particular, how are predictions conveyed? Recently, *in vivo* recordings from GABAergic neurons in the ventral tegmental area (VTA) revealed that these neurons signal the predicted value of cues (Cohen et al., 2012). Given known connectivity between VTA GABA and dopamine

neurons, these findings suggest that VTA GABA neurons could provide dopamine neurons with information they need to calculate prediction errors. We combined optogenetic manipulations with extracellular recordings in behaving mice to test this hypothesis. First, we recorded from light-identified dopamine neurons (n = 40 neurons in 5 mice) while mice received rewards of various sizes. On some trials, an odor cue predicted the timing of reward delivery, while on other trials, reward was delivered unexpectedly. By comparing dopamine response functions to various sizes of reward in these two trial types, we found that expectation suppresses dopamine firing (P < 0.001, t-test), and that this suppression was identical regardless of reward size. In other words, dopamine neurons perform subtraction, a computation that is ideal for reinforcement learning but rarely observed in the brain. Next, we used optogenetics to selectively stimulate or inhibit VTA GABA neurons while simultaneously recording from putative dopamine neurons (n =119 neurons in 12 mice). We found that stimulating VTA GABA neurons subtracts from dopamine reward responses (P < 0.001, t-test), as if reward is more expected, and that inhibiting VTA GABA neurons increases dopamine reward responses (P < 0.001, t-test), as if reward is less expected. Thus, VTA GABA neurons causally contribute to prediction error calculations. Finally, we tested the behavioral relevance of VTA GABA activity by bilaterally stimulating these neurons during a classical conditioning task (n = 12 mice). We found that VTA GABA stimulation dramatically reduced anticipatory licking to conditioned odours (P < 0.001, mixed effects linear model), consistent with an important role for these neurons in reinforcement learning. Together, our results uncover the arithmetic and local circuitry underlying dopamine prediction errors.

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Poster

180. Reward: Dopamine

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Topic: F.03. Motivation and Emotion

Support: NIH grant R01MH095953

NIH grant R01MH095953

Sackler Scholar Programme in Psychobiology

Title: Habenula lesions reveal that multiple mechanisms underlie dopamine prediction errors

Authors: *J. TIAN[1], N. UCHIDA[2];
[2]MCB, [1]Harvard Univ., Cambridge, MA

Abstract: Dopamine neurons are thought to facilitate learning by signaling reward prediction errors (RPEs), or the discrepancy between actual and expected reward. However, how RPEs are calculated remains unknown. Recent studies have found that neurons in the lateral habenula also encode RPEs, but with the opposite sign compared to dopamine neurons. Therefore, it has been hypothesized that dopamine neurons receive RPE signals from the lateral habenula. Here, we tested how lesions of the habenular complex (including media and lateral habenula) affect the response of optogenetically-identified dopamine neurons in mice. Mice were trained in an odor based classical conditioning task. After >10 days training, animals were randomly assigned into control (n=7) or lesion group (n=5). Dopamine neurons (control: n=45; lesion: n=44) were identified based on their responses to light and were recorded while mice performing the conditioning task. We found that habenula lesion impaired specific aspects of RPE signaling in dopamine neurons. In control animals, most of the dopamine neurons showed a significant dip in activity (86.7%; $P < 0.05$, Wilcoxon rank sum test). In lesion animals, however, a much smaller fraction of dopamine neurons showed a dip during the omission of predicted reward (47.7%, $P < 0.05$,). The magnitude of the dip was also reduced in lesion animals (control: -2.6 ± 0.2, spikes/s; lesion: -1.5 ± 0.2, spikes/s; mean ± s.e.m; $P < 0.0001$, Wilcoxon rank sum test). In contrast, inhibitory responses to aversive stimuli, such as air puff-predictive cues or air puff, remained unimpaired. Furthermore, we found that after habenula lesions, dopamine neurons' ability to signal graded levels of positive RPEs became unreliable, yet significant excitatory responses still remained. At the behavioral level, we observed a phenotype that is consistent with a relative reduction of negative, over positive, RPEs. These results demonstrate that the habenula plays a critical role in dopamine RPE signaling but suggest that it is not the exclusive source of RPE signals.

Disclosures: J. Tian: None. **N. Uchida:** None.

Poster

180. Reward: Dopamine

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Topic: F.03. Motivation and Emotion

Support: Sackler Fellow in Psychobiology

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Title: Electrophysiological activity mapping of neurons presynaptic to dopamine neurons

Authors: *M. WATABE-UCHIDA[1], J. TIAN[1], J. Y. COHEN[2], F. OSAKADA[3], E. M. CALLAWAY[4], N. UCHIDA[1];
[1]Ctr. for Brain Science, BL4005, Harvard Univ., Cambridge, MA; [2]Johns Hopkins Univ., Baltimore, MD; [3]Nagoya Univ., Nagoya, Japan; [4]Salk Inst., La Jolla, CA

Abstract: Midbrain dopamine neurons play important roles in learning and motivation but how their activity is regulated remains unclear. In a previous study, we performed whole-brain mapping of neurons presynaptic to dopamine neurons ("input neurons") using modified rabies virus (Watabe-Uchida et al., 2012). However, to understand the role of specific inputs, it is essential to know when and how a population of input neurons modulates their activity in a behavioral context. In the present study, we recorded the activity of input neurons in behaving mice using a modified rabies virus coding channelrhodopsin-2 (ChR2) (Osakada et al., 2011). AAVs were injected to express TVA (receptor for modified rabies virus) and RG (rabies glycoprotein necessary for trans-synaptic spread) in dopamine neurons in the ventral tegmental area (VTA). After recovery, mice were trained in an odor-based classical conditioning task. We then injected modified rabies virus with ChR2 into the VTA, and implanted an optetrode (tetrodes and optic fiber) into one of the input areas. Five to 16 days after rabies injection, we examined the activity of optogenetically-identified input neurons from the major presynaptic areas such as the rostromedial tegmental area, the pedunculo pontine tegmental area, the ventral pallidum, the striatum and the lateral hypothalamus (n = 30 mice in total). We found that input neurons in most of these areas showed task-related activities, such as value-dependent responses to outcome-predictive cues and/or outcome, or sustained responses modulated by reward expectation. In contrast to the prevalent theories of prediction error computations, we found most task-responsive input neurons showed a mixed coding of reward and reward expectation signals in diverse and complicated ways. Furthermore, a small fraction of input neurons already showed relatively intact prediction error coding. New models are needed to explain how dopamine neurons filter complicated inputs to extract robust reward prediction error signals.

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Poster

180. Reward: Dopamine

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Program#/Poster#: 180.05/BB23

Topic: F.03. Motivation and Emotion

Title: A set of projection-specific circuit maps for midbrain dopamine neurons

Authors: *W. MENEGAS[1], J. BERGAN[2], S. K. OGAWA[3], Y. ISOGAI[1], N. UCHIDA[1], M. WATABE-UCHIDA[1];
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Abstract: Midbrain dopamine (DA) neurons are involved in a diverse set of processes such as learning, motivation, and motor control. Although these neurons are known to project widely throughout the forebrain, the functional differences between populations of DA neurons with distinct projection targets are still unknown. We reasoned that DA populations with unique functions are likely to have unique distributions of inputs. Furthermore, inputs common to all populations of DA neurons are likely to be involved in computations that might be performed by all DA neurons, such as reward prediction error (RPE) calculation. Therefore, we infected populations of midbrain DA neurons with rabies-GFP based on their projection target, so that we could compare the inputs of DA neurons that project to the dorsal striatum, ventral striatum, medial prelimbic cortex, lateral orbitofrontal cortex, central amygdala, lateral habenula, and globus pallidus. In order to to compare a large number of total brains (n=81) with high accuracy, we developed a high-throughput pipeline for whole brain image acquisition and analysis. First, we used CLARITY to make brains optically transparent and imaged them at 1um x 1um x 5um resolution using a light-sheet microscope. Then, we aligned these images to a “reference space” and made a 3 dimensional map of this space in order to compare the number of labeled cells (as determined by automated cell counting) in each region. Employing these methods to compare many brains in a single reference space allowed us to identify differences in the distribution of inputs to DA neurons with distinct projection targets.

Disclosures: W. Menegas: None. **J. Bergan:** None. **S.K. Ogawa:** None. **Y. Isogai:** None. **N. Uchida:** None. **M. Watabe-Uchida:** None.

Poster

180. Reward: Dopamine

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Title: Dopamine neurons code reward and aversion in a context-dependent manner

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Abstract: Dopamine is thought to be a key regulator of learning from reward and punishment. It has been proposed that dopamine neurons signal value prediction error (VPE, often referred to as reward prediction error), that is, the difference between the values of actual and predicted outcomes. However, how dopamine neurons integrate information about aversive events remains controversial. Some studies have shown that aversive stimuli inhibit dopamine neurons, while others have suggested that aversive events activate at least some dopamine neurons. One recent study postulated that dopamine neurons largely ignore aversive events (Fiorillo, 2013). In the present study, we performed a series of experiments to resolve these issues. First, we recorded the activity of optogenetically-identified dopamine neurons in the ventral tegmental area in a "mixed-prediction" task in which a single odor predicted both reward and punishment in a complementary and probabilistic manner. We found that dopamine neurons' response to the odor predicting both reward and punishment was larger than their response to the odor predicting only punishment ($n = 26$, $P < 0.05$, paired-sample t test) while smaller than that to the odor predicting only reward ($P < 0.001$). We also found that a vast majority of dopamine neurons were exclusively inhibited by aversive events, and their responses to reward and punishment were reduced when these outcomes were predicted ($P < 0.001$ and $P < 0.05$, respectively). These results demonstrate that dopamine neurons signal VPEs integrating information about both reward and punishment in a common currency. In different experimental contexts, however, many dopamine neurons acquired short-latency excitations that are consistent with "salience prediction error". Furthermore, their responses to air puff-predictive cues were indistinguishable to the responses to nothing-predicting cues, suggesting that the short latency excitations mask inhibitions by aversive events. Together, these results reconcile recent controversies in the field, and demonstrate that dopamine neurons have different modes of signaling, each of which may be adaptive for different environments.

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Poster

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Topic: F.03. Motivation and Emotion

Support: Virginia Foundation for Healthy Youth

Title: Sex-specific lateralization of mesocorticolimbic dopamine receptor mRNAs in adolescent mice

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Abstract: Lateralization of the mesocorticolimbic system plays fundamental roles in motivation and emotion. For example, Davidson and colleagues have shown that left/right asymmetries in D2 dopamine receptors in the striatum and prefrontal cortex (PFC) are correlated with the relative sensitivity of human subjects to positive (attractive) vs. negative (aversive) stimuli. Mesocorticolimbic responses to stress are also lateralized, for example Gratton and colleagues have shown that the right medial PFC (mPFC) of rats plays a predominant role in stimulating the emotional and endocrine responses to stress. Dopamine receptor gene expression in the mesocorticolimbic system undergoes dramatic increases during early to middle adolescence (Teicher and colleagues). Thus adolescent emotional behavior and vulnerability to drug abuse may also be influenced by changes in the levels and lateralization of dopamine receptor expression. Here we show that mice during early adolescence (postnatal day 33) show dramatic and sex-specific lateralization of dopamine receptor gene expression. We used qRT-PCR to measure D1, D2L, and D2S mRNA levels on the left vs. right sides of the mPFC, the ventral striatum (VS), and the ventral tegmentum (VT) of male vs. female mice on postnatal day 33 (p33). We found that p33 female mice tended to have dramatically higher dopamine receptor gene expression (than p33 males), as well as higher lateral differences in all three dopamine receptors, and in all three brain areas examined. Female mice had higher levels of D1, D2L, and D2S mRNAs on the right sides of the mPFC and VS, by 1.9-fold to 5.4-fold (in comparison to the same brain area on the left side). Female mice also had higher levels of D1 mRNA on the right side of the VT by 2.3-fold (in comparison to the same brain area on the left side). Male mice were less asymmetric in their expression of these mRNAs, with significant asymmetries only in D1. Males had a 4.0-fold excess of D1 in the right mPFC, but a 1.9-fold excess of D1 in the left VS (in comparison to the same brain area on the other side). These differences in dopamine receptor levels and lateralization hint at fundamental sex differences in the

development of dopamine signaling in the mesocorticolimbic system, which may be involved in sex differences in emotional behavior, as well as age differences in drug abuse. The response of these genes to nicotine stimulation will be reported elsewhere.

Disclosures: R.L. Murphy: None. **K.J. Fryxell:** None.

Poster

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NIH Grant T32 DA007268 21

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Title: Individual variability in dopamine transporter regulation of neurotransmission and incentive motivation

Authors: *B. F. SINGER[1], B. GUPTAROY[2], C. J. AUSTIN[1], M. E. GNEGY[2], T. E. ROBINSON[1], B. J. ARAGONA[1];
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Abstract: Conditioned stimuli (CSs) have the capacity to powerfully motivate behavior by producing conditioned responses (CRs). There is considerable individual variation in the degree to which a CS exerts motivational control over behavior. For example, in an autoshaping animal model, a lever CS that predicts food reward (unconditioned stimulus, US) becomes attractive, wanted, and elicits an approach CR in a subset of outbred rats termed “sign-trackers” (ST), whereas “goal-tracker” (GT) rats exhibit a CR toward the location where the reward is delivered. Dopamine (DA) neurotransmission in the nucleus accumbens (NAc) core mediates the attribution of motivational meaning to the CS: it is required for lever-directed approach in ST rats, but not conditioned responding in GTs, even though all rats learn the stimulus-reward relationship equally well. Using *in vivo* fast-scan cyclic voltammetry (FSCV) recordings of DA release in the NAc core, we first replicated earlier studies (Flagel et. al. 2011) demonstrating CS-evoked DA responses during lever approach in STs. In contrast to STs, GTs displayed both CS-

and US-evoked DA release during approach to the food-receptacle. A series of experiments were then conducted to better understand what mechanisms may account for differences in DA signaling in the NAc core. It was found that injection of AMPH directly into the NAc core selectively enhanced lever-directed approach in STs, amplifying the incentive value of the CS. This was hypothesized to result from increased DA transporter (DAT) binding of AMPH in STs, as STs showed elevated ventral striatal DAT surface expression compared to GTs. DAT regulation over DA was further investigated using *ex vivo* FSCV recordings of electrically-evoked DA release. STs showed more rapid DA uptake compared to GTs, supporting enhanced DAT function in STs. We propose that greater DAT surface expression in STs increases control over synaptic DA, enhancing the ability of phasic DA to induce plasticity that underlies the encoding of attraction to a reward paired cue (incentive motivation).

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Poster

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Topic: F.03. Motivation and Emotion

Support: Brain and Behavior Research Foundation

Title: *In vivo* optogenetic manipulation of dopamine neurons in a novel behavioral economics based food-seeking task

Authors: *S. SCHELP, G. KRZYSTYNIAK, J. GAGE, D. RAKOWSKI, R. DAS, E. OLESON;
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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). Preliminary 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.

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Poster

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Topic: F.03. Motivation and Emotion

Support: HARMONIA Grant 2012/06/M/NZ4/00143

Title: The role of NMDA receptor-dependent burst activity of dopamine neurons in reinforcement-learning and depressive-like behaviors

Authors: *K. LOPATA[1], M. WALCZAK[2], P. E. CIESLAK[1], &. SZUMIEC[1], M. TURBASA[1], T. BLASIAK[2], J. RODRIGUEZ PARKITNA[1];

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Abstract: Phasic activity of the midbrain dopamine neurons encodes a temporal prediction error and thus underlies reinforcement learning and signaling of incentive salience. Here, we use a transgenic mouse model with inducible deletion of NMDA receptors in dopamine neurons (NR1DATCreERT2 mice) to reduce the burst activity and observe behavioral consequences. As anticipated, iontophoretic application of NMDA had no effect on the activity of dopamine neurons in the ventral midbrain of mutant mice, while it robustly induced bursting in controls. Conversely, the cholinergic agonist, carbachol, had similar effects on neuronal activity in both control and mutant mice, including induction of bursts in subpopulation of dopamine neurons. Thus, the NR1DATCreERT2 mice lack of NMDA receptor-dependent phasic activity of dopamine neurons. We started behavioral experiments from a classic Pavlovian training in which mutant mice correctly associated cue with reward and decreased the time to approach food magazine, however they were impaired in a subsequent conditioned reinforcement test. A second cohort was tested for instrumental responding for food, which was not affected by the mutation, although mutant animals were slower in acquiring this behavior. Moreover, no differences were observed between animals under complex reinforcement schedules. Only under random interval procedure and in instrumental responding for sensory stimuli mutant mice showed deficits. Mutant mice were tested for saccharine preference in an IntelliCage system. While in two cohorts a clear reduction in saccharine preference under FR3 was observed, other experiments have shown normal preference under free access and under gradual increase in FR schedules. To further explore the ability to exert effort to obtain a reward, T-maze test with increasing difficulty to obtain chocolate food pellets was conducted and no changes between mutant and control mice were observed. Finally, based on previous results and literature reports, animals were tested for a depressive-like phenotype. It was shown that mutation reduced the frequency of social interactions as well as increased the time of immobility in the forced swim test, indicating the importance of disrupted phasic DA activity in a negative affect. In summary, the loss of NMDAR-dependent bursting activity in dopamine neurons caused specific impairments in reinforcement learning, with most appreciable changes in cases where rewards were not essential for survival (saccharine, sensory stimuli or conditioned cues) and furthermore, induced depression-related behaviors such as learned helplessness and reduction in social interactions.

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Poster

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Topic: F.03. Motivation and Emotion

Title: Investigating contributions of dopamine D2 and D3 receptors to Pavlovian conditioned approach behaviors

Authors: *K. M. FRASER[1,2,3], J. L. HAIGHT[4], E. L. GARDNER[6], S. B. FLAGEL[2,5,4,3]; [2]Psychiatry, [3]Undergraduate Program in Neurosci., [4]Neurosci. Grad. Program, [5]Mol. and Behavioral Neurosci. Inst., [1]Univ. of Michigan, Ann Arbor, MI; [6]Intramural Res. Program, Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Environmental cues that are repeatedly paired with reward can guide behavior in an adaptive manner, bringing one in close proximity to valuable resources (e.g. food, water, sex). However, cues can also acquire incentive motivational value (i.e. incentive salience) and come to control behavior to the point that it becomes maladaptive. Importantly, individuals differ in the extent to which incentive salience is attributed to reward-paired cues. These individual differences can be captured using a Pavlovian conditioned approach (PCA) paradigm. When a discrete cue (lever) is repeatedly paired with delivery of a food reward, some rats, termed sign-trackers attribute incentive salience to the cue; whereas others, termed goal-trackers, treat the cue only as a predictor of reward. This model, therefore, allows us to parse the incentive from the predictive properties of reward cues. Previous studies utilizing this model have shown that dopamine is critical for the attribution of incentive salience to both food- and drug-associated cues. However, the receptors involved in this form of stimulus-reward learning have yet to be identified. Here we examined the effects of dopamine D2 and D3 receptor antagonism on the expression of sign- and goal-tracking behaviors. Following PCA training, sign- and goal-tracking rats were treated with the D2/D3 antagonist raclopride (0.1 mg/kg), or the selective D3 antagonist, SB-277011A (6 or 24 mg/kg). Non-selective antagonism of D2/D3 receptors attenuated the performance sign-tracking behavior for rats that were previously classified as sign-trackers, and attenuated goal-tracking behavior in rats previously classified as goal-trackers. Interestingly, these effects were specific to the previously acquired conditioned response. In contrast, selective antagonism of D3 receptors had no effect on the expression of either the sign- or goal-tracking conditioned response for either phenotype. The present findings suggest that signaling at the dopamine D2 receptor, or perhaps some combination of synergistic activity at D2/D3 receptors, is critical for the expression of Pavlovian conditioned approach behaviors. Although previous studies demonstrated a specific role for dopamine in learning the sign-tracking response (i.e. the attribution of incentive salience to reward cues), the current findings suggest that the expression of both sign- and goal-tracking behavior can be affected when specific dopamine receptor subtypes are targeted.

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Poster

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Topic: F.03. Motivation and Emotion

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DA038114

Title: Chemogenetic inhibition of mesolimbic dopamine reveals excitability-dependent amphetamine action for behavior

Authors: *S. M. CONWAY[1], M. S. MCMURRAY[1], P. A. GARRIS[2], E. H. CHARTOFF[3], R. A. WHEELER[4], J. D. ROITMAN[1], M. F. ROITMAN[1];
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Abstract: Amphetamine (AMPH) is characterized as a dopamine 'releaser' as it not only blocks dopamine uptake by the dopamine transporter but also enters the presynaptic terminal, causing vesicular depletion and reverse transport of dopamine. These mechanisms are thought to be action potential-independent. Recent work using intact rats has shown that AMPH increases the frequency of phasic dopamine release events, which, in turn, is thought to be action potential-dependent and thus raises questions about the mechanisms of AMPH action. To further probe the dependency of AMPH on dopamine neuron excitability, we injected virus to deliver the Cre-dependent inhibitory (hM4D-Gi) designer receptor exclusively activated by designer drug (DREADD) into the ventral tegmental area (VTA) of transgenic rats expressing Cre recombinase under the control of the tyrosine hydroxlyase promoter (TH:Cre+) and wildtype littermates (TH:Cre-). Following transfection, systemic administration of the hM4D-Gi ligand, clozapine-N-oxide (CNO), should hyperpolarize VTA dopamine neurons, suppress excitability, and attenuate dopamine release in the NAc of TH:Cre+ but not TH:Cre- rats. Using fast-scan cyclic voltammetry we found that CNO suppressed evoked dopamine release in TH:Cre+ but not TH:Cre- rats thus validating our chemogenetic approach. Next, we evaluated the effect of decreased VTA dopamine excitability on AMPH-induced behavior. TH:Cre+ and TH:Cre-, virus-injected rats received were pretreated with either CNO or saline and tested for AMPH-induced locomotion. A second such session was conducted with the other pretreatment in counter-balanced order. Only TH:Cre+ rats exhibited significantly lower AMPH-induced locomotion following pretreatment with CNO versus saline. A separate set of locomotor tests

indicated no effect of CNO on spontaneous locomotion. To examine the effects of AMPH and CNO on a reward-directed behavior, TH:Cre+ and TH:Cre- rats performed a rate-frequency intracranial self-stimulation task. In line with previous work, AMPH decreased the frequency threshold for self-stimulation in all animals while CNO increased threshold only in TH:Cre+ rats. Both AMPH and CNO showed dose-dependency. Furthermore, pretreatment of CNO in TH:Cre+ rats prevented AMPH-induced reductions in threshold. Taken together, these results demonstrate dopamine excitability-dependent behavioral actions of AMPH using a chemogenetic approach validated by electrochemical recordings thus further highlighting the importance of action potential-dependent mechanisms of this psychostimulant.

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Poster

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Concordia University Research Chair to P.Shizgal

Title: Contrasting effects of rewarding electrical and optical stimulation

Authors: *M.-P. COSSETTE, P. SHIZGAL;
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Abstract: Studies of intracranial self-stimulation (ICSS) point to midbrain dopamine (DA) neurons as a critical component of the circuitry underlying reward seeking. Electrical stimulation

of the medial forebrain bundle (MFB) has long been used in ICSS studies. Although such stimulation ultimately excites midbrain DA neurons, the directly stimulated neurons subserving the rewarding effect of MFB stimulation are primarily non-DAergic. Neither the identity of these non-DAergic MFB neurons nor the manner in which they are connected to the DA neurons has been established firmly. As a step towards achieving these goals, we used fast-scan cyclic voltammetry to monitor release of DA in the nucleus accumbens (NAc) in response to electrical MFB stimulation at the level of the lateral hypothalamus (LH) or optical stimulation of neurons in the ventral tegmental area (VTA) that express tyrosine hydroxylase (TH). To render VTA DA neurons optically excitable, we used viral transfection and Cre-Lox recombination to express channelrhodopsin2, tagged with enhanced yellow fluorescent protein (eYFP); an optical fiber was aimed at the viral injection site. To visualize LH to VTA projections, eYFP was expressed in LH neurons under the control of the promoter for the calcium/calmodulin-dependent protein kinase IIa. Whereas unilateral electrical MFB stimulation triggered DA transients in both hemispheres, DA release in the NAc was ipsilateral to the optical fiber when the virus injection had been restricted carefully to one hemisphere. Upon inspection of the VTA to NAc projections via epifluorescence microscopy, we saw no evidence that DAergic fibers cross the midline before terminating in the ventral striatum. In contrast, inspection of LH toVTA projections revealed both fibers confined to the hemisphere ipsilateral to the injection site and fibers that cross the midline en route to the VTA. The latter fibers could have contributed to the observed DA release in the contralateral NAc, as could disynaptic relays via the laterodorsal tegmental and rostromedial tegmental nuclei and pathways that lead to excitation or disinhibition of tonically active, cholinergic interneurons in the ventral striatum. The behavioral consequences of direct optical activation of VTA DA neurons or trans-synaptic activation by electrical MFB stimulation have yet to be worked out.

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Poster

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Topic: F.03. Motivation and Emotion

Support: CAMH Foundation

Title: Repetitive tms of the insula modulates dopaminergic activity: a phno-pet study in healthy subjects

Authors: *S. MALIK[1], M. JACOBS[1], S. CHO[1], I. BOILEAU[1], D. BLUMBERGER[1], A. WILSON[1], Z. J. DASKALAKIS[1], A. STRAFELLA[1], A. ZANGEN[2], B. LE FOLL[1]; [1]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [2]Ben Gurion Univ., Beer-Sheva, Israel

Abstract: Purpose: Modulating the function of the insular cortex could be a novel therapeutic strategy to treat addiction to a variety of drugs of abuse as this region has been implicated in mediating drug reward and addictive processes. The objective of this work was to selectively and bilaterally target the insula with repetitive Transcranial Magnetic Stimulation (rTMS) and subsequently infer changes in dopamine levels using Positron Emission Tomography (PET) with [11C]-(+)-propyl-hexahydro-naphtho-oxazin (PHNO). Methods: This was a within-subject, blinded and sham-controlled pilot study. Seven healthy, right-handed subjects, aged 19-45, underwent 3 PHNO-PET scans preceded by rTMS (sham, 1Hz or 10Hz), on 3 separate days. Sham stimulation (mimicking 1Hz (n=4) or 10Hz (n=4)) was performed on Day 1 followed by real rTMS (1Hz/10Hz, counterbalanced) on Days 2 and 3. PET image analysis was region of interest (ROI)-based. PHNO-specific binding (BPND) was estimated in each ROI using the simplified reference tissue model with the cerebellum as reference region. Statistical comparisons between BPND across conditions in each ROI were done using repeated-measures ANOVA. Tukey post-hoc tests examined differences between paired conditions. Results: Preliminary analysis revealed significant differences in BPND between sham, 1Hz and 10Hz conditions in the substantia nigra, dorsal putamen, dorsal striatum, somatosensory striatum and full striatum (all $P \leq 0.05$). Post-hoc tests primarily showed differences between sham and 1Hz conditions, associated with increases in BPND following 1Hz stimulation. Discussion: Low frequency rTMS (1Hz) targeting the insular cortex significantly decreased dopamine levels in the substantia nigra and striatum relative to the sham condition. Changes in dopamine levels following high frequency stimulation did not appear to statistically differ from the other 2 conditions. Replicating this study in tobacco smokers or alcoholics would be a logical follow-up to assess whether rTMS of the insula can be employed as a treatment option for addiction.

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Poster

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Title: Past exposure to L-dopa accelerates habitual responding in rats

Authors: *T. M. FURLONG[1], A. M. GIBSON[2], K. A. KEEFE[1,2];
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Abstract: Exposure to amphetamine and cocaine alters the brain and cognition to promote further drug-use. Specifically, these dopamine agonists have been shown to accelerate habitual responding in animals in situations unrelated to obtaining drugs. Recent studies show that these drugs have the ability to increase phasic dopamine signaling, and that phasic dopamine signaling is necessary for forming associations underlying reward-based learning. L-dopa is also a dopamine agonist that can increase phasic dopamine signaling by augmenting vesicular dopamine content. However, the impact of L-dopa on behavioral control has yet to be established. Hence, we sought to determine whether L-dopa accelerates habitual responding in animals, similar to drugs of abuse. Initially, we trained rats to press a lever for food-outcome under the influence of L-dopa (25 mg/kg or 50mg/kg, i.p.). We then tested the knowledge of this response-outcome relationship using an outcome-devaluation task, where the food-outcome was devalued by free-feeding it to satiety before testing responding on the lever. Animals that were trained under L-dopa adjusted their behavior according to the current value of the outcome, and, like control animals, reduced responding on the lever when the associated outcome was devalued. Thus, goal-directed responding was not impacted by L-dopa exposure during training. However, when the same animals were then trained to press a different lever for a different outcome without L-dopa administration, goal-directed responding was reduced at test. That is, lever response rates for the animals that had previously received L-dopa were not significantly different between the lever associated with the devalued outcome and the lever associated with the still valued outcome. Further testing also revealed that the L-dopa exposed animals were able to update the response-outcome contingency when it was reversed or degraded, demonstrating that some aspects of flexible behavior were intact. Our findings suggest that deficits in behavioral control associated with the use of L-dopa may result from accelerated habitual responding similar to that seen following exposure to drugs of abuse, and hence L-dopa exposure may also cause similar neuroadaptations to these other dopamine agonists.

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Poster

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Title: Different routes of cocaine administration resolve multiple mechanisms of action to increase phasic dopamine release in the nucleus accumbens

Authors: *A. I. GERTH[1], A. ALHADEFF[2], S. FORTIN[3], H. GRILL[2], M. ROITMAN[3]; [1]Psychology, Univ. of Illinois At Chicago Dept of Psych, Chicago, IL; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Psychostimulants increase dopamine concentration in the nucleus accumbens through the blockade of the dopamine transporter (DAT). However, they also increase the frequency of dopamine release events, a finding that cannot be explained by reuptake blockade alone. Rather, this effect may be mediated by systemic cocaine-induced increases in neural activity in brain regions that project to dopamine cell bodies resulting in increases in dopamine cell excitability. To further explore regionally selective actions of cocaine on phasic dopamine signaling, we administered cocaine into the lateral or fourth ventricles and compared the dopamine response to that of systemically delivered cocaine. Dopamine release in the nucleus accumbens was evoked by electrical stimulation of the ventral tegmental area and measured using fast-scan cyclic voltammetry in urethane anesthetized rats. Stimulation trains were delivered once every 3 minutes and each train resulted in a rapid and pronounced rise in dopamine followed by a rapid decay due to dopamine clearance via the DAT. The magnitude of dopamine release ([DA]max) by each stimulation train as well as the latency to decay to fifty percent of the maximum (t(1/2); index of DAT activity) were recorded. Following stable [DA]max (3 stimulations differing by less than 10%; baseline), rats received an injection of cocaine [systemic: 2.5mg/kg; lateral and fourth ventricle: 50ug in 1ul) or an equal volume of vehicle. All routes of cocaine delivery caused an increase in [DA]max. However, only systemic cocaine caused an increase in t(1/2). That hindbrain-delivered (fourth ventricle) cocaine caused an increase in [DA]max is novel. Thus, we further explored hindbrain sites that may contribute to an increase in dopamine cell excitability by using c-fos immunohistochemistry. Preliminary data suggest that fourth ventricular cocaine delivery caused a robust increase in c-fos immunoreactivity in the nucleus of the solitary tract, a region that has recently been shown to send direct projections to dopamine cell bodies. Together, the data show that cocaine induced effects on phasic dopamine signaling are mediated via actions throughout the brain including the caudal brainstem.

Disclosures: **A.I. Gerth:** None. **A. Alhadeff:** None. **S. Fortin:** None. **H. Grill:** None. **M. Roitman:** None.

Poster

180. Reward: Dopamine

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 180.17/BB35

Topic: F.03. Motivation and Emotion

Title: Collective activity of ventral tegmental area neurons encodes information about ongoing actions

Authors: ***J. WOOD**[1], N. W. SIMON[1], S. KOERNER[2], R. E. KASS[2], B. MOGHADDAM[1]; [1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Statistics, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: In order to plan, organize, and flexibly update behavior, it is necessary to continually monitor actions and feedback. The dopamine systems, and the ventral tegmental area (VTA) are implicated in several processes related to these forms of cognition. Most studies of the VTA or dopamine system focus on how these neurons encode information about reward prediction errors, novelty, and salience during tasks requiring very actions. We were interested in how the collective activity of dopamine and non-dopamine VTA neurons might encode information about repetitive series of instrumental actions performed to earn reward. We performed ensemble recordings while rats executed repetitive actions which were randomly rewarded. Different actions activated different neurons. Some neurons fired predominantly during low numbered actions in a trial, whereas others fired during the medium or high numbered actions. Neural activity was thus, diversely tuned to action execution. The number of actions performed in a trial could reflect effort expenditure, progress toward a goal, or other information necessary for behavioral organization, which is closely linked to dopamine function. Because population averaged neuronal activity did not vary with action number, and owing to the diversity of neuronal tunings to action execution, action number could be encoded via the combined activity of the entire ensemble of neurons. To quantify this, we used linear discriminant analysis (LDA) to classify actions as low (actions 1-7), medium (actions 8-14), or high (15-21). We decoded both population averaged activity, and the collective activity of the entire ensemble of neurons (without averaging or otherwise combining activity across neurons). This analysis revealed that decoding of population averaged activity correctly classified binned action number at chance levels (approximately 33% accuracy). In contrast, the ensemble activity was correctly classified

at approximately 85% accuracy, which was significantly greater than population averaged activity or chance levels. There was no effect of session number, neuron type, or action number bin, demonstrating that this pattern did not significantly differ across sessions, between dopamine and non-dopamine neurons, or between high, medium, and low numbered actions. These results suggest that both dopamine and non-dopamine VTA neurons encode an ensemble signal that provides real-time information about serial action execution. Our data demonstrate a previously unreported ensemble code in the VTA, which encodes novel types of information, which could be used for behavioral organization.

Disclosures: J. Wood: None. **N.W. Simon:** None. **S. Koerner:** None. **R.E. Kass:** None. **B. Moghaddam:** None.

Poster

180. Reward: Dopamine

Location: Hall A

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Topic: F.03. Motivation and Emotion

Support: Internationaal Parkinson Fonds - Grant Esselink Cools 2013

Title: Striatal response during reward and punishment reversal learning in depressed and non-depressed patients with Parkinson's disease

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Abstract: Depression is one of the most common and debilitating non-motor symptoms of Parkinson's disease (PD). Yet, the underlying neurobiological mechanisms are still unclear and treatment is often suboptimal. Affective processing biases characterize depression and presumably contribute to the development of depressed mood. Affective biases have been linked to attenuated striatal function across various tasks. Specifically during reversal learning, depressed individuals demonstrate enhanced punishment and impaired reward learning alongside attenuated striatal responses. Striatal dopamine levels modulate the balance between learning from reward and punishment. Specifically, decreases in dopamine shift the balance to enhanced punishment relative to reward learning, a profile resembling that of depression. Parkinson's disease is characterized by striatal dopamine depletion. However, it is unknown whether

decreases in striatal dopamine and attenuated striatal function contribute to affective processing biases in reversal learning in depression in PD. Here we used functional MRI to compare striatal responses during reward and punishment reversal learning between PD patients without and with (a history of) PD related depression, most not currently depressed. Patients were tested ON and OFF their dopaminergic medication to assess the potential influence of striatal dopamine levels. Irrespective of drug, patients with PD-related depression exhibited impaired reward (but not punishment) reversal learning alongside attenuated valence-dependent striatal BOLD signal. Specifically, whereas unexpected reward elicited significantly greater increases in striatal BOLD signal than unexpected punishment in non-depressed PD patients, this was not observed in depressed PD patients. Furthermore, attenuations in valence-dependent striatal BOLD signal correlated with impairments in reward reversal learning. These findings suggest that attenuated striatal signalling might underlie affective processing biases in reversal learning that putatively contribute to PD-related depressive symptoms.

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Poster

180. Reward: Dopamine

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Topic: F.03. Motivation and Emotion

Support: Grants-in-Aid for Science Research (26710001) to M.M. from the Ministry of Education, Science, Sports, Culture, and Technology of Japan

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Title: Midbrain dopamine neurons signal whether planned eye movements are successfully cancelled during a saccadic stop-signal task

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Abstract: In daily life, we are often required to inhibit planned or ongoing motor actions that would result in unwanted consequences. This ability, called response inhibition, is impaired in patients with damage to the dopamine (DA) system, as well as in pharmacologically DA-disrupted rodents. Although several lines of evidence suggest the DA contribution to response inhibition, it remains unclear what signals DA neurons transmit to attain this ability. To address this issue, we recorded single-unit activity from DA neurons in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) while a monkey was performing a saccadic stop-signal task. Trials began with the appearance of a central fixation point, and the monkey was required to fixate the point. After 1000 ms, 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. In the remaining 30%, on the other hand, the fixation point reappeared as a "stop signal" with a random delay (stop-signal delay) after the onset of the saccadic target. The monkey was then required to cancel the planned saccade. When the stop-signal delay was short, the monkey easily cancelled planned saccades. As the stop-signal delay was extended, the monkey increasingly failed to cancel the saccades. We recorded the activity of 40 DA neurons. Of these, 16 neurons (40%) exhibited a significant excitation that was aligned at the onset of the stop signal (Wilcoxon signed-rank test, $P < 0.05$). This excitatory response decreased when the monkey failed to cancel planned saccades, and increased as the stop-signal delay was extended (i.e., as the task became more demanding). Notably, the DA neurons activated with the stop signal were distributed dorsolaterally in the ventral midbrain, corresponding probably to the SNc rather than the VTA. In order to investigate the causal relationship between DA signals and response inhibition, we next pharmacologically disrupted DA signals with intramuscular administration of haloperidol, a D2 receptor antagonist, while the monkey was performing the stop-signal task. We found that the pharmacological disruption impaired the ability to cancel planned saccades. Our data suggest that DA neurons transmit a signal that is necessary to achieve response inhibition, and that these neurons are located in a particular area of the ventral midbrain.

Disclosures: **T. Ogasawara:** None. **M. Takada:** None. **M. Matsumoto:** None.

Poster

180. Reward: Dopamine

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

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Topic: F.03. Motivation and Emotion

Support: NIAAA Intramural Research Program

Title: Real-time dopamine release during discrimination and reversal learning in mice

Authors: *A. K. RADKE[1], Y. MATEO[2], A. KOCHARIAN[1], D. M. LOVINGER[2], A. HOLMES[1];
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Abstract: The dopamine system is well known for its role in goal-directed behavior. Phasic dopamine release has been observed in response to novel stimuli as well as to unexpected presentation of rewards, yet few studies have monitored dopamine release over the course of multiple stages of learning and as reward contingencies change. To address this gap, we used fast-scan cyclic voltammetry with chronically implanted electrodes to measure sub-second dopamine release in the nucleus accumbens during a touchscreen-based visual discrimination and reversal learning task in C57BL/6J mice. During initial discrimination learning, we detected significant increases in dopamine release after mice made a correct choice, and dopamine decreases following an error. Interestingly, the increase on correct trials was dependent on the outcome of the previous trial, such that dopamine release was only increased when a correct choice followed an error. These signals abated as discrimination learning progressed to criterion levels of performance. When stimulus-reward contingencies were reversed, such that the previous incorrect choice was now unexpectedly rewarded, a large dopamine signal in the nucleus accumbens re-emerged in response to the unexpected reward. As in discrimination, this response diminished as the new contingences were relearned to criterion. These results support theoretical models that dynamic changes in striatal dopamine signaling guide successful learning.
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Poster

180. Reward: Dopamine

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Topic: F.03. Motivation and Emotion

Support: Ontario Problem Gambling Research Centre #3132

Title: Repeated exposure to uncertain rewards induces sensitization and increases risky decision-making: implications for modelling gambling disorder in rats

Authors: *F. D. ZEEB[1], Z. LI[1], D. C. FISHER[1], M. H. ZACK[1,2], P. J. FLETCHER[1,3,4];
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Abstract: Gambling opportunities are increasing and gambling disorder (GD) is a growing public health concern. An animal model could advance our understanding of GD and aid in the development of effective treatment strategies. Pathological gamblers, compared to healthy controls, demonstrate greater dopamine (DA) release in the striatum and midbrain in response to amphetamine. We hypothesize that repeated exposure to gambling scenarios induces sensitization of the DA system, similar to chronic exposure to drugs of abuse. Here, male rats responded for saccharin reward on a predictable fixed ratio (FR, n=8) or unpredictable variable ratio (VR, n=8) schedule of reinforcement. Rats yoked to receive unpredictable reward according to the FR or VR group were also included (Y, n=16). Animals were trained twice daily for 28 days, beginning with a FR/VR1 schedule which increased to a FR/VR20 schedule. Locomotor activity following an injection of saline or amphetamine (AMPH; 0.5 mg/kg) was then assessed. There was no significant difference between the two yoked groups. Compared to the FR group, rats in the VR and Y groups demonstrated increased hyperactivity following AMPH. Therefore, similar to Singer et al (2012), animals exposed to an uncertain reward schedule demonstrate increased sensitivity of the DA system. Rats were then tested on the rat gambling task (rGT), a rodent analogue of the Iowa Gambling Task (IGT). Risky decision-making may be a hallmark feature of GD and pathological gamblers choose disadvantageously on the IGT. During the rGT rats have 30 min to maximize gains. Animals choose among four options, each associated with the delivery of a different number of rewarding pellets and a different frequency and duration of timeout periods during which reward cannot be earned. These frustrating timeouts decrease long-term gains, equivalent to losses in the IGT. Like the IGT, the optimal strategy is to favour options that yield smaller immediate reward but less loss and avoid risky options associated with greater rewards, but greater long-term loss. All groups learned which options were advantageous and animals in the FR group and Y groups significantly preferred the advantageous options over the disadvantageous options. However, rats in the VR group did not show a significant preference for the advantageous options. Locomotor activity following an injection of saline or AMPH (0.5 mg/kg) was again assessed following rGT training. Compared to the FR group, only the VR group showed greater hyperactivity following AMPH. In sum, actively responding for uncertain reward sensitizes the DA system and impairs the ability to make optimal decisions, possibly similar to patients with GD.

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Poster

180. Reward: Dopamine

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Topic: F.03. Motivation and Emotion

Support: NIH Grant NS36999

Title: Fast dopamine transients in the medial prefrontal cortex enhance stimulus discrimination

Authors: *A. T. POPESCU, M.-M. POO;
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Abstract: Phasic dopamine (DA) release is believed to guide associative learning. Although extensively studied in the ventral tegmental area (VTA) to striatum pathway, the precise action of DA in other target regions remains unclear. We examined DA function in the medial prefrontal cortex (mPFC), and found that mice receiving repetitive temporal pairing of an auditory conditioned stimulus (CS) with optogenetically-induced DA release in mPFC learned faster a subsequent task involving the same CS. During and after CS-DA pairing, mPFC neurons increased firing in response to CS, and blocking DA receptors in mPFC during learning impaired stimulus discrimination. Furthermore, endogenous DA transients in mPFC showed a gradual shift towards the onset of reward-predicting stimuli during learning, in correlation with anticipatory behavior. Thus, DA transients tune mPFC neurons for the recognition of behaviorally relevant events during learning.

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Poster

180. Reward: Dopamine

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Topic: F.03. Motivation and Emotion

Support: NSF Grant 0953106

Title: Neurochemistry of pair bonding

Authors: *N. NEVÁREZ[1], I. K. WOHL[2], C. W. CARTER[2], C. J. AUSTIN[2], S. L. RESENDEZ[2], B. J. ARAGONA[2];
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Abstract: In the socially monogamous prairie vole (Microtus orchrogaster), activation of D2-like dopamine (DA) receptors results in the development of a selective social attachment, known as a 'pair bond'. However, the specific receptor subtype(s) (i.e. D2 or D3) responsible for this are currently not known. Because of the high density of D2 and D3 receptors within the nucleus accumbens (NAc) shell, we tested the involvement of these receptors in partner preference formation in male prairie voles. Here, we replicate findings showing that quinpirole, a mixed D2/D3 agonist, when infused into the NAc shell, induces a robust partner preference. We then tested whether selective activation of D2 or D3 receptors in the NAcc shell, via infusions of the D2R selective agonist 5,6,7,8-Tetrahydro-6-(2-propenyl)-4H-thiazolo[4,5-d]azepine-2-amine dihydrochloride (BHT-920; 1.5ng, 3ng) or the specific D3R agonist R(+)-2-Dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (7-OH-DPAT; 1.5ng, 3ng), influenced the development of partner preferences. Preliminary data suggest that activation of both receptors is involved in partner preference formation. In addition to its initial regulation of pair bond formation, DA transmission within the NAc shell is also substantially altered in voles with fully formed pair bonds. Specifically, pre-synaptic DA release is robustly enhanced and D1Rs are upregulated. However, nothing is known about the regulation of DA transmission in pair bonded voles. We are, therefore, investigating DA autoregulation and uptake in discrete regions of the NAc shell in bonded and non-pair bonded voles using fast scan cyclic voltammetry. Together, these studies will significantly extend our understanding of DA regulation of prairie vole pair bonding.

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Poster

180. Reward: Dopamine

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Program#/Poster#: 180.24/BB42

Topic: F.03. Motivation and Emotion

Title: Sexual experience results in morphological changes in ventral tegmental area dopamine neurons that project to nucleus accumbens and not medial prefrontal cortex

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Abstract: Sexual experience in male rats causes neural alterations in the nucleus accumbens (NAc) and ventral tegmental area (VTA) that change d-amphetamine and morphine reward. Specifically, sexual experience reduces soma size of VTA dopamine neurons, which is dependent on mu opioid receptor activation in the VTA, is transient, and is essential for long term maintenance of memory for sexual incentive salience. VTA dopamine neurons project to numerous brain areas, including NAc and medial prefrontal cortex (mPFC). However, it is unknown if sexual experience alters dopamine neurons projecting to each of these brain areas. In the current study we tested the hypothesis that sexual experience causes soma size reduction in specific subpopulations of VTA dopamine neurons with projections to the NAc or PFC. Adult male Sprague Dawley rats received unilateral cholera toxin b (Ctb) in NAc and Fluorogold (FG) injections in ventral mPFC, followed by 5 consecutive days of mating (sexually experienced) or handling (sexually naïve). One week later, males were perfused following either mating behavior or exposure to the mating or handling environment. Brains were sectioned, and VTA sections were immunoprocessed for tyrosine hydroxylase (TH), Ctb and FG using triple immunofluorescence. Injection sites for Ctb and FG were verified. Ctb and FG were found throughout the VTA, with approximately 28% of Ctb and 27% of FG cells co-expressing TH. No colocalization of Ctb and FG was detected. Area and perimeter for Ctb/TH+ and FG/TH+ neurons in the VTA were analyzed based on TH immunoreactivity and averaged per animal. Sexually experienced males had decreased cell body area and perimeter in Ctb/TH+ cells (n=5) but not FG/TH+ cells (n=7), compared to sexually naïve rats (n=4, n=7, resp). In addition, VTA sections were stained for mating-induced cFos and Ctb. Animals that received mating or mating-associated cues had a higher number of cFos/Ctb+ neurons, suggesting that NAc-projecting neurons were activated by mating or exposure to mating cues. In conclusion, sexual experience reduced soma size of TH neurons that project to the NAc, but not the ventral mPFC. Since VTA dopamine soma size reduction has been associated with reduction in trafficking and release of dopamine, sexual experience may thus influence dopamine release in the NAc, but not mPFC. The mechanism by which sexual behavior can result in selective effects on NAc-projecting dopamine neurons is unclear but these results suggest that mu opioid receptor activation during sexual reward may preferentially influence NAc projecting neurons in the VTA.

Disclosures: L.N. Beloate: None. **L.M. Coolen:** None.

Poster

180. Reward: Dopamine

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Topic: F.03. Motivation and Emotion

Title: Correlation of conditioned place preference to high fat/sugar food with nucleus accumbens c-Fos and p-DARPP-32 protein expression

Authors: *G. C. LOPEZ, L. PEREZ, T. SONGTACHALERT, L. MININBERG, S. PIERCE, J. SCHROEDER;
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Abstract: We have previously demonstrated that conditioned place preference to high fat/sugar food (Oreos) is comparable to conditioned reward behavior associated with cocaine or morphine. Exposure to drugs of abuse and other rewarding stimuli is associated with nucleus accumbens neuronal activation. The current study was an attempt to correlate reward behavior measured using conditioned place preference with c-Fos and p-DARPP-32 protein expression in the nucleus accumbens. An 8-day biased CPP paradigm was used to assess reward behavior in response to high fat/sugar food (Oreo cookies) consumption. Behavioral assessment was followed by immunohistochemical assessment of c-Fos and p-DARPP-32 expression. Results indicate that the reward behavior associated with consuming Oreos is equivalent to previous studies that measured conditioned place preference to cocaine or morphine. The magnitude of conditioned place preference to cocaine and high fat/sugar food was positively correlated with nucleus accumbens c-Fos expression. Immunohistochemcial evaluation of p-DARPP-32 expression is ongoing. These findings suggest that high fat/sugar foods and drugs of abuse trigger similar behavioral and neurobiological brain addictive processes and lend support to the hypothesis that maladaptive eating behaviors contributing to obesity can be compared to drug addiction.

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Poster

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Topic: F.03. Motivation and Emotion

Support: NIH grant DA022340

Title: Cannabinoid type 1 receptors facilitate conditioned reinforcement evoked by optogenetic stimulation of dopamine release

Authors: *J. F. CHEER[1,2], D. P. COVEY[1];
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Abstract: An ability to predict and exploit one's environment supports the rational and efficient pursuit of rewards and therefore survival. While the goals differ, reward seeking behaviors are all powerfully driven by reward-predicting cues. Mesolimbic dopamine projections from the ventral tegmental area (VTA) to nucleus accumbens (NAc) play a fundamental role in cue-directed reward seeking. Accordingly, dysregulation of this circuit is thought to promote aberrant modes of reinforcement, such as substance abuse and addictions. Alterations in cannabinoid type 1 (CB1) receptor signaling potently regulate conditioned reinforcement, such that increasing or decreasing CB1 signaling potentiates or curtails cue-directed reward seeking, respectively. While this is thought to arise from altered CB1-mediated regulation of VTA to NAc dopamine release, a direct and causal link has not been determined. We first confirmed that VTA-evoked NAc dopamine release supports positive reinforcement by demonstrating that optogenetic activation of channelrhodopsin 2 (ChR2)-expressing dopamine neurons in the VTA of DAT-Cre mice promotes vigorous intracranial self-stimulation (ICSS). Fast-scan cyclic voltammetry recordings demonstrated consistent NAc dopamine release time-locked to dopamine neuron self-stimulation. Additionally, when a predictive cue signaling reward availability was introduced, latency to lever press decreased over consecutive trials while cue-elicited NAc dopamine release increased. Thus, VTA dopamine neuronal firing sufficiently supports NAc dopamine release accompanying reward prediction. Systemic administration of the indirect CB1 receptor agonist JZL-184, which inhibits degradation of the endocannabinoid 2-arachidonoylglycerol (2AG), facilitated cue-directed ICSS as evidenced by a decrease in cue-response latency. Therefore, raising tissue levels of 2AG facilitates behaviors reinforced exclusively by VTA-evoked dopamine release. In separate experiments, we confirmed that blocking CB1 receptors with AM-251 inhibited cue-evoked NAc dopamine release and reward seeking in a food-reinforced task. Importantly, pairing cue presentation with optical stimulation of VTA dopamine neurons reversed the effects CB1 blockade, suggesting that deficits in cue-directed reward seeking arise exclusively from CB1 receptor antagonists suppressing VTA-evoked dopamine release. Collectively, this work confirms and refines current understanding of the canonical role of CB1 receptor signaling in VTA-evoked NAc dopamine release and conditioned reinforcement.

Disclosures: J.F. Cheer: None. **D.P. Covey:** None.

Poster

180. Reward: Dopamine

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Topic: F.03. Motivation and Emotion

Support: DA030676

DA013429

Title: Enhanced extinction of cocaine conditioned place preference via glutamate transporter activation is associated with reduced nucleus accumbens c-Fos expression

Authors: *J. W. PICKEL[1], V. SVYSTUN[1], T. HAVICAN[1], L. MININBERG[1], L. PEREZ[1], S. PIERCE[1], A. DILISIO[1], M. REILLY[1], J. A. SCHROEDER[1], S. M. RAWLS[2];
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Abstract: Ceftriaxone is a β-lactam antibiotic that activates glutamate transporter subtype 1 (GLT-1), Clavulanic acid is a structurally-related β-lactamase inhibitor that retains the β-lactam core required for glutamate transporter activity but displays more patient-friendly characteristics such as enhanced brain penetrability, minimal antibacterial activity, and oral activity. We have previously shown that both drugs attenuate the rewarding aspects of cocaine by enhancing extinction from cocaine conditioned place preference (CPP). C-Fos is a proto-oncogene that is expressed within neurons following depolarization. The current study was an attempt to correlate these behavioral effects with neurochemical changes by evaluating nucleus accumbens c-Fos protein expression. Animals that underwent a 5-day extinction period following cocaine CPP during which ceftriaxone, clavulanic acid or saline was administered daily were challenged with a final cocaine exposure in their drug-paired environment before being euthanized. Brains were sectioned and processed for c-Fos immunohistochemistry. Results indicate that c-Fos expression in the shell and the core of the nucleus accumbens was reduced significantly in animals that received either ceftriaxone or clavulanic acid during extinction from cocaine CPP. These findings suggest that pharmacologic enhancement of glutamate clearance disrupts the *in vivo* actions of cocaine which are correlated with reduced expression of a proto-oncogene marker of neuronal activity.

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Poster

181. Song Circuit and Motor Control

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Title: Analyzing the population dynamics underlying a complex motor act

Authors: *M. A. PICARDO[1,2], K. A. KATLOWITZ[1,2], D. E. OKOBI[1,2], S. E. BENEZRA[1,2], R. C. CLARY[1], J. MEREL[3,4], L. PANINSKI[3,4], M. A. LONG[1,2];
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Abstract: The courtship song of the zebra finch is a well-studied learned complex behavior that has yielded important insights concerning the mechanisms of vertebrate skill learning and motor performance. The forebrain regions that are necessary for singing are clearly defined and several lines of evidence suggest that a single premotor region called HVC (proper name) plays an important role in the production of song. Indeed, early lesion studies demonstrated that this region is necessary for song production, and focal cooling of HVC results in the stretching of the song without affecting its structure suggesting that this region contains the motor program for the song. Electrophysiological recordings during singing reveal that single HVC premotor neurons exhibit a high-frequency firing pattern at a precise and specific moment during the song, but the representation of the song at the network level is still unclear. Optical methods can enable large scale recordings of neuronal circuits and *in vivo* 2-photon imaging combined with fluorescent activity indicators has been used in the anesthetized zebra finch to study auditory responses to song playback (Graber et al., 2013; Peh et al., 2015). Here we introduce a novel head-fixed singing preparation that has enabled us to study the motor representation of singing behavior at an ensemble level within HVC. Projection neurons exhibited calcium transients that reflected the song-related spiking activity previously observed with electrophysiology. To better understand the contribution of individual neurons to this behavior, we developed an analytical approach that

could integrate information across multiple trials to increase our temporal resolution and to more precisely estimate the onset of HVC neurons. We fitted the calcium trace corresponding to each burst as a double-exponential with additive noise that was matched to our electrophysiological calibration curves. We used a Markov chain Monte Carlo inference method, and then we inverted this generative model to estimate the onset times for each trial. In many cases, we were able to establish a posterior uncertainty that was less than the duration of a single song-related burst event (~10 ms), suggesting that this approach can be used to resolve circuit dynamics related to premotor sequences.

Disclosures: M.A. Picardo: None. **K.A. Katlowitz:** None. **D.E. Okobi:** None. **S.E. Benezra:** None. **R.C. Clary:** None. **J. Merel:** None. **L. Paninski:** None. **M.A. Long:** None.

Poster

181. Song Circuit and Motor Control

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Topic: F.04. Neuroethology

Support: NIH Grant NS075044

NIH Grant NS084767

Title: Connectomic analysis of local HVC circuitry in the zebra finch

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Abstract: The zebra finch song is a complex motor sequence with important parallels to human skilled behavior, such as speech and musical performance. Lesions to the cortical nucleus HVC abolish singing behavior, suggesting that this region plays a critical role in the production of song (Nottebohm et al., 1976). Single premotor neurons in HVC exhibit a high frequency burst of action potentials at one time point during singing, and different cells are often active at different moments in the song (Hahnloser et al., 2002; Long et al., 2010; Vallentin and Long, 2015). Cooling HVC slows song speed across all timescales, suggesting that connectivity within HVC is important for generating these premotor sequences (Long and Fee, 2008). Despite

previous electrophysiological studies (Mooney and Prather, 2005; Kosche et al. 2015), we know very little about the local wiring rules within that nucleus, in part because of an almost total lack of knowledge concerning the fine structure of single HVC premotor neurons (Gurney and Katz, 1981; Mooney, 2000). To address this, we conducted an anatomical study of these cells using a strategy that combines light (LM) and electron microscopy (EM) approaches. We filled 15 premotor neurons *in vivo* with single-cell Neurobiotin injections and reconstructed the entirety of these processes throughout the nucleus. We also used serial block-face EM to examine the synaptic properties of labeled premotor neurons within a 150x150x60 μm volume. Together, these reconstructions enabled us to quantify the distribution and identity of presynaptic inputs along the dendrites and to estimate the total number of synapses received by single premotor neurons. To examine the number of postsynaptic targets for each HVC premotor neuron, we quantified bouton distribution along axon collaterals in LM. EM reconstructions were used to assess the extent to which boutons reflect the presence of a synapse. Furthermore, we were able to distinguish the cell identity of many confirmed postsynaptic targets. Because a prominent model of HVC sequence generation relies upon strong feedforward monosynaptic connections between premotor neurons to form a 'synaptic chain' (Long et al., 2010), we were especially interested in examining synapses of this nature. Our approach enabled us to identify a large number of premotor-premotor contacts and to characterize their distribution along various dendritic branches. Taken together, these data will inform computational models of information flow within HVC and help to establish a mechanistic understanding of the circuitry underlying other forebrain sequence generating circuits.

Disclosures: S. Benezra: None. **J. Kornfeld:** None. **R.T. Narayanan:** None. **M. Oberlaender:** None. **W. Denk:** None. **M. Long:** None.

Poster

181. Song Circuit and Motor Control

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Topic: F.04. Neuroethology

Support: NIH 1U01NS090454-01

NIH R01 - NS089679-01

Title: Stability and drift of motor sequencing in the songbird HVC

Authors: *W. A. LIBERTI, III[1], D. C. LIBERTI[2], J. E. MARKOWITZ[1], T. GARDNER[1]; [1]Biol., [2]Ctr. for Regenerative Med. (CReM),, Boston Univ., Boston, MA

Abstract: Nearly all living creatures maintain learned motor skills over long timescales- for days, years or even decades. However, little is known about the mechanistic basis of this stability. Some propose that while motor skills can remain stable for years, the individual neurons controlling them may significantly change their firing properties over the course of hours. Others contend the tuning of individual neurons is as stable as the motor skill itself. Merging these two viewpoints, the central hypothesis of this presentation is that the brain encodes learned behaviors on two distinct levels - a mesoscopic level that is highly stable, and a microscopic level in which single neurons can be influenced by a recent history of reward or punishment. In other words, the stability of a memory is rooted not in single neuron stability, but in the stability of network patterns that persist in spite of drifting individual neuron dynamics. Here, we examine the question of motor coding stability in one of the most stable of all animal behaviors: birdsong. The neural circuits that underlie song are well defined, extensively studied, and in key respects homologous to the cortico-basal ganglia circuits underlying sensory-motor learning in mammals. The extreme precision of vocal behavior in zebra finches, coupled with the long-term stability of song structure, presents a unique opportunity to observe how motor memories are maintained at the network level. A zebra finch will sing the same learned song with great precision for years. Indeed, we observe that both inhibitory interneuron firing patterns and local field potentials in premotor cortical area HVC persist for weeks to months. However, the story appears to be different for at least one class of projection neuron in HVC. Here we use genetically encoded calcium indicators and miniature head-mounted microscopes, to longitudinally track the activity of HVC projection neurons in the freely behaving zebra finch. In contrast to stable LFPs and inhibitory neuron activity, preliminary data indicates that individual projection neurons in HVC can shift their tuning properties over time-scales of days. We discuss these results in light of various models for the maintenance of stable motor actions.

Disclosures: W.A. Liberti: None. **D.C. Liberti:** None. **J.E. Markowitz:** None. **T. Gardner:** None.

Poster

181. Song Circuit and Motor Control

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Program#/Poster#: 181.04/BB49

Topic: F.04. Neuroethology

Support: HHF 23103

Title: Left and right HVC independently control song respiratory amplitude

Authors: *C. M. URBANO[1], B. G. COOPER[2];
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Abstract: Birdsong is a learned vocal behavior, like human speech, that requires formation of long-term auditory memory and precise coordination between the respiratory and syringeal (vocal organ) motor systems to reproduce learned song. In songbirds, HVC (proper name) is critically important for auditory-motor integration. Bilateral HVC ablation in Waterslager canaries (*Serinus canaria*) leads to a complete loss sound production, yet males still assume a singing posture. Subsyringeal air sac pressure measures the combined effects of respiratory muscle activity and syringeal gating of airflow. The respiratory pattern of subsongs produced by in juvenile zebra finches (*Taeniopygia guttata*) have demonstrated the importance of coordinated motor output and suggest that acoustic characteristics of song are refined by improving respiratory and syringeal motor coordination. This refinement can be disrupted in juvenile songbirds through HVC inactivation, which causes young birds to return to producing subsong. In a juvenile zebra finch housed adjacent to a live tutor, we recorded air sac pressure during the development of the bird's song (phd 65-85). In an adult zebra finch, we temporarily inactivated HVC using reverse dialysis of tetrodotoxin and recorded changes in subsyringeal air sac pressure. These replications of previous work (Aronov, et al., 2008; Veit, et al., 2011) confirm that the respiratory pattern produced by juveniles is similar to adult song during HVC inactivation. These results are consistent with the view that HVC enables the coordination of respiratory and syringeal gating of airflow during song. To further study the role of HVC in regulating air pressure during song, we used electrolytic lesions to perform a unilateral, followed by bilateral, HVC ablation. We studied Bengalese finches (*Lonchura striata domestica*) because sound production in Bengalese finches is lateralized at the level of the syrinx but respiratory activity is symmetrical. In three male Bengalese finches, bilateral lesion electrodes were implanted in HVC. Air sac pressure and song were recorded, and then either left (n=2) or right (n=1) electrolytic HVC lesion was performed. The resultant changes in air pressure were measured after each lesion. We found that the sequential lesion procedure resulted in an approximately linear decline in air sac pressure amplitude of song syllables ($p = 0.01$) and strongly suggests that the two HVC nuclei independently contribute to song respiratory control. The emerging view from this research is that HVC is critical for generating the driving air pressure amplitude necessary for phonation via coordinating respiratory and syringeal motor systems.

Disclosures: C.M. Urbano: None. **B.G. Cooper:** 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.; HHF 23103, Hearing Health Foundation.

Poster

181. Song Circuit and Motor Control

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 181.05/BB50

Topic: F.04. Neuroethology

Support: NSF

Title: A computational model that can reproduce the effects of partial HVC ablation on the zebra finch song

Authors: *D. GALVIS[1], D. FLORES[1], M. BASISTA[2,3], W. WU[3,4], R. HYSON[2,3], F. JOHNSON[2,3], R. BERTRAM[1,3];
[1]Mathematics Dept., [2]Psychology Dept., [3]Program in Neurosci., [4]Statistics Dept., Florida State Univ., Tallahassee, FL

Abstract: The temporal ordering of acoustic elements in zebra finch song is controlled by the pre-motor nucleus HVC. Recently, we have shown that bilateral ablation or silencing (TTX) of distinct regions in HVC have differing effects on adult song. Lateral HVC ablation results in syllable omission, but the ordering of the remaining syllables is consistent with the song prior to ablation. Medial HVC ablation, on the other hand, results in misordered song sequences interspersed with bouts of the normal sequence. We present a computational neural network model that can account for these ablation effects. In this model, the "medial" neural population determines note sequence and consists of co-existing chains of neurons coupled by inhibition so that not all chains can fire. The "lateral" neural population is divided into subpopulations that must fire for a specific syllable to be produced. Output from the two populations comes together at a layer of RA (robust nucleus of the arcopallium) neurons. Medial ablation, modeled as the removal of a subset of the medial chains, reduces the inhibition on other chains, allowing them to fire. Lateral ablation, modeled as a reduction in the number of syllable-specific neurons, effectively eliminates the RA firing pattern representative of a specific syllable, while having no effect on the ordering of other syllables. While this model is preliminary, it demonstrates one possible network structure that can account for general features of the ablation data, and uncovers challenges in constructing networks that are consistent with the data.

Disclosures: D. Galvis: None. **D. Flores:** None. **M. Basista:** None. **W. Wu:** None. **R. Hyson:** None. **F. Johnson:** None. **R. Bertram:** None.

Poster

181. Song Circuit and Motor Control

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Topic: F.04. Neuroethology

Support: NSF Grant IOS 1146607

Title: Using dynamic clamp to explore the current properties of HVC neurons in the zebra finch

Authors: *D. FLORES, M. T. ROSS, J. TABAK, R. L. HYSON, F. JOHNSON, R. BERTRAM;
Florida State Univ., Tallahassee, FL

Abstract: The dynamic clamp technique has become a widely used tool for the study of neural systems at the cellular and circuit levels. One of the many applications of this technique is to evaluate the influence of various ionic currents by injecting channel conductances into neurons that are based on mathematical models (Prinz et al., 2004). From previous current clamp experiments, we developed conductance-based models for the different neurons in the HVC (Daou et al., 2013), an area that is known to have a central role in controlling the remarkable temporal structure of birdsong (Long et al., 2010). Using our mathematical models and a pharmacological approach we were able to determine the contribution of the Ih and ICa-T currents in the production of the sag and the rebound firing respectively, features that are present in the interneurons and X-projecting neurons. Also, we found that the IA current is responsible for the long delay to spiking in one of the two RA-projecting neuron types (Daou et al., 2013). Here, we use dynamic clamp to manipulate these three specific ionic currents in the living cell in real-time without the use of any drug. Subtracting these currents confirmed their contribution to the firing patterns and the accuracy of our models. Unlike the purely subtractive effects of pharmacological manipulations, dynamic clamp also gives us the ability to add currents to neurons to test hypotheses about their contributions to the firing pattern. For example, adding T-type Ca2+ current to an RA-projection neuron produced rebound action potentials following a hyperpolarizing current pulse. This behavior is not typically seen in these neurons. Dynamic clamp also allowed us to test more subtle aspects of our models, such as the effects of varying the activation and inactivation time constants of the currents. This combined approach of modeling and hypothesis-testing using dynamic clamp is leading to a more detailed understanding of the biophysical properties of HVC neurons, overcoming the limitations of pharmacological manipulations.

Disclosures: **D. Flores:** None. **M.T. Ross:** None. **J. Tabak:** None. **R.L. Hyson:** None. **F. Johnson:** None. **R. Bertram:** None.

Poster

181. Song Circuit and Motor Control

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Topic: F.04. Neuroethology

Support: 1U01NS090454-01

R01 - NS089679-01

Title: Chronical electrophysiological recordings using carbon fiber microarrays in a songbird premotor brain region

Authors: *S. MOORMAN, T. J. GARDNER;
Biol., Boston Univ., Boston, MA

Abstract: Many studies, conducted in a range of different species, indicate that motor skills improve when the subjects are allowed to sleep after learning the task. However, the mechanisms that underlie this effect remain largely unclear. To investigate the role of sleep in motor performance, activity patterns need to be tracked in the same group of cells across several days and nights. Prevailing electrophysiological methods cannot track the same neuron over a timescale of several days or longer in freely behaving animals. The major limitation of chronic recording is an immune response that encapsulates conventional electrodes and kills nearby neurons or attenuates their electrical signals. The problem is particularly acute for small, densely packed neurons found in small animals such as mice and birds. Here, we use minimally invasive electrode arrays that were recently developed in the Gardner lab to record neural activity in singing zebra finches over long timescales. The arrays consist of 5 μm diameter, flexible carbon fibers arranged in a bundle, such that they splay apart when inserted in the brain. Using these arrays, we were able to stably record from single neurons in a premotor brain region of freely behaving adult male zebra finches across multiple sleep wake-cycles.

Disclosures: S. Moorman: None. **T.J. Gardner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Circuit Formation, Inc.

Poster

181. Song Circuit and Motor Control

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Topic: F.04. Neuroethology

Support: NSF grant IOS -1146607

Title: A role for the Ih current in the production of song in zebra finches

Authors: *M. T. ROSS, D. FLORES, R. BERTRAM, F. JOHNSON, R. L. HYSON; Neurosci., Florida State Univ., Tallahassee, FL

Abstract: The I_h current is important in many neuronal systems requiring precise timing of firing. We have previously shown the existence of the I_h current in nucleus HVC (proper name) of adult zebra finches (Daou et al. 2013). The HVC is critical in the learning and production of vocalization as it is thought to be responsible for the timing of the vocal pattern (Roberts et al. 2012, Long et al. 2010). The I_h current has a major role in shaping the physiology of the three main classes of HVC neurons. Here, we use *in vitro* patch clamp electrophysiology to record from cells in the HVC across stages of vocal development and use our biophysical models to identify changes in a variety of ionic currents. We see an increase in the rectifying response to hyperpolarizing currents as the bird ages, implicating a possible role of the I_h current in the development of vocal behavior. Interestingly, the interneurons have adult-like physiology earlier than the projection neurons but show increased variability in the magnitude of the rectifying response across cells in earlier stages of development. Given that the changes in I_h seemed to be linked to the development of the singing behavior we then explored the role the I_h current has in the production of song in the adult bird by infusing the I_h channel blocker, ZD7288, into selective subregions of HVC in awake singing animals and monitoring singing behavior. The application of the drug either selectively deleted notes or altered note timing depending on the location of infusion. The magnitude of the effects gradually decay throughout the day as the drug's effect weakens. Together, the results suggest that changes in I_h across development may represent a form of nonsynaptic plasticity that is required for the emergence of the adult song pattern. The results allow for a greater understanding of the interplay between cell physiology and the process of learning.

Disclosures: **M.T. Ross:** None. **D. Flores:** None. **R. Bertram:** None. **F. Johnson:** None. **R.L. Hyson:** None.

Poster

181. Song Circuit and Motor Control

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Program#/Poster#: 181.09/BB54

Topic: F.04. Neuroethology

Support: NSF Grant IOS 1146607

Title: Encoding and integration of birdsong syllable repertoire and sequencing

Authors: *M. J. BASISTA[1], M. T. ROSS[1], W. WU[2], R. BERTRAM[3], R. L. HYSON[1], F. JOHNSON[1];
[1]Psychology, [2]Statistics, [3]Math, Florida State Univ., Tallahassee, FL

Abstract: Many motor actions that animals perform consist of a learned series of movements. How the brain generates these movements and imposes a sequence on them is one of the fundamental unanswered questions of neuroscience. Recent ablation studies in the zebra finch have shown that the avian premotor nucleus HVC (proper name) can be divided along at least two anatomically adjacent and functionally parallel pathways, one that encodes syllable repertoire and another that encodes syllable sequence. It was also found that these pathways converge at the avian vocal-motor cortex, the robust nucleus of the arcopallium (RA). To further explore this parallel organization we developed a surgical technique to bilaterally target infusion cannulae to each parallel pathway within HVC. By systematically varying cannula position between birds as well as the volume of tetrodotoxin (TTX) infused within birds we will test how vocal elements in the repertoire- and sequence-encoding regions of HVC are mapped. Preliminary results demonstrate the reversibility of TTX infusion effects (normal song within hours) and replicate previous ablation findings - inactivation of the repertoire-encoding portion of HVC results in wholesale omission of syllables while inactivation of the sequence-encoding portion of HVC elicits atypical transitions between syllables. Additionally, the physiology of cells within the two parallel pathways is being investigated using patch-clamp recordings. Current experiments are aimed at determining how the outputs from the parallel pathways of HVC interact at the level of RA.

Disclosures: **M.J. Basista:** None. **M.T. Ross:** None. **W. Wu:** None. **R. Bertram:** None. **R.L. Hyson:** None. **F. Johnson:** None.

Poster

181. Song Circuit and Motor Control

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Topic: F.04. Neuroethology

Support: Leiden Institute for Brain and Cognition

Title: Zebra finch song phonology and structure across populations and continents - a computational comparison

Authors: *S. M. TER HAAR[1,2,3,4], R. LACHLAN[5], C. A. A. VAN HEIJNINGEN[2,3], C. TEN CATE, senior[3,2];
[1]Utrecht Univ., Utrecht, Netherlands; [2]Leiden Inst. for Brain and Cognition, [3]Behavioural Biol., [4]Leiden Univ. Ctr. for Linguistics, Leiden Univ., Leiden, Netherlands; [5]The Sch. of Biol. and Chem. Sci., Queen Mary Univ. of London, London, United Kingdom

Abstract: Birdsong is a widely used model for behavioral and neural research on vocal learning. Learned bird songs are often characterized by variation between individuals and sometimes populations, while at the same time maintaining species specificity. The evolution of such songs will depend on the balance between plasticity and constraints. Captive populations provide an opportunity to examine signal variation and differentiation in detail. Zebra finches (*Taeniopygia guttata*) are commonly used by many songbird scientists as a model species. Surprisingly, only few attempts have been made to quantitatively study the variation of sounds across populations. Here, we analyzed adult male zebra finch songs recorded from 13 populations across the world, including one sample of songs from wild-caught males in their native Australia. Cluster analyses were performed on both element and syllable level. Element analysis suggests that zebra finch song units broadly belong to universal categories, probably linked to constraints in vocal production and non-song parts of the vocal repertoire. The strongest clustering indicates two universal clusters: high frequency element versus all other elements. There is also slightly weaker evidence for element clustering at 10 element types and 7 syllables types. Across populations, songs also show some similarity in syntactical structure; although any song unit can be placed anywhere within the song, some combinations are more likely to occur than others. Differentiation between populations is statistically significant, but the effect size is very small, and its communicative significance dubious. Probably, duration of isolation for each population is not enough for strong divergence in constraints. In addition to duration of isolation, the weak cultural divergence in zebra finches might be explained by relatively weak song imitation, creating strong within population variation. Zebra finches therefore serve as an example of a system where frequent learning errors may rapidly create within-population diversity, within broad phonological and syntactical constraints. This may prevent the formation of long-term cultural traditions that allow populations to diverge.

Disclosures: **S.M. Ter Haar:** None. **R. Lachlan:** None. **C.A.A. van Heijningen:** None. **C. ten Cate:** None.

Poster

181. Song Circuit and Motor Control

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Topic: F.04. Neuroethology

Support: ONR MURI (N00014-12-1-0850)

Title: Factors determining the degeneracy of the relationship between the parameters and dynamics of conductance model neurons

Authors: ***P. MALONIS**[1], A. DAOU[1], N. KADAKIA[2], U. MORONE[2], H. ABARBANEL[2,3], D. MARGOLIASH[1];
[1]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [2]Physics, Univ. of California at San Diego, La Jolla, CA; [3]Scripps Inst. of Oceanography, La Jolla, CA

Abstract: Conductance models are powerful tools for qualitatively characterizing the mechanisms of electrical activity in single neurons. However, using these models to estimate biological parameters is complicated by the fact that a range of parameters can appear to reproduce the measured behavior of a neuron. Here, we investigate the degree to which this degeneracy in the relationship between model parameters and model fit is an artifact of the method used to compare the model output to recorded biological activity, as well as the type of activity that is compared. We are modeling the responses to somatic current injection in whole-cell voltage patch recordings of Area-X projecting neurons in a brain slice preparation of the forebrain nucleus HVC of zebra finches (see Daou, Malonis, Margoliash abstr). In that study, the magnitude of the principle intrinsic ionic conductances in a previously derived, pharmacologically verified Hodgkin-Huxley model of HVC(X) (see Daou et al., J.Neurophys, 2013) were manually fit by one of us (AD), while holding all other parameters at fixed, biologically plausible values determined from the literature. This resulted in excellent fit to the neuron's response to the injected current, and - importantly - excellent prediction of other injected currents, both step functions and chaotic currents. It is of biological importance to determine whether the maximal conductances for each neuron, and observed similar conductances for some sets of neurons, arise because the manually-derived estimates are indeed near to global best fit to the data. Our strategy for addressing this question involves using a comparison function that takes into account spike timing, subthreshold activity, and several

features of the spike shape. We are investigating the sensitivity of model fit to the features of the comparison function. In order to identify best-fitting parameter sets we employ variational data assimilation methods. Moreover, since the number of principal ionic conductances being varied is small (5), we are able to visualize the entire error function using brute-force searches on a supercomputer. Using these approaches, we are also investigating techniques to eliminate spurious minima of the error function that yield good fits but poor predictions. The results will enhance the ability to use conductance models to make predictions and characterize physiological differences between different classes of neurons.

Disclosures: **P. Malonis:** None. **A. Daou:** None. **N. Kadakia:** None. **U. Morone:** None. **H. abarbanel:** None. **D. Margoliash:** None.

Poster

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Topic: F.04. Neuroethology

Support: NSF DGE0903637

NIH R01DC012859

Title: Premotor population dynamics predict muscle activity in zebra finch song

Authors: ***K. J. BROWN**[1], D. MARGOLIASH[1,2];
[1]Committee on Computat. Neurosci., [2]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Song control requires rapid and precise transitions in motor state, which has been termed vocal gestures. Gesture transitions occur non-uniformly and are necessarily unique for each song. We hypothesize that the timing of gesture transitions is causally related to song production, and therefore the encoding of these times is over-represented in the activity of neurons in cortical areas such as sensorimotor area HVC. Here, we are studying singing in zebra finches. We are using a mathematical model of vocal production, which creates artificial song using physiological parameters pressure and tension. By inverting the model, we can predict pressure and tension from recorded birdsong. Previously, gesture transitions identified using this model were found to predict the timing of activity in HVC. While some gesture transitions are clear from the acoustic features of recorded song as assessed by spectrographic analysis, such as

onsets and offsets of phonation, other gesture transitions may be less apparent. In this work, we record from the syringeal (vocal) muscles directly to 1) evaluate and extend the model of vocal production and 2) provide a model-independent test of HVC gesture transition coding by simultaneously recording from HVC and the vocal muscles. In sleeping birds, song is spontaneously replayed throughout the motor system, from bursting in HVC to activation of the vocal muscles. We find that multi-unit population activity collected with silicon arrays from sleeping birds in HVC is correlated with ventral syringeal muscle (vS) EMG activity with short latency, which is consistent with state-dependent coding in HVC (μ=18.5ms, σ=8ms, units=150). Summed multi-unit activity across recording shanks was significantly correlated with sleeping spontaneous muscle activity, with a mean Pearson's R value of 0.17 (σ=0.2, N=24, $p<.001$). Our current data is likely to be primarily interneurons, as our recorded neurons tend to have a high spontaneous firing rate. We believe this correlation should exist in downstream projecting neurons due to the strong interactions between the interneuron and projection neuron populations. These results are consistent with HVC encoding the timing of gesture transitions in the syringeal muscles. While this data has been collected during sleep, we are currently testing these results in singing birds.

Disclosures: K.J. Brown: None. **D. Margoliash:** None.

Poster

181. Song Circuit and Motor Control

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Topic: F.04. Neuroethology

Support: TCU RCAF 60710

Hearing Health Foundation 2301

Title: A comparison of vocal organ morphology between Bengalese finches and white-rumped munias

Authors: C. M. URBANO[1], K. OKANOYA[2], *B. G. COOPER[1];
[1]Psychology, Texas Christian Univ., Fort Worth, TX; [2]Univ. of Tokyo, Tokyo, Japan

Abstract: Song learning and sound production in oscine birds is constrained by the central nervous system and the anatomy of the vocal organ (syrinx). Fundamental frequency is controlled by the vibratory characteristics of the sound generating tissues in the syrinx, and the

left and right sides of the oscine vocal organ differentially contribute to the fundamental frequency range. Across several songbird species, the morphologic asymmetry in the size and number of cellular layers of the vibratory tissues is a significant predictor of fundamental frequency range. We sought to explore the role of peripheral versus neural constraints in song learning in Bengalese finches (*Lonchura striata domestica*) because cross-fostering studies have revealed that white-rumped munias (*Lonchura striata*) are less likely to imitate higher frequency, tonal syllables sung by Bengalese finch tutors, whereas Bengalese finches are able to imitate the syllable phonology of adult munia tutors. We predicted that if peripheral constraints restrict learning acoustic features of song, then Bengalese finches should have a greater degree of morphological vocal tissue asymmetry compared to white-rumped munias. Whereas, if neural constraints limit song learning, then munias should show a similar morphologic asymmetry in their vocal tissues as compared to Bengalese finches. We measured the surface area and volume of syringeal structures relevant to sound production (labial tissue, bronchial cartilage, muscle) in both species. We found that the right lateral labium in both Bengalese finches (n=6) and white-rumped munias (n=4) is at least twice as large as the left (BF: $p < 0.001$; WRM: $p < 0.05$, paired t-test). Bronchial semi-rings, A1 and A2, are also larger in the right sound source for the Bengalese finch, but not in white-rumped munias. The right medial labium approached a significant surface area difference compared to the left ($p = 0.06$), in white-rumped munias; no differences in medial labia length in Bengalese finches were found. Muscle volume and area were not lateralized in either species. These results suggest that white-rumped munias do not have a significant anatomic constraint that limits the copying of acoustic features of the song. Instead, these data are consistent with the interpretation that neural constraints limit the acquisition of tonal song syllables in white-rumped munias.

Disclosures: **C.M. Urbano:** None. **K. Okanoya:** None. **B.G. Cooper:** 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.; 60710, RCAF.

Poster

181. Song Circuit and Motor Control

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 181.14/BB59

Topic: F.04. Neuroethology

Support: FNU grant Sapere Aude 2

Title: Modulation Dynamics of the Syringeal Skeleton *in vitro* and *ex vivo*

Authors: D. N. DÜRING[1], *C. P. ELEMANS[2];
[1]Biol., Univ. of Southern Denmark, Odense, Denmark; [2]Univ. of Southern Denmark, Odense M, Denmark

Abstract: Birdsong is an important neuroethological model system for understanding learned vocal behavior. Although many components of the involved neural circuitry have been identified, we lack mechanistic insights into the function of the motor circuits that generate song. The small size and inaccessibility of the avian vocal organ, the syrinx, make it difficult to investigate how its structure relates to function in freely singing birds. We currently lack biomechanical insights how the musculature actuates the syringeal skeleton and sound producing structures to generate vocalizations. Here we use combined *in vitro* (excised) and *ex vivo* (perfused organ) paradigms to study motor control of the syringeal skeleton and sound producing structures. The setup enables high-speed imaging of labial and skeletal motion under controlled pressure conditions combined with direct actuation of muscle attachment sites or muscle stimulation. These data allow for the quantification of 3D motion of syringeal elements, estimates of the forces required for modulating syringeal elements, and effects of individual muscle recruitment on mechanical properties of sound producing structures.

Disclosures: D.N. Düring: None. **C.P. Elemans:** None.

Poster

181. Song Circuit and Motor Control

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 181.15/BB60

Topic: F.04. Neuroethology

Title: Small perturbation of subglottal pressure affect call types differently

Authors: *T. RIEDE;
Midwestern Univ., Glendale, AZ

Abstract: The coordination between breathing and motor control of a sound source is critical for normal vocal production. In most mammals it is the fine-tuned integration between sufficient lung pressure, appropriate glottal opening and vocal fold tension which produces different vocal types. Studies in laboratory rats had demonstrated flexibility in the laryngeal-respiratory motor coordination producing ultrasonic calls. In order to elucidate the source of this flexibility, I tested

if small pertubations of subglottal pressure affect call production. Awake and spontaneously vocalizing male Sprague-Dawley rats were tested while interacting with an estrous female. Subglottal pressure was measured under normal and under experimental conditions during which subglottal pressure was raised by 0.5 kPa by injecting a small amount of compressed air into the trachea through a custom-built implant. One calltype (22 kHz calls) was associated with a large increase in call duration when the subglottal pressure was raised. This effect was not observed or only mildly present in another calltype (50kHz calls). The experimental manipulation of lung pressure did not cause an earlier inspiratory termination but an expiratory prolongation. The call-type specific response could be caused by different laryngeal-respiratory control mechanisms paralleling different brain areas implicated in the generation of the two call types.

Disclosures: T. Riede: None.

Poster

182. Genetic Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 182.01/BB61

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Creation of a neuroscience toolbox: neuron-specific Cre and conditional fluorescent protein and opsin rat lines

Authors: *Z. LIU, G. ZHAO, X. CUI;
Horizon Discovery, Saint Louis, MO

Abstract: The Cre-LoxP system has been widely applied in the mouse, providing spatiotemporal control of gene expression. However, its adoption in the rat has been hindered by technical challenges until the recent development of nuclease-mediated genome editing technologies, such as zinc finger nucleases and CRISPR/Cas9, which allow site-specific insertion of Cre expression cassette and loxP sites in rat genome (Brown et al., 2013). We have previously created two dopaminergic neuron-specific Cre lines, tyrosine hydroxylase-Cre (Th-cre) and dopamine active transporter-Cre (DAT-Cre), by inserting an IRES-Cre cassette directly in front of the stop codon of Th and DAT gene, respectively, with zinc finger nuclease technology and shown that the expression of Cre recombinase reflects that of the endogenous Th and DAT gene. Here we report that by employing CRISPR/Cas9 technology, we have created additional neuron-specific rat Cre lines, including Tph2-Cre (Tryptophan hydroxylase 2, serotonergic neurons), Sst-Cre (Somatostatin, somatostatin-expressing neurons), Slc32a1-Cre (VGAT or VIAAT, GABAergic neurons), Calb2-Cre (Calbindin 2 or calretinin, calbindin 2-expressing interneurons), VIP-Cre

(Vasoactive intestinal polypeptide, VIP-expressing GABAergic interneurons) and HTR3A-Cre (5-hydroxytryptamine receptor 3A, serotonergic neurons), CamK2a-Cre (Calcium/Calmodulin-dependent protein kinase II alpha), Pvalb-Cre (Parvalbumin, GABAergic interneurons). Furthermore, we created a fluorescent reporter line and two conditional opsin lines, where the expression of the reporter or opsins relies on the excision of an upstream floxed stop sequence by Cre. We will report the preliminary characterization of these lines as well. We believe these rat lines will not only provide the research community useful tools but also encourage researchers to join force to make more Cre rats.

Disclosures: **Z. Liu:** A. Employment/Salary (full or part-time):; Horizon Discovery. **G. Zhao:** A. Employment/Salary (full or part-time):; Horizon Discovery. **X. Cui:** A. Employment/Salary (full or part-time):; Horizon Discovery.

Poster

182. Genetic Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 182.02/BB62

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Neurological Foundation of NZ

Health Research Council of NZ

Title: Neuroprotective efficacy of a calpain-dependent gene regulation system expressing X-linked Inhibitor of Apoptosis Protein in a model of Parkinson's disease

Authors: *J. NAIDOO, D. FONG, A. MURAVLEV, D. YOUNG;
Univ. of Auckland, Auckland, New Zealand

Abstract: Unregulated gene expression is a key issue limiting clinical translation of promising gene therapies. We have developed a novel bicistronic autoregulatory gene expression system that relies on cell stress-induced activation of calpain to drive transgene expression. We have compared the therapeutic efficacy of constitutive expression of the anti-apoptotic gene X-linked Inhibitor of Apoptosis Protein (XIAP) under the control of the chicken beta-actin/CMV hybrid promoter (CBA) to XIAP expression under the control of our regulatory sytem in a neurotoxin based model of Parkinson's disease. Regulated plasmids (Calpain-XIAP, a control Calpain-GFP) and constitutive plasmids (CBA-XIAP and CBA-GFP) were packaged into AAV vectors and injected into the SNc of male Sprague Dawley rats (200-300g). Subgroups of rats (n=6 per vector

per treatment) received a unilateral intrastriatal injection of 20µg 6-hydroxydopamine (6-OHDA) to induce calpain activation or PBS 3 weeks later. Amphetamine-induced rotation and cylinder testing were performed 4 and 8 weeks later followed by euthanasia and removal of brains for immunohistochemical (IHC) analysis. 6-OHDA lesioned animals did not display a significant difference in mean ipsilateral rotational bias or forepaw preference across treatment groups. Lower transgene expression levels were observed in Calpain-XIAP and Calpain-GFP rats compared with CBA-XIAP and CBA-GFP rats, as assessed by IHC against a C-terminal myc tag on XIAP and GFP, respectively. Unbiased stereological counting of tyrosine hydroxylase immunoreactive cells (TH-ir) spaced 240µm apart revealed that compared to the contralateral hemisphere, rats injected with Calpain-XIAP vector had 50.4% TH-ir cells remaining compared with only 18.8, 29.6 and 27.0% in rats injected with the CBA-GFP, CBA-XIAP and Calpain-GFP vectors, respectively ($P<0.05$). No significant differences in TH cell number were observed between vector treatments in PBS rats. Immunostaining for the neuronal marker, HuC/D reflected the extent of cell loss. Calpain-XIAP rats displayed less of an inflammatory response following 6-OHDA challenge compared to CBA-GFP, CBA-XIAP and Calpain-GFP rats, as assessed by changes in glial fibrillary acidic protein and CD11b expression, indicators of astrogliosis and microglial activation, respectively. Our analyses suggest that expressing XIAP under the control of our calpain-regulated system provides superior protection of cell bodies and reduces inflammation in comparison to constitutive transgene expression in the 6-OHDA model of Parkinson's disease. Supported by Marsden Fund, NZ HRC, NZ Neurological Foundation

Disclosures: **J. Naidoo:** None. **D. Fong:** None. **A. Muravlev:** None. **D. Young:** None.

Poster

182. Genetic Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 182.03/BB63

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R01NS079268

Title: Step-function luminopsin for prolonged activation of neurons by bioluminescence

Authors: *K. BERGLUND[1], C.-A. GUTEKUNST[2], J. TUNG[2], U. HOCHGESCHWENDER[3], R. E. GROSS[2];
[1]Neurosurg. and Anesthesiol., [2]Neurosurg., Emory Univ., Atlanta, GA; [3]Central Michigan Univ., Mt Pleasant, MI

Abstract: Over the past decade, optical tools for controlling neuronal activity have vastly expanded. However, there are still unmet needs which require development and refinement of optogenetic probes. For example, light delivery into the brain is still a major practical challenge that hinders potential translation of optogenetics in human patients. In addition, it would be advantageous to manipulate neuronal activity acutely and precisely as well as chronically and non-invasively, using the same genetic construct in animal models. We have addressed these challenges by employing bioluminescence as a light source for optogenetic activation. Luminopsin was created by fusing a light-sensing channelrhodopsin with a light-emitting *Gaussia* luciferase (*PLoS ONE* e59759). To expand applications of luminopsins, we incorporated *Chlamydomonas* channelrhodopsin 2 with step-function mutations (SFLMO). Bioluminescence-induced depolarization in transfected neurons in culture lasted longer than the bioluminescence signal due to very slow inactivation of the channel. In addition, bioluminescence was able to activate almost all the channels on the cell surface due to extremely high sensitivity of the channel to light. As a result, SFLMO unilaterally transduced by a viral vector into neurons of the substantia nigra was able to induce circling behaviors in rats *in vivo* upon intravenous injection of the luciferase substrate. Thus, step-function luminopsin expands the current approaches for manipulation of neuronal activity in the brain and adds more versatility and practicality of optogenetics in freely behaving animals.

Disclosures: K. Berglund: None. **C. Gutekunst:** None. **J. Tung:** None. **U. Hochgeschwender:** None. **R.E. Gross:** None.

Poster

182. Genetic Techniques

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Program#/Poster#: 182.04/BB64

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant MH101525

NSF Grant 1464686

Research Incubator Award Duke Institute for Brain Science

Title: Luminopsins allow neuronal activation over a range of spatial and temporal scales

Authors: L. WEN[1], S. PARK[1], K. A. CLISSOLD[2], K. BERGLUND[3], H. H. YIN[2], G. J. AUGUSTINE[1], *U. HOCHGESCHWENDER[4];

[1]Lee Kong Chian Sch. of Med., Nanyang Technological Univ., Singapore, Singapore; [2]Duke Univ., Durham, NC; [3]Emory Univ., Atlanta, GA; [4]Neurosci., Central Michigan Univ., Mt Pleasant, MI

Abstract: Luminopsins, LMOs, are fusions of a luciferase, which emits biological light in the presence of a small molecule substrate, and an optogenetic actuator, which translates the light into neuronal activation or silencing (PLoS ONE 8: e59759). LMOs take advantage of the superior versatility of opsins as current conductors and preserve their functionality, while adding chemical genetic access to the entire arsenal of optogenetic elements. By matching the light source - physical or biological - to the experimental question, LMOs allow interrogation of neuronal circuits at different temporal and spatial resolutions in the same experimental animal. Here we applied this approach to analysis of mouse neurons and circuits. An AAV vector containing a human Synapsin promoter-driven Gaussia luciferase fused to Volvox channelrhodopsin 1 (VChR1) was unilaterally injected into the substantia nigra pars reticulata (SNr). The luciferase substrate coelenterazine (CTZ) was injected peripherally into a tail vein. Activation of SNr neurons by bioluminescence caused mice to make ipsilateral turns, consistent with the expected motor effects of unilateral activation of the SNr. Using whole-cell patch clamp in brain slice preparations we recorded responses in individual cells to activation of afferents of these neurons. Small regions were illuminated by a laser light spot in a random fashion to activate local excitatory synapses onto the neurons, assembling a photostimulation map (PNAS 104: 8143). These experiments demonstrate the flexibility of using LMOs to interrogate neuronal circuits by complementing behavioral testing at the macrocircuit level with photostimulation mapping at the local microcircuit level.

Disclosures: **L. Wen:** None. **S. Park:** None. **K.A. Clissold:** None. **K. Berglund:** None. **H.H. Yin:** None. **G.J. Augustine:** None. **U. Hochgeschwender:** None.

Poster

182. Genetic Techniques

Location: Hall A

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Program#/Poster#: 182.05/BB65

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH R01 NS079268

NIH R01 NS079757

NIH NRSA NS086433

Title: Inhibitory luminopsins: genetically encoded bioluminescent opsins for versatile, scalable, and hardware independent optogenetic inhibition

Authors: *J. K. TUNG[1,2], C.-A. GUTEKUNST[2], K. BERGLUND[2], R. E. GROSS[2,1];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Emory Univ., Atlanta, GA

Abstract: Optogenetic techniques have become mainstream in the neuroscience community because they offer scientists an unprecedented ability to precisely manipulate neural activity in the context of complex neural circuitry. Although the toolbox of optogenetic probes continues to expand at a rapid pace with more efficient and responsive reagents, light delivery into the brain is still a major practical challenge that needs to be addressed. We have bypassed the challenges of external light delivery by directly coupling a bioluminescent light source (a genetically encoded Renilla luciferase) to an inhibitory opsin (Natronomonas halorhodopsin) as a single fusion protein, which we term an inhibitory luminopsin (iLMO). iLMO was shown to suppress action potential firing and synchronous bursting activity *in vitro* in response to both physical light and luciferase substrate. iLMO was further shown to suppress single-unit firing rate and local field potentials in the hippocampus of anesthetized animals. Finally, expression of iLMO was scaled up to multiple structures of the basal ganglia to modulate rotational behavior of freely moving animals in a hardware-independent fashion. This novel class of optogenetic probes demonstrates how conventional opsins can be converted to luminopsins to achieve non-invasive inhibition of neural activity, adding to the versatility, scalability, and practicality of optogenetic applications in freely behaving animals.

Disclosures: **J.K. Tung:** None. **C. Gutekunst:** None. **K. Berglund:** None. **R.E. Gross:** None.

Poster

182. Genetic Techniques

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Program#/Poster#: 182.06/BB66

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant

Michael J Fox Foundation Grant

Brain Research Foundation Grant

Title: Multi-timescale *in vivo* regulation of the thalamic reticular nucleus using bioluminescent optogenetics (BL-OG)

Authors: *B. HIGASHIKUBO[1], E. MCDONNELL[2], U. HOCHGESCHWENDER[3], C. MOORE[2];
[1]Moore Lab, Brown Univ., Providence, RI; [2]Neurosci., Brown Univ., Providence, RI; [3]Neurosci., Central Michigan Univ., Mt Pleasant, MI

Abstract: The thalamic reticular nucleus (TRN) provides inhibitory input to thalamic relay nuclei and is thus poised to gate the transmission of sensory information and to modulate activity in the thalamocortical circuit. The involvement of the TRN in diverse processes like sleep and epilepsy makes it an attractive target for modulation in experimental and clinical contexts. In order to combine the cell-type specificity of optogenetics with the systemic administration associated with pharmacology, we developed a novel approach that uses bioluminescence to drive light-sensitive molecules. Using Luminopsin 3, a fusion of Gaussia luciferase and VChR1, we demonstrated the ability to modulate TRN firing and rhythmicity on the order of seconds to ~45 minutes with a single peripheral injection. Intravenous delivery of the luciferase substrate coelenterazine caused a short-lasting increase in spike rate within seconds, while intraperitoneal delivery drove a more subtle effect lasting 30-60 minutes post injection.

Disclosures: B. Higashikubo: None. **E. McDonnell:** None. **U. Hochgeschwender:** None. **C. Moore:** None.

Poster

182. Genetic Techniques

Location: Hall A

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Program#/Poster#: 182.07/BB67

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant NS072948

NIH Grant EY024890

Title: PV inhibitory neuron subtype specificity in cortex from low titer adeno-associated virus (AAV) containing a Fugu parvalbumin promoter

Authors: Y. LIU[1], H. SHAO[1], R. JAPPELLI[2], *D. C. LYON[1];
[1]Anat. & Neurobio., Univ. of California, Irvine, CA; [2]Lab. of Genet., Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: In the visual cortex of transgenic mice, several recent studies have established that the somatic-targeting parvalbumin (PV) positive inhibitory neuron subtype has a distinct role in

shaping visual responses. However, investigation of the roles of any inhibitory neuron subtype in non-transgenic mammals with more complex visual systems remains elusive. An earlier attempt to target PV interneurons with viral vectors, used AAV 2/1 and a 'short' (2.4 Kb) Fugu rubripes (pufferfish) PV promoter fragment driving EGFP, but expression was not restricted to PV neurons (Nathanson et al 2009, Front Neural Circuits 3:19). Promoter size was kept short to stay under the 5.2 Kb packaging capacity of AAV. However, studies have shown that AAV can effectively package larger gene constructs (up to 8.7 Kb) with only a moderate reduction in gene expression (Hirsch et al 2010, Mol Ther 18:6-8). In support of this, we found previously that AAV 9 with a 3.1 Kb GAD1 promoter and a total gene construct of 7.4 Kb selectively transduced GABAergic neurons while yielding high gene expression (Liu et al 2013 Curr Biol 23, 1746-1755). In this same vector backbone, we have now cloned a longer (2.9 Kb) version of the PV promoter in place of GAD1 to make AAV 9 at a titer of 1.33E+11. To test for cell type specificity we made 0.6 µl injections at 2-3 depths in visual cortex of mouse and cat. On average, transduced cells were found over a 500 µm radius from the injection center. Nearer the injection track, <350 µm, PV selectivity was not high (55% were PV+; with ~10% of PV- cells showing pyramidal morphology). However, beyond 350 µm PV selectivity was dramatically higher, ranging from 80-92%, with 0 pyramidal neurons identified. Furthermore, at this distance the number of transduced neurons was still high, so that for example in a single section 360 µm from the injection track in one mouse there were 95 transduced neurons, 84 of which (88%) were PV+. We next tested for PV selectivity at lower titer and found 75-90% selectivity for concentrations reduced to 33% and below, with only 2 pyramidal cells identified out of hundreds. Further investigation is needed to determine whether the PV- transduced neurons represent other inhibitory neuron subtypes or whether this is due to incomplete PV antibody penetration. Either way, this approach using low titer AAV9 with a 'long' PV promoter shows promising specificity for PV inhibitory neurons in non-transgenic animals, and because our AAV construct includes genes necessary for infection and spread of a modified rabies virus this will provide a unique opportunity for targeted circuit tracing of an inhibitory neuron subtype.

Disclosures: **Y. Liu:** None. **H. Shao:** None. **R. Jappelli:** None. **D.C. Lyon:** None.

Poster

182. Genetic Techniques

Location: Hall A

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIDA Intramural Research Program

NIMH Intramural Research Program

Title: Development of CRISPR toolkit for knock-in transgene targeting to the rat rosa26 locus

Authors: *B. K. HARVEY[1], L. FORTUNO[1], Y. ZHANG[1], J. PICKEL[2], C. RICHIE[1];
[1]NPR Section, NIDA - NIH, Baltimore, MD; [2]NIMH - NIH, Bethesda, MD

Abstract: The advent of CRISPR-based genome modification has lowered the investment cost required for the creation of highly-customized model organisms. Here we describe the development of a Cas9-based genetic toolkit for the rat, which streamlines the targeted insertion of transgenes into the Rosa26 locus. We have constructed a set of plasmids which express the Cas9(D10A) nickase (Cas9n) and a pair of rat Rosa26-targeting guide RNAs (gRNAs) along with a selectable marker. When transfected into rat adrenal gland pheochromocytoma (PC-12) cells, these plasmids induce the formation of double-strand breaks and thereby lead to the accumulation of small insertion/deletions (InDels) between the gRNA binding sites. These plasmids also increase the efficiency of homology-directed insertion of a transgene (donor template) when delivered by cotransfection. The verified Rosa26-targeting gRNAs were transcribed and purified *in vitro*, and microinjected alongside Cas9n mRNA into Long-Evans rat embryos. InDel frequency at the Rosa26 locus in the resulting pups was assessed using a T7 endonuclease assay. For three rounds of injections, the frequency of InDels detected was (2 out of 16), (7 out of 12), and (10 out of 15). The ability to enhance homology-directed insertions *in vivo* was also validated by coinjecting these gRNAs with single-stranded oligonucleotides (ssODN) containing a novel loxP site flanked on each side by 72 basepairs of Rosa26 sequence. A single round of injections produced 15 pups, 4 of which carried the loxP allele. Next, a single round of injections employing a plasmid-based donor template with 1 kilobase homologous arms produced 7 pups, 1 of which carried the transgene. The use of this rat Rosa26 CRISPR toolkit will simplify the creation of "defined" transgenic rat models and should reduce the inconsistencies attributed to copy number- and position-effects when dealing with randomly integrated transgenes.

Disclosures: B.K. Harvey: None. **L. Fortuno:** None. **Y. Zhang:** None. **J. Pickel:** None. **C. Richie:** None.

Poster

182. Genetic Techniques

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Grant-in-Aid for Scientific Research (A)

Grant-in-Aid for Young Scientists (A)

PRESTO program

Title: Nanowire transgene injector arrays

Authors: ***Y. KUBOTA**, A. GORYU, R. NUMANO, M. ISHIDA, T. KAWANO;
Toyohashi Univ. of Technol., Toyohashi Aichi, Japan

Abstract: It is possible to modify the function of cells by transferring the DNA into the cells. As the DNA injection into the cells, nanoscale probes such as carbon nanotubes (CNT) and atomic force microscopy (AFM) are powerful methodologies, in terms of multisite and pinpoint DNA injections into cells. However, the cell injecting deep within a brain tissue is still problematic because of the short length of the nanowire/tube devices (< 10 μm in length). To realize multisite and deep area injections, here we propose the injection using an array of vertically aligned nanoscale-tipped wire (NTW) arrays. An array of NTWs with a high aspect ratio is fabricated by vapor-liquid-solid (VLS) growth of silicon-microwire and the nanotiop formation. The length of the NTW varies from 25 μm to 100 μm, depending on the injection applications (cultured cells, brain tissue with a thickness). As DNA trapping sites, these NTWs are metalized with 100 nm-thick gold layer by sputtering, while the sidewalls of the NTWs are covered with 1-μm-thick parylene insulator. Transgene plasmid DNA encoding an FP (Venus: mutant Yellow FP) was injected into HEK293 cells and a fibroblast cell lines using a 20 × 20 NTW-array (wire length = 25 μm, gap = 100 μm). Cells containing yellow fluorescent signals were separated by a gap, which was consistent with the layout of the NTW array. The observation indicated that plasmid DNA containing the FP transgene were transfected into the multiple-cells by the NTW-array injection, without damage to the cells. With 100-μm long NTW array, a plasmid containing a gene encoding the FP (Venus with a negative charge) was injected into cells within a ~400-μm-thick brain slice of mouse suprachiasmatic nucleus (SCN). After slice incubation up to 4 days, confocal microscope observation of the slice confirmed that the FP signals were located at sites of NTWs in a deep area of the brain slice.

Disclosures: **Y. Kubota:** None. **A. Goryu:** None. **R. Numano:** None. **M. Ishida:** None. **T. Kawano:** None.

Poster

182. Genetic Techniques

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Program#/Poster#: 182.10/BB70

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH/NINDS R01NS088645

MDA

Title: New nsc-derived motor neuron model for als generated using crispr/cas9 reveals involvement of dna damage response

Authors: *J. MITRA[1], V. M. VASQUEZ[2], M. L. HEGDE[2];
[1]Radiation Oncology, Houston Methodist Res. Inst., The Houston Methodist Hosp., Houston, TX; [2]Dept. of Radiation Oncology and Neurology,, Houston Methodist Res. Inst., Houston, TX

Abstract: The recent breakthrough of creating patient-derived induced pluripotent stem cells (iPSC) has allowed researchers to study disease mechanism, therapeutic development and patient-specific drug screening in the context of identical genetic information. Particularly in the perspective of neuroscience, these iPSC lines are invaluable resources for scientists. At the same time, challenges exist with regard to transfections and generating disease-linked mutant cell lines. In case of a progressive motor neurodegenerative disease, called Amyotrophic Lateral Sclerosis (ALS), several mutations in the RNA/DNA binding Tar DNA Binding protein 43 (TDP-43) has been implicated in disease development and progression. Till date no such patient-derived iPSC lines are available carrying etiologically linked mutant TDP-43 protein for studying the disease mechanism and therapeutic approach. Here, utilized the recently developed gene editing technology (CRISPR/Cas9 technique) to establish TDP-43 knock-out iPSC - derived neural progenitor stem cells (NSCs). We also generated Q331K mutant TDP-43 carrying progenitor neuronal cells. These two cell models provided tools for proof-of-principle in-depth mechanistic study of TDP-43 - associated ALS pathology mimicking identical genetic background. To reduce the off-target hitting of CRISPR/Cas, we have employed cell penetrating peptide (CPP) tagged Cas9 protein and packaged the sgRNA with a highly cationic CPP, along with a short single-strand DNA as a template for gene editing. Moreover, we have created a cellular condition where homologous recombination would over-weigh non-homologous end joining after CRISPR/Cas mediated double-strand break generation enhancing the possibilities of "clean" gene editing in neural stem cells. Finally, these novel ALS cell models reveal that genome damage and DNA damage response (DDR) are not only involved in TDP-43 pathology-mediated cell death, but could also be potential therapeutic targets.

Disclosures: J. Mitra: None. **V.M. Vasquez:** None. **M.L. Hegde:** None.

Poster

182. Genetic Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 182.11/BB71

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Institutional Funds

Title: Dried blood spot RNA sequencing (DBS-RNA-Seq): A novel approach for the identification of circulating biomarkers

Authors: *A. WOLFE[1], A. SINIARD[2], R. RICHHOLT[2], I. SCHRAUWEN[2], M. HUENTELMAN[2];
[1]Tgen, Phoenix, AZ; [2]TGen, Phoenix, AZ

Abstract: There is growing interest in the use of cross sectional RNA biomarkers to determine personalized risk for the development of disease, yet many of these biomarker panels are still in need of independent validation. Typical biospecimens used for neurological disease are either peripheral blood or more frequently, cerebrospinal fluid. Due to the invasiveness of collecting these biospecimens, most research using them is limited to the investigation of a single time point. It is likely that the statistical power of novel biomarker assessment would be improved through the use of longitudinally collected samples. To collect biospecimens with higher frequency and use them during longitudinal analysis, we developed a dried blood spot based (DBS) next generation RNA sequencing approach (DBS-RNA-Seq). Single drops of blood (~30ul) were collected onto RNA-stabilizing filter papers (RNASound) and allowed to dry. We demonstrated the ability to isolate on average 10ng of total RNA from one DBS. This RNA was of sufficient quality and quantity to generate Illumina transcriptome and miRNA-Seq libraries, resulting in the assay of 35,000 transcripts and ~500 miRNAs when sequenced to a depth of ~2 million or 650,000 counts, respectively. RNA-Seq analysis of DBS that were stored for 1, 7, 30, and 60 days demonstrated the presence of no significantly differentially expressed transcripts, suggesting that RNA species collected in this fashion are stable for long periods of time. In summary, these studies demonstrated the feasibility and appropriate performance of the DBS-RNA-Seq approach. As proof of concept, we attempted to utilize the DBS-RNA-Seq to develop a biomarker panel associated with 1 hour of aerobic exercise. Each Monday for 4 consecutive weeks, DBS were collected from one study participant at the following time points: 5am (first wake), 9:30am, every 10 minutes during exercise for a total of 6 collections, and then 1, 2, 3, and 4 hours following exercise. Exercise consisted of a 17-mile road cycling ride (165W average power) on the exact same route. Total RNA was isolated from each DBS and utilized for Illumina transcriptome. Surprisingly, analysis demonstrated no significantly differentially expressed transcripts between 5am and 9:30am. However, several transcripts known to be induced by exercise, and many new transcripts, were identified during exercise as well as during

the hours immediately following exercise. In total, this demonstrated the utility of DBS-RNA-Seq to identify biomarkers associated with a "condition." Therefore, we propose that this approach could be utilized to characterize longitudinal RNA biomarkers in larger cohorts of disease cases and controls.

Disclosures: A. Wolfe: None. **A. Siniard:** None. **R. Richholt:** None. **I. Schrauwen:** None. **M. Huentelman:** None.

Poster

182. Genetic Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 182.12/BB72

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: KAKENHI from MEXT and JSPS

Brain/MINDS

CREST-JST

PRESTO-JST

SICPME-JST

CBSN

Strategic Research Program for Brain Sciences

Title: Rational design of ultrafast, high-affinity red calcium indicator for monitoring neuronal activity

Authors: *M. INOUE[1,2], A. TAKEUCHI[3], S.-I. HORIGANE[1,2], H. FUJII[1], S. KAMIJO[1,2], S. TAKEMOTO-KIMURA[1,4], M. OHKURA[5], K. GENGYO-ANDO[5], M. KANO[3], J. NAKAI[5], K. KITAMURA[6,4], H. BITO[1,2];
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Abstract: Fluorescent Ca2+ reporters are widely used as readouts of neuronal activities. We recently developed R-CaMP2, a high-affinity red genetically encoded calcium indicator (GECI) with a Kd for Ca2+ < 70 nM and a Hill coefficient near 1. One major breakthrough was the successful use of a mutated Ca2+/CaM-sensing domain of CaMKK-α/β (ckkap sequence) to replace the calmodulin-binding sequence of M13 sequence which was previously used in all G-CaMP derivatives and their color variants. Thus, R-CaMP2 resulted in three-fold faster rise and decay times of Ca2+ transients than R-CaMP1.07. These features allowed resolving single action potentials (APs) and recording fast AP trains in the 20-40 Hz range, with similar efficacy as with previously reported sensitive green GECIs in acute cortical slices and *in vivo*. Here, we show improvement of R-CaMP performance, based on rational mutagenesis of the ckkap sequence of R-CaMP2. Through screening *in vitro* and in acute cortical slices, we identified R-CaMP variant with a further two-fold on-rate amelioration (time-to-peak around 30 msec) while maintaining a relatively high Ca2+ affinity (Kd ~ 100 nM). In acute cortical slices, spike trains over 50 Hz were resolved, consistent with improved linear response properties as compared with R-CaMP2. The characteristics of these next generation red GECIs will provide enormous advantage in measuring activities of fast-spiking neurons that critically regulate cortical local circuits.

Disclosures: M. Inoue: None. **A. Takeuchi:** None. **S. Horigane:** None. **H. Fujii:** None. **S. Kamijo:** None. **S. Takemoto-Kimura:** None. **M. Ohkura:** None. **K. Gengyo-Ando:** None. **M. Kano:** None. **J. Nakai:** None. **K. Kitamura:** None. **H. Bito:** None.

Poster

182. Genetic Techniques

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Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 182.13/BB73

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: R01 NS 078331

Title: A novel plasmid, NASTIE, for distinguishing neurons and astrocytes during calcium imaging experiments in rat hippocampus

Authors: *M. GIBBONS[1,2], S. W. A. TITEN[3], P. TVRDIK[3], M. R. CAPECCHI[3], J. A. WHITE[5], K. S. WILCOX[2,4];
[2]Interdepartmental Program in Neurosci., [3]Dept. of Human Genet., [4]Dept. of Pharmacol. and Toxicology, [1]Univ. of Utah, Salt Lake City, UT; [5]Dept. of Biomed. Engin., Boston Univ., Boston, MA

Abstract: Astrocytes are now recognized to be active participants in neurotransmission. Deciphering their importance in this role is integral to an in-depth understanding of signaling in the healthy and diseased brain. The recent availability of genetically encoded calcium indicators, such as the GCaMP family of proteins, have made it possible to monitor the activity of neurons and astrocytes in large brain networks. However, no genetically encoded tool exists to selectively distinguish astrocyte processes from neuronal processes in a single animal, seriously limiting our ability to assess local interactions between these cells. We recently developed a novel transgene dubbed Neuron Astrocyte Specified Targeting with *In utero* Electroporation (NASTIE). This transgene encodes the ubiquitous CAG promoter driving expression of the newest membrane tethered calcium indicator, Lck-GCaMP6f, followed by the red fluorescent protein, tdTomato, flanked by lox2272 sites, and then followed by the blue fluorescent protein, Cerulean. It also encodes the neuron-specific promoter, synapsin, driving expression of the site-specific recombinase Cre. By placing Cre expression under the control of synapsin, Cre mediates the excision of the lox2272-flanked tdTomato only in synapsin-expressing cells, leaving these cells to fluoresce blue. To obtain functional and stable transfection of the adult rat brain, we used *in utero* electroporation (IUE) to introduce the NASTIE piggyBac transposon and a plasmid carrying the transposase source into the brains of embryonic day 14 rats. To characterize NASTIE transgene functionality in juvenile and adult rat brains, we dissected and post-fixed brains from postnatal day 9 and 35 rats that had previously undergone IUE. Immunohistochemistry was performed on the tissue to label NeuN (neuronal marker), GFAP (astrocyte marker), and GFP (GCaMP marker). Future directions for this project include investigating calcium activity in reactive astrocytes in acute brain slices prepared from adult rats treated with kainic acid in order to evaluate neuronal-glial signaling in the epileptogenic brain.

Disclosures: **M. Gibbons:** None. **S.W.A. Titen:** None. **P. Tvrdik:** None. **M.R. Capecchi:** None. **J.A. White:** None. **K.S. Wilcox:** None.

Poster

182. Genetic Techniques

Location: Hall A

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Program#/Poster#: 182.14/BB74

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH/NIMH IRP

Title: Establishing the genetic background of marmosets for transgenic production

Authors: A. DALLSTREAM[1,2], B. GOLD[3], B. KOLACHANA[4], *J. PICKEL[1];
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Abstract: Transgenic primate models offer new insights into the study of inherited complex behavior and neurophysiology. Researchers have produced primate models in rhesus macaque, cynomolgus monkeys, and marmosets. Our laboratory has generated a transgenic marmoset using a lentiviral construct and used CRISPR/Cas9 genome engineering to target genes in marmoset embryos. As the demand for transgenic marmoset models of cognitive, behavioral, and social phenomenon grows, it becomes imperative to establish a thorough genetic background of marmosets used in research. In this study, we have begun to characterize the genetic background of marmosets used in our colony for breeding and production of transgenic animals. DNA was extracted from different tissue and bodily fluid sources. Chimerism poses a unique challenge in genetic studies of marmosets. Due to the fusion of placental tissue and shared circulation in gestation, chimerism has been shown to be present in the hematopoietic tissue of marmosets. Chimerism was also observed in buccal swab samples in this study. More reliable and less invasive DNA extraction methods are needed in order to accrue better data in the future. Published microsatellite markers were used to establish a preliminary genetic background and determine relatedness in the colony. The established microsatellites used in our study are reproducible in our colony. However, our results show that some microsatellites have a greater range of alleles in our population creating an overlap in sizes and that a change of parameters in PCR reactions using multiple primers is necessary. Microsatellite analysis reveals the distribution of alleles among family groups and these results will help guide breeding strategies. This preliminary genotyping data serves as a foundation to future studies to characterize marmosets using clinically relevant genetic markers. Establishing a genetic background of the marmosets in research colonies is imperative for any future studies of marmosets that study behavior in family or social settings as well as any transgenic studies that manipulate social behavior.

Disclosures: **A. Dallstream:** None. **B. Gold:** None. **B. Kolachana:** None. **J. Pickel:** None.

Poster

182. Genetic Techniques

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Program#/Poster#: 182.15/BB75

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: KAKENHI Grant Number 25560436

Title: Survival of corticostriatal neurons by rho gtpase signaling pathway

Authors: *K. KOBAYASHI[1], H. SANO[2], S. KATO[3], K. KAIBUCHI[4], K. KOBAYASHI[3]; [2]Div. of Syst. Neurophysiol, [1]Natl. Inst. For Physiological Sci., Okazaki, Japan; [3]Dept of Mol Genet, Fukushima Med. Univ., Fukushima, Japan; [4]Dept of Cell Pharmacol, Nagoya Univ., Nagoya, Japan

Abstract: Corticostriatal neurons are widely distributed in the cortex and play an essential role in various higher brain functions. However, the molecular mechanism underlying the survival of corticostriatal neurons remains unknown. The small GTPase Rho is well known to control various cellular functions. In this study, we investigated the role of Rho in the survival of corticostriatal neurons using newly developed gene transfer system combined neuron-specific retrograde gene transfer lentiviral (NeuRet) vector and adeno-associated virus (AAV) vector. We injected NeuRet vector carrying Cre recombinase gene into the striatum in mice to induce the gene expression in neuronal populations innervating the striatum. AAV vector containing a double-floxed inverted open reading frame encoding a gene of C3 transferase, which is known as a general Rho inhibitor, was injected into the cerebral cortex of NeuRet vector injected mice to suppress the activity of Rho specifically in corticostriatal neurons. Expression of C3 transferase caused the remarkable decrease in the number of corticostriatal neurons. These results indicate that Rho signaling pathway plays a crucial role in the survival of corticostriatal neurons.

Disclosures: K. Kobayashi: None. **H. Sano:** None. **S. Kato:** None. **K. Kaibuchi:** None. **K. Kobayashi:** None.

Poster

182. Genetic Techniques

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Program#/Poster#: 182.16/BB76

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: RNA-lipid nanoparticles: a robust and potent tool for gene knockdown and expression in primary neurons

Authors: A. S. ANSARI[1], D. ZWAENEPOEL[1], A. K. WHITE[2], C. L. WALSH[1], A. THOMAS[1], T. LEAVER[1], A. WILD[1], Y. LI[3], Y. WANG[3], J. R. TAYLOR[1], *E. RAMSAY[1], C. HANSEN[4],

P. CULLIS[5];
[1]Precision Nanosystems Inc., Vancouver, BC, Canada; [2]Genome Sci. and Technol., [3]Brain Res. Ctr., [4]Physics and Astronomy, [5]Biochem. and Mol. Biol., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: There is an unmet need for an efficient delivery tool to facilitate the use of RNA to manipulate gene expression in the mammalian central nervous system. Here, we describe a solid-core lipid nanoparticle (LNP) system, developed by employing the microfluidics-based NanoAssemblr platform, capable of delivering RNA into neurons *in vitro* and *in vivo* with high efficiency and low toxicity. The lipid nanoparticles are taken up by 96% of primary neurons within the first 4 hours of treatment. This rapid uptake provides flexibility in experimental design and downstream procedures. Primary neurons exhibit > 85% gene knockdown, which is sustained for 21 days after LNP administration, even at a dose as low as 100 ng/ml of siRNA-LNP. When exposed to mRNA-LNP, primary neurons exhibit > 80% reporter gene expression. A single treatment of 500 ng/ml of mRNA-LNP results in sustained expression of the reporter gene for 7 days post transfection. Investigation of mRNA-LNP induced protein expression at various timepoints with different particle exposure times showed expression of the protein after just 4 hours of LNP administration. This allows for the integration of this system with other procedures in time-sensitive studies. The performance of these RNA-LNP systems is further characterized by looking at their effect on individual cells, in order to gain insight into the response seen in a bulk population. This is facilitated by the use of a microfluidic device capable of carrying out high-throughput single-cell digital PCR and hence precise quantification of RNA levels in hundreds of individual cells. A 9.8-fold mean reduction in mRNA abundance was observed, when the number of GAPDH mRNA transcripts were measured at the single cell level, in response to treatment with GAPDH siRNA-LNP. Single-cell digital PCR also confirmed the presence of reporter gene mRNA in >99% of cells 24 hours after mRNA-LNP administration. These single-cell studies are important to understand cell-to-cell heterogeneity in terms of quantity of delivered RNA, and its correlation within a cell to changes in gene expression. Examination of cell-to-cell variability, kinetics, and efficiency of using this lipid nanoparticle technology for nucleic acid delivery, demonstrates the potency of the developed RNA-LNP system as well as provides an informed basis for optimizing the manipulation of gene expression in neuronal cells. This technology, thereby, offers a simple and versatile solution to enable loss- and gain-of-function studies in difficult-to-transfect primary neurons.

Disclosures: **A.S. Ansari:** None. **D. Zwaenepoel:** None. **A.K. White:** None. **C.L. Walsh:** None. **A. Thomas:** None. **T. Leaver:** None. **A. Wild:** None. **Y. Li:** None. **Y. Wang:** None. **J.R. Taylor:** None. **E. Ramsay:** None. **C. Hansen:** None. **P. Cullis:** None.

Poster

182. Genetic Techniques

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Neurological Foundation of NZ

Title: Generation of region-specific induced neural precursors following SOX2/PAX6 transfection of adult human fibroblasts

Authors: *R. LIU, E. FIRMIN, K. JONES, B. CONNOR;
Univ. of Auckland, auckland, New Zealand

Abstract: Somatic cell reprogramming is an innovative field with considerable potential to enhance our understanding of developmental processes. We previously demonstrated that adult human dermal fibroblasts (aHDFs) can be directly converted to neural precursors by over-expression of the transcription factors SOX2 and PAX6, using non-viral plasmid transfection1. Induced neural precursors (iNPs) express a wide range of neural stem/precursor and pro-neural genes and upon differentiation give rise to GFAP+ astrocytes and functionally mature neurons expressing TUJ1, MAP2, NSE, and subtype specific markers TH, calbindin, DARRP32 and GAD65/67. The aim of the current study was to investigate the temporal profile of neural stem and pro-neural gene expression during the conversion of aHDFs to iNPs to determine whether reprogrammed iNPs express the same sequential gene profile as per human neural development, or if they represent defined regions of the developing brain. Expression of genes controlling neural induction, forebrain, midbrain and hindbrain specification were examined through qPCR at weekly time points throughout iNP reprogramming. During early expansion (days 38-45 post transfection), iNPs predominantly expressed the anterior forebrain marker LHX2 and the LGE markers GSH2 and DLX2. The iNPs from late expansion (days 52-66 post transfection) expressed the forebrain markers LHX2, FOXG1, SIX3, and the LGE marker GSH2. The MGE markers NKX2.1 and ASCL1, and the midbrain/hindbrain markers OTX2 and GBX2 were not expressed at any stages of reprogramming. This demonstrates that aHDF-derived iNPs do not express the gene expression profile observed in hESCs or iPSCs, possibly due to the stochastic process of direct reprogramming of iNPs. However, iNPs do exhibit gene expression representative of anterior neuroectoderm initially, and then a telencephalic glutamatergic and GABAergic phenotype with preference for ventral LGE fate. To support this, we observed that differentiation of iNPs retained their transcriptional identity and generated a population of neurons expressing the subtype specific markers GAD65/67, DARRP32 and vGLUT. Overall, these results indicate that direct reprogramming of aHDFs to iNPs by plasmid transfection of SOX2 and PAX6 generates a heterogeneous population of neural precursor cells. 1.Maucksch,

C., E. Firmin, et al. (2012). "Non-Viral Generation of Neural Precursor-like Cells from Adult Human Fibroblasts." Journal of Stem Cells & Regenerative Medicine 8: 162-170.

Disclosures: **R. Liu:** None. **E. Firmin:** None. **K. Jones:** None. **B. Connor:** None.

Poster

182. Genetic Techniques

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Program#/Poster#: 182.18/BB78

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: HHMI

Title: Rapid block of unaltered endogenous receptors *in vivo* with a genetically-targeted drug

Authors: P. F. APOSTOLIDES, C. KIM, B. C. SHIELDS, J. BROWN, E. KAHUNO, S. VAIDYA, J. T. DUDMAN, J. C. MAGEE, L. D. LAVIS, *M. R. TADROSS;
HHMI Janelia Farm, Ashburn, VA

Abstract: Drugs are fundamental to molecular neuroscience and neurotherapeutics. Because drugs act broadly within a tissue, their cell-type-specific mechanisms of action have been elusive−a limitation exaggerated by the enormous diversity of cell types in the brain. Here, we describe a straightforward approach to restrict a broadly-active drug to genetically-specified cells in awake-behaving mice. The technique manipulates unaltered endogenous receptors within minutes, affording a combination of protein specificity, cell-type specificity, and speed of onset not previously achieved. In healthy mice, targeting the same drug to spiny projection neurons of the direct (dSPN) versus indirect (iSPN) class yields opposing effects on locomotion. In hemiparkinsonian mice, dSPN drug targeting has remarkably little behavioral consequence, whereas iSPN targeting yields substantial therapeutic benefit−findings relevant both to the molecular etiology and treatment of the disorder. The approach is modular, and may generalize to many drugs with significance to neural circuits and therapeutics.

Disclosures: **P.F. Apostolides:** None. **C. Kim:** None. **B.C. Shields:** None. **J. Brown:** None. **E. Kahuno:** None. **S. Vaidya:** None. **J.T. Dudman:** None. **J.C. Magee:** None. **L.D. Lavis:** None. **M.R. Tadross:** None.

Poster

182. Genetic Techniques

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Program#/Poster#: 182.19/BB79

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

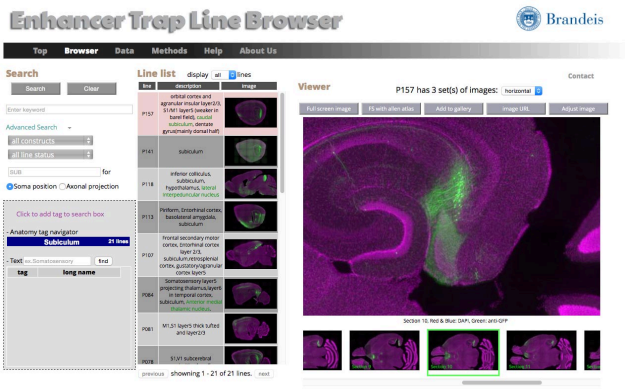
Support: NEI

NINDS

Title: Highly restricted mouse driver strains via transposon-based enhancer trapping

Authors: *Y. SHIMA[1], C. HEMPEL[1], K. SUGINO[1], P. TANEJA[1], J. BULLIS[1], C. LOIS[2], S. NELSON[1];
[1]Biol., Brandeis Univ., Waltham, MA; [2]Biol., Caltech, Pasadena, CA

Abstract: There is a continuing need for driver strains to enable cell type-specific manipulation in the nervous systems. Establishing transgenic lines has been the most reliable method to access restricted neuronal populations. However, most current mouse transgenic strategies are designed to copy endogenous gene's expression patterns (promoter-based methods). These methods, such as BAC transgenesis and homologous recombination, have successfully generated many useful lines using specific marker genes associated with specific neurotransmitters and brain regions. However, for many cell types, specific markers are not known, while for others the available markers label a very large number of populations in the brain limiting usefulness for some applications. . Here we explore the alternative method of enhancer trapping. This has been widely used in model animals such as fly and zebrafish but few large-scale enhancer trap screens have been performed in mice, in part because of the low efficiency of traditional methods of transgenesis. We developed an efficient enhancer trapping method using the PiggyBac transposon. Each cross between an animal carrying a single-copy PiggyBac transposon and an animal carrying the transposase can generate new transgenic lines with novel PiggyBac insertion sites. Our enhancer trap probes contain tet-transactivator and a tet-dependent fluorescent reporter to enable expression screening without crossing with reporter lines. Through our screening over 200 founders have been established and most had expression in brain. We have obtained various lines with restricted labeling such as specific cell types in cerebellum, sublayers of cerebral cortex, and thalamic nuclei. We have confirmed most of our lines keep stable expression patterns over many generations. Tet-dependent AAV can drive cell-type specific expression of genes of interest. Images and data of our lines can be browsed at our web site (http://enhancertrap.bio.brandeis.edu/).



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Poster

182. Genetic Techniques

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: This research was supported by the Intramural Research Program of the NINDS, National Institutes of Health.

Title: Generation of transgenic marmosets expressing genetically encoded calcium indicator GCaMP

Authors: ***J. PARK**[1], X. ZHANG[1], S.-H. CHOI[1], J. PICKEL[2], J. OKAHARA[3], E. SASAKI[3], A. C. SILVA[1];
[1]CMS, LFMI, NINDS, NIH, Bethesda, MD; [2]NIMH, NIH, Bethesda, MD; [3]Dept. of Applied Developmental Biol., Central Inst. for Exptl. Animals, Kawasaki, Japan

Abstract: The chronic monitoring of neuronal activity in the living brain has become feasible as a result of continued development of optical imaging techniques and genetically encoded calcium indicators (GECIs). The green fluorescent protein based GECIs, GCaMPs, have been considered a visible marker of neural activity as these molecules fluoresce upon calcium binding. They can be targeted to specific cell types when used in combination with cell type-specific promoters and can be delivered through transgenic techniques. The common marmoset (Callithrix jacchus) is an important nonhuman primate model in neurophysiological research.

Recently, the successful demonstration of germline transmission confirmed transgenic marmoset has brought greater interest in this species. Here we report the generation of transgenic marmosets that express GCaMPs under the control of either CMV or hSyn promoter. High-titer lentiviral vectors expressing GCaMP under the control of the CMV or hSyn promoter were produced and injected into marmoset embryos that were collected by nonsurgical uterine flushing or laparotomic follicular aspiration followed by *in vitro* maturation and fertilization. Single cell to morula stage embryos injected with lentiviruses encoding CMV-GCaMP5G, CMV or hSyn-mKO-GCaMP6S were transferred to 14, 10 and 20 surrogate mothers, respectively. Twelve of them (three CMV-GCaMP5G, three CMV-mKO-GCaMP6S, and six hSyn-mKO-GCaMP6S) became pregnant and 6 transgenic babies were born. The transgene was detected by PCR using genomic DNA extracted from the mouth swabs and hair roots of six babies. Flow cytometric analysis of peripheral blood samples showed GCaMP positive cells in transgenic marmosets with CMV promoter. The spinal cord tissue was collected from one transgenic marmoset with neuron specific promoter that survived for less than a day, and neuronal GCaMP expression was detected via immunohistochemical image analysis. These results are important steps in developing transgenic marmosets that robustly express calcium sensors for functional optical imaging of neural activity.

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Poster

182. Genetic Techniques

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NINDS 1R01S081716

NIMH 5R00MH085944

PEW FOUNDATION

ALFRED SLOAN FOUNDATION

Title: MicroRNA-guided neuron tags (mAGNETs) exploit a novel framework for genetically targeting neural subtypes in the mammalian brain

Authors: *M. K. SAYEG, B. H. WEINBERG, S. S. CHA, M. GOODLOE, S. SHAIKH, W. W. WONG, X. HAN;
Biomed. Engin., Boston Univ., Boston, MA

Abstract: The ability to genetically modify specific neural subsets is essential in elucidating their contributions to brain computation and disease. Recently, we developed a novel strategy for targeting transgene expression to neuronal subtypes by exploiting endogenous microRNA (miRNA) regulation, which we call miRNA-guided neuron tags (mAGNETs). miRNAs are small (~20 nt), non-coding RNAs that inhibit gene expression by hybridizing to complementary recognition sites within mRNA transcripts. Because different miRNAs are upregulated in specific cell types, we are able to target gene expression by including “signature” miRNA recognition sites at the end of mAGNET transcripts. Exploiting miRNA regulation to target gene expression is an attractive technique for brain research due to the small footprint of miRNA sites (which facilitates viral packaging), the potential to engineer combinations of miRNA sites to tune selectivity, and the possibility of targeting the many neuron types in the brain for which no cell type specific promoters have been identified. As a proof of principle demonstration, we designed and tested mAGNETs that target EGFP expression to cortical inhibitory neurons in the mouse brain.

Disclosures: **M.K. Sayeg:** None. **B.H. Weinberg:** None. **S.S. Cha:** None. **M. Goodloe:** None. **S. Shaikh:** None. **W.W. Wong:** None. **X. Han:** None.

Poster

183. Optical Methods I

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Topic: G.04. Physiological Methods

Support: Canadian Institutes of Health Research Grant MOP119432

Henry Farrugia Research Fund

Canadian Foundation for Innovation

Natural Sciences and Engineering Research Council Doctoral Postgraduate Scholarship

Glaucoma Research Society of Canada

Vision Science Research Program Student Award

Title: *In vivo* photoacoustic imaging of cerebrospinal fluid spaces in mice

Authors: *E. MATHIEU[1,2,3], M. FIRAS[3,4], C. HUPPLE[5], Y. YUCEL[1,2,3,4];
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Abstract: Introduction: Cerebrospinal fluid (CSF) fulfills various roles in the central nervous system and its improper drainage is implicated in a number of neurological disorders. At present, there is a lack of available *in vivo* techniques for studying CSF flow in mice, a particularly useful species due to the availability of transgenic animals. The purpose of this study was to establish a novel *in vivo* assay for detection of CSF spaces and clearance in mice using a near-infrared fluorescent tracer and multispectral photoacoustic (optoacoustic) imaging. **Methods**: Male CD1 mice (n=4) received CSF injections of IRDye800CW (LI-COR Biosciences, NE, USA) conjugated to 7.5 kDa polyethylene glycol, followed by photoacoustic imaging of the head with a Multispectral Optoacoustic Tomography system (MSOT, iThera Medical, Germany). Under general isoflurane anesthesia (2% in O_2), the head and neck were shaved and hair was removed with depilatory cream. Mice were positioned in a stereotaxic frame and 3 μL of tracer was injected into the CSF of the cisterna magna through a suboccipital incision. The injection site was closed with cyanoacrylate tissue glue, the head and neck were coated in a thin layer of ultrasound gel, and mice were placed in a dorsal recumbent position in the scanning chamber. A 13 mm scan region was defined between the posterior orbits and start of the spinal cord. Mice were scanned prior to CSF injection and then continuously every 2.5 minutes after injection for 28 (n=2), 35 (n=1), or 40 (n=1) minutes. Multispectral photoacoustic data was reconstructed (ViewMSOT, iThera Medical, Germany) and linear regression was used for unmixing spectral components to visualize IRDye 800 signal distribution. Tracer signal was quantified over time in a region of interest corresponding to the subarachnoid space (SAS) of the hindbrain. **Results**: The photoacoustic signal of near-infrared tracer was readily identified in the cerebral subarachnoid space, ventricles and basal cistern. Time-course quantification of the IRDye 800 signal in the hindbrain SAS showed an initial increase in signal with a plateau 15-20 minutes after injection, followed by a steady decrease until imaging was discontinued. **Conclusions**: These preliminary results provide the first evidence that multispectral photoacoustic tomography can be used as a novel *in vivo* assay to qualitatively and quantitatively assess CSF spaces in mice. This technique may be a valuable tool for the study of cerebrospinal fluid clearance in the healthy mouse and in various models of neurological disorders.

Disclosures: **E. Mathieu:** None. **M. Firas:** None. **C. Hupple:** A. Employment/Salary (full or part-time):; Employee of iThera Medical GmbH. **Y. Yucel:** None.

Poster

183. Optical Methods I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 183.02/BB83

Topic: G.04. Physiological Methods

Support: MIUR FIRB (RBAP11X42L)

EU-FP7 (DESIRE)

NIH, Brain Initiative (U01-NS090576)

Title: Cell-specific optical perturbation of electrical activity *in vivo*

Authors: A. FORLI, C. MORETTI, S. BOVETTI, *T. FELLIN;
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Abstract: The ability to determine the spatial and temporal patterns of neuronal activation in response to external sensory stimuli using well-established tools as *in vivo* two-photon microscopy is revolutionizing our understanding of how sensory inputs are processed at the cellular level. However, to causally investigate how these spatial and temporal patterns of neuronal activity encode information about the external stimulus, we lack a complementary method that makes it possible to perturb the activity of many neurons with cellular resolution. To achieve this goal, we combined two-photon patterned illumination to control the size and shape of the two-photon focal volume with photostimulation experiments to perturb the spiking activity of single neurons expressing the excitatory opsin channelrhodopsin-2 (ChR2). Patterned illumination was obtained using a commercial liquid crystal spatial light modulator which was placed in a plane optically conjugated to the objective back aperture. In simultaneous photostimulation and two-photon-guided juxtasomal recordings from ChR2-positive cells in the neocortex of anesthetized mice, we found reliable increase in the firing rate of recorded neurons upon patterned two-photon (λ = 920 nm) illumination with extended shapes. Cellular response increased with light intensity (average power, 10-50 mW) and ChR2-positive neurons up to a maximal depth of 150-200 μm could be reliably photostimulated within this power range. The spatial resolution of photostimulation was ~ 30 μm in the radial direction and ~ 40 μm in the axial direction. Experiments are currently ongoing to investigate the effects of illumination with

different shapes on the suprathreshold response of individual neurons and to characterize these different stimulation protocols in terms of efficiency and spatial resolution. By precisely manipulating the activity of neuronal networks in space and time, this technique provides the basis for the investigation of how neuronal patterns of activation can encode information.

Disclosures: **A. Forli:** None. **C. Moretti:** None. **S. Bovetti:** None. **T. Fellin:** None.

Poster

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Program#/Poster#: 183.03/BB84

Topic: G.04. Physiological Methods

Support: BBSRC

Title: A calcium transient detection algorithm optimized for GCaMP6 kinetics, tested in Layer 2/3 of mouse somatosensory cortex

Authors: ***C. S. COPELAND**[1], S. REYNOLDS[2], J. OÑATIVIA[2], L. A. ANNECCHINO[3], P. L. DRAGOTTI[2], S. R. SCHULTZ[3];
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Abstract: Accurate detection of action potentials is key to the study of neuronal networks. Intracellular free calcium concentration is a reliable indicator of neuronal activity, and calcium-sensitive fluorescent indicators have proven useful tools. Indeed, detecting action potentials using 2-photon calcium transient imaging offers advantages over standard electrophysiological approaches as it enables up to thousands of spatially and immunohistochemically defined neurons to be recorded from simultaneously. Recently, we introduced a novel approach to calcium transient detection by making use of finite rate of innovation (FRI) theory (Vetterli et al 2002 IEEE Trans. Signal Process. 50:1417-28). This method enabled us to retrieve the timing of action potentials from calcium transient time series using both surrogate data and real data (obtained by simultaneous electrophysiology and 2-photon imaging of calcium dye signals in cerebellar Purkinje cell dendrites) (Oñativia et al 2014 J. Neural Eng. 50:046017). We have now extended and optimised this algorithm to detect calcium transients with kinetics corresponding to the genetically encoded calcium indicators GCaMP6s and GCaMP6f and the calcium-sensitive fluorescent dye Oregon Green BAPTA (OGB). Upon performing test simulations using surrogate data, the FRI algorithm was able to achieve a high spike detection rate for each fluorescent

indicator, regularly detecting above 90% of spikes. No false positives were produced in 81% of surrogate data (for a spike rate of 0.25Hz, false positive rates were OGB: 8.7x10-3Hz; GCaMP6S: 7.1x10-3Hz; GCaMP6f: 3.4x10-4Hz). The temporal location of GCaMP6s spikes is estimated with the highest accuracy, which can be attributed to its comparatively high signal-to-noise ratio. However, if the comparison of spike detection performance is made linear in terms of signal-to-noise ratio, the fastest decaying pulse, that of GCaMP6f, outperforms both OGB and GCaMP6s (by average spike detection rate margins of 2.1% and 4.3%, respectively). We are now introducing real data from single-neuron and population responses to vibrissae deflection in Layer 2/3 of identified columns in mouse somatosensory (barrel) cortex, using *in vivo* 2-photon imaging to validate the algorithm. Implementation of this algorithm will prove a useful tool for analysis of neuronal network dynamics both within the somatosensory cortex, and across additional cortical areas associated with other sensory modalities (eg. vision, audition, proprioception).

Disclosures: C.S. Copeland: None. **S. Reynolds:** None. **J. Oñativia:** None. **L.A. Annecchino:** None. **P.L. Dragotti:** None. **S.R. Schultz:** None.

Poster

183. Optical Methods I

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Program#/Poster#: 183.04/BB85

Topic: G.04. Physiological Methods

Title: Toward voltage imaging in the live mouse brain with enhanced Rhodopsin based voltage sensors

Authors: *Y. ADAM[1], Y. ZHAO[2], M. A. MOSTAJO-RADJI[1], P. ARLOTTA[1], R. E. CAMPBEL[2], A. E. COHEN[1];
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Abstract: Optical detection of action potentials in the intact mammalian brain will open new frontiers in neuroscience research. Archaerhodopsin-based voltage sensors exhibit voltage-sensitive fluorescence, with a sensitivity and speed of response far greater than any other protein- or dye-based voltage indicator. Although they perform well in cultured cells, these indicators are difficult to implement *in vivo* due to their dim fluorescence and poor membrane trafficking. To address these limitations, we designed a series of modified sensors, based on the QuasAr backbone previously developed by our lab. Our panel of constructs included chimeras with other rhodopsins, different fluorescent fusion proteins and linkers, additional signal peptides as well as

rational point mutations. These constructs were then expressed in primary hippocampal neurons and evaluated by measuring their brightness and their signal to noise ratio (SNR) for optically induced action potentials. Constructs that showed significant improvement were then expressed *in vivo* using *in utero* electroporation and tested in acute brain slices. Our screen resulted in new variants of QuasAr, which show significantly improved membrane trafficking as well as brighter fluorescence. These constructs allow high fidelity recording of single spikes in acute brain slices at single cell resolution, and they are currently under evaluation in the live mouse brain.

Disclosures: Y. Adam: None. **Y. Zhao:** None. **M.A. Mostajo-Radji:** None. **P. Arlotta:** None. **R.E. Campbel:** None. **A.E. Cohen:** None.

Poster

183. Optical Methods I

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Topic: G.04. Physiological Methods

Support: Ministry of Education, Culture, Sports, Science, and Technology of Japan (YM)

Special Postdoctoral Researchers Program in RIKEN (YY)

Ministry of Education, Culture, Sports, Science, and Technology of Japan (KM)

Title: *In vivo* chronic macroimaging of sensory representation in marmosets

Authors: *Y. MATSUMOTO[1,2], Y. YAMADA[1,2], K. MIKOSHIBA[1,2];
[1]Brain Sci. Institute(bsi), RIKEN, Saitama, Japan; [2]Central institute for Exptl. Animal, Kawasaki, Japan

Abstract: Marmosets represent an attractive primate species for neuroscience research due to 1) ease of handling with the small body size; 2) high reproductive rate and short gestational period; 3) applicability of transgenesis technology. Based on these advantages, several neurological disorder models (e.g. Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis (ALS)) have been recently established with marmosets. To understand the underlying neuronal mechanism of these models, the experimental technique to chronically track the neuronal ensemble activity has been required. Here we established epifluorescent macroimaging of flavoptotein as well as genetically encoded Ca^{2+} indicator GCaMP in marmosets. We have successfully revealed a somatotopic map in the primary somatosensory cortex (area 3b) using electrical stimulation applied to contralateral body parts. Similar activation patterns were

observed with flavoprotein and GCaMP, the latter displaying better signal to noise ratio. The somatotopic map could be stably recorded up to 8 weeks. The responsiveness to sensory stimuli and spontaneous activity rate were found to be much higher under awake state than under anesthetized state. Our experimental system can be applied to other cortical areas, and will be useful for studying the evolution of brain dysfunction underlying neurological disorder.

Disclosures: **Y. Matsumoto:** None. **Y. Yamada:** None. **K. Mikoshiba:** None.

Poster

183. Optical Methods I

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Topic: G.04. Physiological Methods

Support: JSPS KAKENHI Grant Number 26249051

Title: Visualizing neuronal activities of the deep brain in a freely-moving mouse by using implantable micro imaging devices

Authors: ***Y. OHTA**, M. MOTOYAMA, M. HARUTA, H. TAKEHARA, T. NODA, K. SASAGAWA, T. TOKUDA, J. OHTA;
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Abstract: The mesolimbic dopamine system concerning reward pathway like VTA-NAc circuit has been studied with interest, but few studies have reported direct visualization of neuronal activities. Imaging neuronal activities in a mouse deep brain area such as VTA or NAc is, however, extremely difficult without disturbing behavior of an awake mouse. In order to address this issue, we have developed an implantable micro imaging device [1]. We have successfully visualized activities of GFP-labeled dopamine neurons and GABAergic neurons in the deep brain region of a mouse which was administrated with ethanol acutely. Our device has micro needle shape (width: 0.7mm, thickness: 0.2mm) so as to be suitable for the implantation into deep brain with minimal invasiveness. The device consist of a dedicated CMOS (Complementary Metal Oxide Semiconductor) image sensor and one or two blue LEDs (center wavelength: 460nm) placed on the sides of the sensor to excite GFP. The sensor has 40 x 90 or 40 x 120 pixels with the pixel size of 7.5 μm square. The sensor surface is covered with a filter to cut off the excitation light and to pass the fluorescence of GFP (center wavelength: 520nm). In the experiment, we visualized activity of DP neurons in freely moving TH (tyrosine hydroxylase) transgenic mice expressing GFP in the majority of midbrain dopamine neurons [2]. We

anesthetized a TH-GFP mouse and implanted our device into VTA, in the depth of 4.5mm from the brain surface. In the next day of the implantation, we have demonstrated to visualize the activities of somas of DP neurons in VTA for six hours after oral alcohol administration and successfully measured the intensity change of the GFP luminescence. Since the device is small and light, it can be applied to a multi-point measurement. We have visualized the activity of the GABAergic neurons by a similar method to observe the network of the nerve of the reward system. Two devices were implanted into both NAc shell region and lateral part of the central nucleus of the amygdala in GAD67 (glutamic acid decarboxylase 67) -GFP transgenic mice [3] and successfully visualized network of brain functions at the same time under freely behaving. Since our device has already been confirmed operation in a mouse brain over three months, we are planning an experiment to observe the long term change of neuronal activities in the same mouse. [1] J. Ohta et al., Sensors 2009; 9(11), 9073 -93. [2] N. Matsushita et al., J Neurochem. 2002 Jul; 82(2):295-304. [3] N. Tamamaki et al., J Comp Neurol 2003; 467:60-79.

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Poster

183. Optical Methods I

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Program#/Poster#: 183.07/BB88

Topic: G.04. Physiological Methods

Title: Temperature changes in the brain slice by NIR optical stimulation

Authors: ***S. MIN**, M. YOO, S. KIM;
Gwangju Inst. of Sci. and Technol. (GIST), Gwangju, Korea, Republic of

Abstract: For more than a decade, optical stimulation has been studied as a new tool that can replace the conventional electrical stimulation method to modify neuronal activities. Since temperature increases by optical stimulation have been proven to be the main reason for neuronal modulation, how the optical stimulation parameters would control temperature increases and heat distribution in the brain tissue needs to be investigated to utilize optical stimulation as an efficient tool to stimulate only targeted neurons. In the present study, we studied thermal changes in brain slices by applying various modes of optical stimulation. A male C57BL/6 mouse was decapitated and the brain was extracted. Brain slices were prepared by dissecting the brain with a thickness of 400 μm using a vibratome (VF-200, Precision Instruments). Optical stimulation

using near infrared laser with a wavelength of 808nm and intensities from 1.72 to 35.4 ㎽ was applied to the brain slices in continuous and pulsed modes. The core diameter of the optical fiber was 62.5 μm. The temperature change in the brain slice under laser illumination was measured using an infrared camera (FLIR SC5000, FLIR Systems, Boston, MA). Using continuous waves with intensities of 35.4 and 1.72 mW, the temperature in the brain tissue increased by 1.42℃ and 0.1℃, respectively. The size of the heated area was observed to be around 2.08 mm with the stimulation power of 35.4 ㎽. On the other hand, when pulsed laser with the width of 5 ㎳ and the pulse train duration of 100 ㎳ at the repetition rate of 5 Hz was applied, the affected area was observed to be about 180.2 μm at the same stimulation power of 35.4 mW, and the temperature increased by 1.8℃. This result suggests that pulsed waves provided a better spatial resolution by limiting heat transfer within the restricted area. It is concluded that pulsed laser stimulation affects a significantly smaller area thermally, suggesting that it would provide a better thermal spatial selectivity than that of continuous wave and the possibility that the size of affected areas could be controlled by controlling the pulse width. Also, we found that as the power of optical stimulation increased, larger temperature increases were observed, implicating that the degree of neuronal modulation would be controllable by controlling the laser power level. As further study, we will measure the changes in neural activity by using simultaneous electrophysiological recording and calcium imaging that would provide both detailed temporal and spatial information while optical stimulation is applied.

Disclosures: **S. Min:** None. **M. Yoo:** None. **S. Kim:** None.

Poster

183. Optical Methods I

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Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) to M.M. and T.Y.

Title: Two-photon calcium imaging using genetically-encoded calcium indicator in primate neocortex

Authors: *O. SADAKANE[1,2], Y. MASAMIZU[3], A. WATAKABE[1,2], S.-I. TERADA[3,4], M. OHTSUKA[1,2], M. TAKAJI[1,2], H. MIZUKAMI[5], K. OZAWA[5,6], H. KAWASAKI[7], M. MATSUZAKI[3], T. YAMAMORI[1,2];
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Abstract: The combination of two-photon microscopy with genetically-encoded calcium indicators enabled researches to chronically monitor neuronal population activity in behaving animals, opening up an avenue to investigating the organization of neural circuits and its plasticity. This technique has been successfully applied to a variety of invertebrates and vertebrates including flies, fish, and rodents. However, thus far, the application of this technique to the primate brain has had severe limitations in terms of the number of simultaneously imaged neurons and its ability to follow the same neuron population over a long time period. One reason of this difficulty is that stronger fluorescent signals are required for clear visualization of calcium signals, because primate brains are less transparent than rodent brains. Here, we present a novel system to chronically image the activity of cortical neurons of the adult marmoset (Callithrix jacchus), a small New World primate. In this system, the subject is head fixed under a two-photon microscope in anesthetized state. A chronically implanted optical window provides access to the cortical surface. The activities of cortical neurons are monitored by GCaMP6f, expressed from an AAV vector-based high expression system. Using this system, we succeeded in monitoring spontaneous neuronal activities of cortical neurons up to 3 months. Importantly we were able to simultaneously image more than a hundred of neurons three dimensionally at high speed, which proved the effectiveness of our system. In conclusion, our system has made it possible to monitor neuronal population activities from primate neocortex with unprecedented quality, and it will be applicable to functional analysis of neuronal population activity in behaving marmosets.

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Poster

183. Optical Methods I

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Topic: G.04. Physiological Methods

Title: Light-controlled activation of neurons via a graphene-based biocompatible optoelectronic interface

Authors: E. MOLOKANOVA[1], G. B. BRAUN[2], A. ALMENAR-QUERALT[3], A. ZARETSKI[4], L. S. B. GOLDSTEIN[3], M. MERCOLA[5], *A. SAVTCHENKO[5];
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Abstract: A light signal is the perfect trigger to reversibly control functional activity of a neuron in a spatially resolved and temporally precise manner. Optogenetics uses genetically encoded light-sensitive proteins to achieve this goal, but in certain cell systems (e.g., stem cell-derived neurons), it is not desirable to express exogenous proteins due to potential side effects. Thus, there is a need for an external light-controlled triggering platform for remote activation of intact non-modified neurons. Considered “the wonder material of the 21st century”, graphene has a unique combination of properties - broadband absorption, high electrical conductivity, high charge carrier mobility, and high transparency, making it very attractive for the proposed graphene-based biointerface for neuronal activation. By rapid generation of free charge carriers under light illumination, we expect a capacitive coupling effect to occur between the neuronal membrane and the local graphene surface resulting in membrane depolarization and subsequent action potential generation. Here we developed a novel graphene-based biointerface and studied its effect on human iPSC-derived neurons. First, using fluorescent and scanning electron microscopy, we confirmed exceptional compatibility of these biointerfaces with long-term cell culture. Then we demonstrated the ability of the graphene-based biointerface to activate neurons by intermittently illuminating them with light while performing electrophysiological recordings. Lastly, taking into account high transparency of the graphene-based biointerface, we explored its utility for all-optical electrophysiology that combines light-controlled activation with optical recording methods. Indeed, in our experiments, we were able to trigger action potentials in

neurons by light illumination of the graphene-based biointerface, and simultaneously monitor the resulting changes in intracellular calcium concentrations using fluorescent calcium indicators. In summary, we present a novel graphene-based optoelectrical biointerface for activation of neurons via external light-controlled electric field. This biointerface is expected to be beneficial for studies of activity-driven neuronal maturation, long-term effects of environmental, pharmacological or genetic factors, and activity-driven corrections in "disease-in-a-dish" models.

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Poster

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Support: NIH Grant R21

NIH Grant R24

Title: A compact multifunctional laser scanning imaging system developed on open source hardware

Authors: *X. LI, K. BORGES, G. M. G. SHEPHERD;
Physiol., Northwestern Univ., Chicago, IL

Abstract: To investigate the functions of neural circuits in the mouse motor system, we are developing an economical, compact, multifunctional 2-photon laser scanning imaging system combined with a portable behavioral task monitoring system. The microscope is developed on optical, mechanical, and electronic parts available as stock items from commercial suppliers, with a small number of custom-made components. The frame of the microscope is built around a horizontal platform. A rotating head allows *in vivo* imaging off the vertical axis, a useful feature for imaging more laterally located cortical areas such as auditory cortex. The beam path can be switched between two different laser scanning modules: (1) a pair of mirror galvanometers (G-G scanner; Cambridge Technology), or (2) a resonant-galvanometer (R-G) scanner (Sutter). Switching is executed by an Arduino based remote control motorized switch. An optogenetic laser scanning photostimulation module can be added in parallel with the 2P imaging modules. The structure of the microscope is very flexible and expandable, new 2D photostimulation

modules such as a digital mirror device (DMD) and spatial light modulator (SLM) can easily be attached. An electrically focus-tunable lens can be attached to the objective to enable fast 3D imaging and photostimulation. The hardware is operated by ScanImage software, and interfaced via National Instrument DAQ boards. In the portable behavior rig, a mouse's locomotion drives a trackball or treadmill, which in turn is interfaced with an Arduino that is programmed with the task paradigm. This Arduino also does basic real time data analysis and triggers the imaging and/or photostimulation from the microscope. A single-board computer (e.g. Raspberry Pi, BeagleBone) as the host controller sets the parameters of the behavior paradigm, and collects, stores, and visualizes the mouse behavioral data streamed from the behavior Arduino. The software is developed in Labview, Matlab, Arduino (and AVR C), and Python.

Disclosures: **X. Li:** None. **K. Borges:** None. **G.M.G. Shepherd:** None.

Poster

183. Optical Methods I

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Topic: G.04. Physiological Methods

Support: Craig H. Neilson Foundation 296332

Title: *In vivo* three-photon excited fluorescence imaging of neural activity in the spinal cord of awake mice

Authors: ***Y.-T. CHENG**[1], I. M. BASTILLE[2], J. CRUZ HERNANDEZ[2], D. G. OUZOUNOV[3], T. WANG[3], X. LI[2], N. NISHIMURA[2], J. R. FETCHO[1], C. XU[3], C. B. SCHAFFER[2]; [1]Neurobio. and Behavior, [2]Biomed. Engin., [3]Applied and Engin. Physics, Cornell Univ., Ithaca, NY

Abstract: Imaging of calcium-sensitive indicators using nonlinear microscopy enables minimally-invasive studies of neuronal activity and circuit dynamics at high spatial and temporal resolution. Due to strong optical scattering from myelinated axons in the dorsal spinal cord, traditional two-photon excited fluorescence (2PEF) imaging is able to visualize cell structure or neural activity only in superficial regions. Current approaches to investigate neuronal circuits in deeper mid/ventral regions of the spinal cord rely primarily on explanted spinal cord preparations, so are unable to capture the full complexity of the system. We recently showed that three-photon excited fluorescence (3PEF) microscopy enables significantly increased penetration into scattering samples as compared to 2PEF imaging (Horton NG, et al; 2013 Nat Photonics).

Here, we demonstrate 3PEF imaging deep into the spinal cord of mice using both 1300 and 1700-nm excitation wavelengths. We implanted the long-term spinal cord imaging chamber we previously developed (Farrar MJ, et al; 2012 Nat Methods) into C57Bl/6 mice. In some mice, the vasculature was labeled with intravenous injections of Fluorescein- (1300 nm excitation) or Texas Red-dextran (1700 nm excitation) and we were able to visualize vascular structure and quantify blood flow in individual vessels at depths of up to 500 μm, or about 50% of the way through the spinal cord. In other mice, we injected adeno-associated viral vectors to drive expression of the genetically encoded calcium sensitive fluorescent protein, GCaMP6, in spinal cord neurons. We delivered electric stimulation to the hind paw while the animals were anesthetized and observed robust neuronal responses (normalized change in fluorescence > 50%) in dorsal horn neurons at a depth of ~200 μm. Finally, we developed a spinning disk treadmill that enables awake mice to be held fixed under the microscope by the spinal cord chamber while able to walk and run on the treadmill. When combined with quantitative tracking of limb motion, this capability for 3PEF imaging of cell-resolved neuronal firing in the spinal cord of awake, moving mice could enable detailed studies of, for example, the patterns of neural activity in central pattern generator circuits that underlie locomotor function.

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Poster

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Support: K25NS083754

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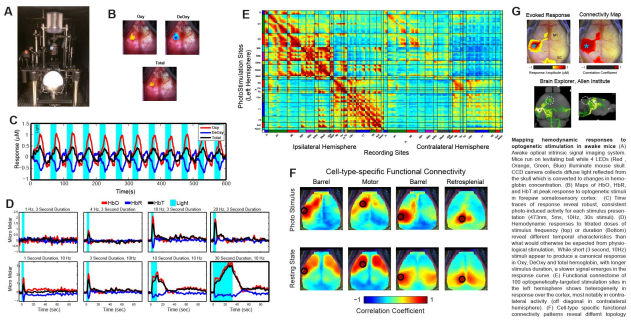
P01NS080675

Title: Mapping large-scale functional connections with chr2-evoked hemodynamic signals

Authors: *A. Q. BAUER[1], G. BAXTER[2], A. KRAFT[3], M. BRUCHAS[4], J.-M. LEE[3], J. CULVER[2];

[1]Radiology, Washington Univ. In St. Louis, Saint Louis, MO; [2]Radiology, [3]Neurol., [4]Anesthesiol., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: A major goal of neuroscience is the development of a connectome for understanding structural and functional connections of the brain. Resting-state functional connectivity (RS-FC) analysis using fMRI has been important for mapping the brain's functional architecture through analysis of spontaneous hemodynamic fluctuations. However, because these oscillations reflect meso-scale activity from ensembles of different types of neurons, FC mapping with fMRI lacks the specificity for measuring how FC within a subpopulation of neurons contributes to the entire network. Optogenetic methods are a natural approach for probing local and global brain circuitry. But, mapping the entire cortex with fMRI optogenetically is challenging due to space constraints, and signal to noise limitations prevent linking these functional subcurcuits to RS-FC patterns. To satisfy this need, we combined optogenetic mapping with optical intrinsic signal imaging in 5 awake Thy1-ChR2 mice. (Fig.1A). We first established that we could elicit hemodynamic responses (HRs) in these mice (Fig. 1B, C), and then titrated optical stimuli to define a regime over which stimulated circuits produce linear HRs (Fig. 1D). These optical stimuli were then scanned over the left hemisphere to generate a functional connectome for Thy1-based circuits (Fig. 1E). Photostimulation of left barrel cortex (S1BC) produces hemodynamic responses in S1BC as well as highly correlated activity in motor cortex (M1) (Fig. 1F, Barrel) that agrees with anatomy (Fig. 1G). Interestingly, while stimulation of M1 evokes a reciprocal connection with S1BC (Fig. 1F, Motor), the time course of S1BC activity to M1 stimulation is less coherent. Further, RS-FC maps of S1BC or of M1 exhibit less specific FC patterns, and do not show the same functional topography as ChR2-based fc maps (Fig. 1F bottom). Understanding how neurons integrate multiple inputs, and the function of coordinated population activity within the larger scope of a functional circuit may help deduce impaired functional relationships between cortical areas in disease models.



Disclosures: **A.Q. Bauer:** None. **G. Baxter:** None. **A. Kraft:** None. **M. Bruchas:** None. **J. Lee:** None. **J. Culver:** None.

Poster

183. Optical Methods I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 183.13/CC1

Topic: G.04. Physiological Methods

Support: UK EPSRC EP/1005439/1

UK EPSRC EP/F500378/1

UK BBSRC BB/J015369/1

Title: High-resolution recording of neural activity by transforming intrinsic optical signals

Authors: ***M. J. WALL**, M. J. E. RICHARDSON, M. G. THOMAS, J. A. COVINGTON, M. S. TURNER;
Univ. of Warwick, Coventry, United Kingdom

Abstract: Understanding functional network dynamics in brain tissue requires chronic recording at high spatial and temporal densities, potentially across large areas of tissue. Existing experimental tools afford either a small number of high temporal-density channels at the cellular level which become overly invasive at high density or instead have recording durations limited by dye bleaching. It has long been known that the intrinsic optical properties of neural tissue are activity-dependent and thus have formed the basis of a non-invasive imaging technique. However, slow temporal dynamics and lack of correspondence with on going network activity, have limited their usefulness. A detailed quantitative analysis of this intrinsic optical signal (IOS) using a high frame rate camera (250 fps) has allowed us to develop a mathematical model that accurately reproduces the IOS from network activity for a range of network activities including: field EPSPs, population spikes, seizure-like bursts and spreading depression. Inversion of this forward model provides a simple procedure for converting IOS imaging data into a transformed IOS (tIOS) that mimics the local-field potential (LFP). This approach markedly increased the temporal resolution of IOS imaging from a timescale of minutes down to tens of milliseconds and also radically improved upon the spatial resolution. Using a standard camera (25 fps) the tIOS was used to characterise seizure-like activity in the neocortex and made it possible to localise the seizure onset zone for infrequent events within ~20 um. This would be very difficult to achieve using dye-imaging techniques because of their limited recording duration. Using tIOS requires little modification of standard electrophysiological imaging hardware, can be carried out with transmitted or reflected light and can be used on-line during experiments, thus has the potential for widespread adoption as an efficient method for monitoring network activity.

Disclosures: **M.J. Wall:** None. **M.J.E. richardson:** None. **M.G. Thomas:** None. **J.A. Covington:** None. **M.S. Turner:** None.

Poster

183. Optical Methods I

Location: Hall A

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Program#/Poster#: 183.14/CC2

Topic: G.04. Physiological Methods

Support: NIH Grant R01NS078223

NIH Grant K25NS083754

Title: Non-invasive functional neuroimaging in the mouse using diffuse optical tomography

Authors: *M. REISMAN, A. BAUER, Z. MARKOW, G. BAXTER, J. CULVER;
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: The study of correlated spontaneous activity in functionally related brain regions using functional connectivity magnetic resonance imaging (fcMRI) has allowed comprehensive mapping of distributed brain networks in humans. Extending fcMRI to the mouse has been challenging due to necessary trade-offs between high resolution and high signal-to-noise in the small mouse brain volume. Instead, optical intrinsic signal (OIS) techniques have provided most of the observations of fc in the mouse brain. While effective, OIS requires scalp retraction and is limited to superficial cortical tissues. Diffuse Optical Tomography (DOT) provides non-invasive imaging, but current DOT systems are either too sparsely sampling to match the cortical resolution of OIS or are too slow for capturing functional response in the mouse brain. Here we develop a DOT system that combines the spatial sampling of camera-based systems with the rapid-imaging of structured light illumination to map brain activity in the mouse. The DOT system is comprised of a sCMOS camera and a digital micromirror device (Fig 1A) that produce ~10^6 source-detector pair measurements with 0.15mm spacing at a speed of ~10Hz. This system increases the spatial sampling by >10x over existing human functional neuroimaging DOT and increases the speed by >10x over previous CCD-based mouse DOT. Activations in the somatosensory cortex upon electrical stimulation of the forepaw are seen non-invasively, through the intact scalp (Fig 1B). Extending the technique to imaging spontaneous activity reveals the expected resting state fc maps within the cortex as previously observed using OIS, but with improved depth sensitivity (Fig 1C). Establishing analogous functional imaging in both mouse and man is one of the most promising strategies for providing clinical translation. We have developed a method for optical imaging of the mouse brain using DOT with structured light source patterns. This method extends our previous mouse neuroimaging techniques to image through the scalp and with increased sensitivity to deeper regions of the mouse cortex.

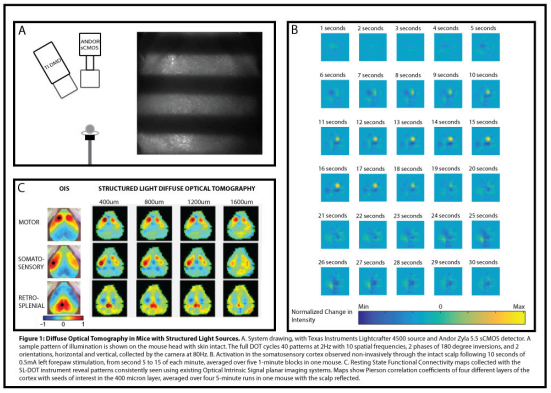

Figure 1: Diffuse Optical Tomography in Mice with Structured Light Sources. A. System drawing, with Texas Instruments Lightcrafter 4500 source and Andor Zyla 5.5 sCMOS detector. A sample pattern of illumination is shown on the mouse head with skin intact. The full DOT cycles 40 patterns at 2Hz with 10 spatial frequencies, 2 phases of 180 degree inversions, and 2 orientations, horizontal and vertical, collected by the camera at 80Hz. B. Activation in the somatosensory cortex observed non-invasively through the intact scalp following 10 seconds of 0.5mA left forepaw stimulation, from second 5 to 15 of each minute, averaged over five 1-minute blocks in one mouse. C. Resting State Functional Connectivity maps collected with the SL-DOT instrument reveal patterns consistently seen using existing Optical Intrinsic Signal planar imaging systems. Maps show Pierson correlation coefficients of four different layers of the cortex with seeds of interest in the 400 micron layer, averaged over four 5-minute runs in one mouse with the scalp reflected.

Disclosures: **M. Reisman:** None. **A. Bauer:** None. **Z. Markow:** None. **G. Baxter:** None. **J. Culver:** None.

Poster

183. Optical Methods I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 183.15/CC3

Topic: G.04. Physiological Methods

Support: KAKENHI 26870577

Title: Sparse MAP inference of cell shapes and spike trains from calcium imaging data with non-negative constraints

Authors: ***T. TAKEKAWA**[1], M. SATO[2], N. OHKAWA[3], K. INOKUCHI[3], Y. HAYASHI[2], T. FUKAI[2];
[1]Fac. of Informatics, Kogakuin Univ., Tokyo, Japan; [2]RIKEN BSI, Wako, Japan; [3]Dept. of Biochem., Univ. of Toyama, Toyama, Japan

Abstract: Calcium imaging technique is a powerful method for simultaneous recording the activity of large number of neurons. However, inferring the spike trains of neurons from calcium imaging movie data is often difficult, due to the noise, the low temporal resolution, unknown biophysical mechanism and parameters of calcium dynamics. Moreover we need further consideration of overlaps of cell position in the frames and signal crosstalks between these neurons especially in analysis of spike coincidence. Therefore many algorithms using different

approaches have been proposed to infer the approximately most likely spike train of each neuron from calcium imaging data. Recently, Vogelstein et al. (2010) propose an idea to sort calcium imaging data based on a simple outer product model of cell shape and fluorescence intensity change. However, their formulation had some problems about stability and convergence of algorithm. In this paper, we proposed a improved statistical model with prior of cell shape and spike frequency and a efficient implementation of the MAP inference algorithm to the model. The model assumes that calcium imaging movies can be reconstituted to the sum of fluorescence intensity of individual cells using a modified non-negative matrix factorization algorithm. Fluorescence intensities of each cell can be also assumed to be deconvoluted to spatial filter that represent the position and shape of cell and time variation derived from spiking activities. Each spike derives transient elevation of fluorescence intensity with double-exponential shape. Spatial filters and spike timings are estimated by two iterative steps. In first step of the algorithm, we prepare tentative spatial filters and estimate spike trains corresponding to respective filters by least-square approach with non-negative restraint condition. Consequently, spacial filters are also estimated using least-square method on condition that estimated spike trains are feasible. In addition, we introduced L1 sparse regularization derived from priors which represent typical cell size and spike frequency. Lastly we evaluated the method using both simulated data and actual calcium imaging data.

Disclosures: **T. Takekawa:** None. **M. Sato:** None. **N. Ohkawa:** None. **K. Inokuchi:** None. **Y. Hayashi:** None. **T. Fukai:** None.

Poster

183. Optical Methods I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 183.16/CC4

Topic: G.04. Physiological Methods

Support: Bleser Endowed Chair of Neurology (HTW)

Baumann Research Endowment (HTW)

Title: Basic principle of Near Infrared Spectroscopy: Application in monitoring cerebral oxygenation and neuronal energy metabolism

Authors: K. SANNAGOWDARA[1], M. RANJI[3], *H. T. WHELAN[2];
[1]Pediatric Neurol., [2]Bleser Professor of Neurol., Med. Col. of Wisconsin, Milwaukee, WI; [3]Univ. of Wisconsin- Milwaukee, Milwaukee, WI

Abstract: We are exploring the possibility of combining regional cerebral oxygen saturation with neuronal energy metabolism utilizing near-infrared spectroscopy (NIRS) in conditions such as epilepsy, migraine, stroke and postural orthostatic tachycardia syndrome. Cytochrome c oxidase, also known as cytochrome aa3, is the final electron transporter enzyme in the mitochondrial respiratory chain. The redox state of cerebral mitochondrial cytochrome c oxidase (CCO) monitored with near-infrared spectroscopy (Δ[oxCCO]) is a signal with potential as a bedside biomarker of cerebral metabolic status. The relevant light wavelengths for Cytochrome c oxidase at approximately one microMolar brain tissue concentration is 600 nm for reduced-CCO, 650nm for oxidized-CCO as 620 nm serves as isosbestic point. The cyto-oximeter is a NIRS device, that will be used to detect cytochrome c oxidase redox state as well as regional cerebral oxygen saturation in-vivo and non-invasively. The oximeter uses light at multiple wavelengths to determine the relative changes in tissue oxygenation and blood volume. Near infrared (NIR) light is used for oximetry measurements because if its ability to penetrate tissues. For a 2-wavelength calculation, one below (short- 735 nm for deoxy-Hb) and one above (long- 850 nm for oxy-Hb) the isosbestic wavelength (805 nm) are used to calculate reduced and oxygenated hemoglobin. The short wavelength is attenuated more by reduced hemoglobin and the long by oxygenated hemoglobin. Measurements in absorption units (ΔOD) can be used to calculate the relative changes in the concentrations of reduced and oxygenated hemoglobin. The probe head contains a dual wavelength LED (light source) and four photodiodes (detectors). The detectors are arranged in a circle, each 2 cm from the LED and separated by 90° The LED and detectors are contained in a molded rubber probe head, which will be affixed to the patient. A daqboard will control the swithcing of the LED's and synchronize the readings from the multi-pixel photon counters (MPPC's). A software (labview) program will record the data generated by the sensors and perform basic signal processing to display the data in real time. The realtive optical densities of each of the wavelengths will be used to estimate the concentrations of oxidized & reduced cytochrome c oxidase (CCO). Available in two or four data channels, clinicians can conveniently monitor multiple brain and body areas Supported by Bleser Endowed Chair of Neurology (HTW) & Baumann Research Endowment (HTW)

Disclosures: K. Sannagowdara: None. **M. Ranji:** None. **H.T. Whelan:** None.

Poster

183. Optical Methods I

Location: Hall A

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Program#/Poster#: 183.17/CC5

Topic: G.04. Physiological Methods

Support: NIH Grant NS085729

Title: Simultaneous high speed, sensitive, sodium and calcium imaging from dendrites

Authors: *W. N. ROSS, K. MIYAZAKI;
New York Med. Col., Valhalla, NY

Abstract: High speed calcium imaging has been an important tool in examining synaptic events in dendrites. It can reveal information about events at locations in the dendrites that cannot be reached by patch electrodes and can aggregate information from many sites in the arborization. Sodium imaging can, in principle, reveal complementary information about dynamic processes that involve [Na+]i changes. However, there are far fewer studies using this technique, possibly because the fluorescence changes using currently available indicators are harder to detect than the [Ca2+]i changes. To take advantage of the potential information that sodium imaging can reveal we designed a system (a) with greater sensitivity than previous systems, (b) that can detect these signals at high speed (250 Hz or faster), and (c) can detect these changes at the same time as [Ca2+]i changes at the same locations. With this system we could simultaneously detect localized subthreshold synaptically activated [Na+]i and [Ca2+]i changes at multiples locations in the dendrites. In some cases these locations could be as small as individual dendritic spines. To achieve high speed and sensitivity we used a microscope with an Olympus 60X, 1.1 NA lens and a RedShirtImaging NeuroCCD 80x80 camera operated at 500 Hz. To detect [Na+]i and [Ca2+]i changes we injected cells with combinations of indicators that have separated excitation bands (either SBFI and OGB-1, or Bis-fura-2 and ANG-2 (a newly developed Na+ indicator from Teflabs)). High intensity LEDs, with excitation bands sharpened with narrow filters, provided illumination at the appropriate wavelengths. The LEDs we used had approximately 10X the intensity at key wavelengths as 75W Xenon lamps often used in similar experiments. Custom designed dichroic and emission filters inserted in a single filter cube allowed alternate detection of fluorescence from the two indicators when the LED source was changed. Quasi-simultaneous illumination was achieved by switching between LEDs every 2 ms with light pulse durations of about 1.8 ms. Alternate camera frames, synchronized with the switching of the LEDs, detected emission separately from each indicator. Control experiments showed that this switching protocol did not introduce additional noise into the system. Noise in each channel was dominated by the shot noise of the detected light. Software then separated the signals into two streams that were displayed along with simultaneously recorded membrane potential from the patch electrode. Localized responses from a single synaptic stimulus were often detected. The main limitation in the experiments was photodynamic damage from the high intensity illumination.

Disclosures: W.N. Ross: None. **K. Miyazaki:** None.

Poster

183. Optical Methods I

Location: Hall A

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Program#/Poster#: 183.18/CC6

Topic: G.04. Physiological Methods

Support: David Geffen School of Medicine Dean's Fund

R37 AG013622

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

1DP1MH104069

Title: Open-source miniaturized fluorescence microscope for imaging large-scale neural activity in freely behaving animals

Authors: *D. AHARONI[1,2,3,4,5], T. SHUMAN[1,4,5], D. J. CAI[2,3,4,5], J. LOU[1,4,5], M. SONG[2,3,4,5], B. WEI[2,3,4,5], I. KIM[2,3,4,5], B. S. KHAKH[2,5,6], A. J. SILVA[2,3,4,5], P. GOLSHANI[1,4,5];
[1]Dept. of Neurol., [2]Neurobio., [3]Psychiatry and Psychology, [4]Integrative Ctr. for Learning and Memory, [5]Brain Res. Inst., [6]Dept. of Physiol., UCLA, Los Angeles, CA

Abstract: Over the past decade, advancements in optical sensors and Genetically Encoded Calcium Indicators (GECIs) have led to new developments in optical recording techniques. These developments include wide-field and two photon head mounted miniaturized microscopes. Miniaturized microscopes have the potential to open up new avenues of research in neuroscience, but the specialized skills required to design and fabricate them and the high cost of purchasing them commercially has thus far limited their use. Here, we present an open-source, low cost, miniaturized wide-field microscopy system which includes all the necessary hardware, electronics, software, and analyses needed to record large-scale neural activity in freely behaving animals. The custom data acquisition (DAQ) hardware and software simultaneously records behavioral and neural video streams and syncs external devices through digital input/output connections. The DAQ software, which is multithreaded and built with Open Computer Vision (OpenCV) libraries, is capable of online image processing and thus real-time feedback. We have developed a novel, fully automated, segmentation algorithm for identifying individual cells based on the correlation of pixel intensity surrounding calcium events. Using amplitude based image registration, our system can track the activity of hundreds of identified segments (i.e., neurons) over weeks, maintaining a stable field-of-view and focal plane across recording sessions. By imaging hippocampal CA1 neurons expressing GCaMP6f in freely behaving mice, we have investigated the overlap of neural populations encoding novel contexts and followed the stability of place fields over time. Our system is easily assembled, affordable, and built around

an open-source platform to facilitate collaboration and encourage sharing of future developments.

Disclosures: **D. Aharoni:** None. **T. Shuman:** None. **D.J. Cai:** None. **J. Lou:** None. **M. Song:** None. **B. Wei:** None. **I. Kim:** None. **B.S. Khakh:** None. **A.J. Silva:** None. **P. Golshani:** None.

Poster

183. Optical Methods I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 183.19/CC7

Topic: G.04. Physiological Methods

Support: NIH LRP L30 NS063811

National Headache Foundation

Title: Optical imaging of baseline and stimulus-induced changes in facial perfusion

Authors: ***M. CORTEZ**[1], N. REA[2], L. HUNTER[2], J. PETERSON[2], J. THERIOT[2], K. BRENNAN[1];
[1]Univ. of Utah Neurol., Salt Lake City, UT; [2]Univ. of Utah, Salt Lake City, UT

Abstract: Objective: Trigeminal and autonomically mediated perfusion changes in the face (e.g. flushing, pallor) can be discerned with the naked eye and can reflect emotional, sensory and other environmental stimuli modulated by the autonomic nervous system. We combine optical imaging techniques developed for animal models of migraine with psychophysical testing and physiological monitoring to characterize craniofacial autonomic outflow. Methods: Subjects were positioned supine and imaged under green filtered light using a high sensitivity CCD camera. Skin reflectance was recorded before, during and after ammonia vapor inhalation. Heart and respiratory rate was monitored throughout the procedure. Additional testing in selected cases included correlation with pharmacological blockade, sensory and emotional stimuli and temperature challenges. Anatomically consistent regions of interest were analyzed in ImageJ for percent-change in skin reflectance. Results: Data was collected in non-headache controls and migraine subjects (a disorder known to have facial autonomic features and altered sensory thresholds). At baseline, low frequency hemodynamic fluctuations in skin reflectance were observed; these fluctuations were distinguishable from reflectance changes due to heartbeat. Oscillation amplitude and frequency increased significantly following ammonia inhalation, compared to baseline. Ammonia-induced craniofacial hemodynamic changes were associated

with a brief, non-sustained (<30 sec) increase in heart rate in most, but not all, individuals. Induced alterations in baseline oscillations were also noted due to non-painful sensory and emotional stimuli, though these differences were not significantly different between migraine and non-migraine subjects. Conclusions: Our optically-based measures of craniofacial trigeminovascular autonomic function appear to provide information independent of systemic cardiovascular function and fill a gap in current autonomic testing. Furthermore, our pilot data suggest that migraineurs and controls may be distinguishable based on baseline and stimulus-evoked facial hemodynamic oscillations. These findings expand on the current data supporting autonomic dysfunction in migraine.

Disclosures: M. Cortez: None. **N. Rea:** None. **L. Hunter:** None. **J. Peterson:** None. **J. Theriot:** None. **K. Brennan:** F. Consulting Fees (e.g., advisory boards); Advisory Board, Eli Lilly, 2015 (unrelated).

Poster

183. Optical Methods I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 183.20/CC8

Topic: G.04. Physiological Methods

Support: Burroughs Wellcome Fund

University of Cincinnati

Title: Double-sided voltage sensitive dye imaging of the nervous system of the leech

Authors: *Y. TOMINA[1], D. A. WAGENAAR[2];
[2]Dept. of Biol. Sci., [1]Univ. of Cincinnati, Cincinnati, OH

Abstract: Studies of neuronal mechanisms of sensory processing and motor control are greatly facilitated by technologies that permit recording of the activity of a large population of neurons at once. The medicinal leech *Hirudo verbana* is a classic experimental animal for comprehensive analysis of the function of neural circuits. Voltage-sensitive dye (VSD) imaging combined with electrophysiology is an increasingly powerful technology to assist in such studies. One recent dye in particular, the VoltageFluor VF2.1(OMe).H, is sensitive enough to record subthreshold events even in small cells. In the leech ganglion, all cell bodies are located on the surface of a flattened sphere, making all of them potentially accessible to imaging. However, thus far, imaging experiments have been limited to recording from one side of a ganglion. If we were able

to simultaneously image the other side, we could record the activity of all the neurons in a ganglion, allowing comprehensive analysis of the neuronal activity in the entire system. We have developed a double-sided microscope to do just this, and compared to last year's prototype, we greatly improved the ratio of signal to noise by removing vibrational noise from the CCD cameras. From simultaneously imaged neural activity on both (dorsal and ventral) surfaces of isolated leech ganglia, we successfully reconstructed stereotyped electrical activity associated with local bending behavior in response to P-cell stimulation. The technique should extend readily to other situations where researchers require simultaneous recording of activities from two distinct layers of neurons.

Disclosures: Y. Tomina: None. **D.A. Wagenaar:** None.

Poster

183. Optical Methods I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 183.21/CC9

Topic: G.04. Physiological Methods

Support: Simons Foundation Grants to MS

US National Institutes of Health (R01EY007023, R01EY018648, U01NS090473), the National Science Foundation (EF1451125)

US National Science Foundation (EF1451125)

Title: Two photon imaging with genetically -encoded calcium indicators in new world primates

Authors: *J. SHARMA[1], R. LANDMAN[2], F. YOSHIDA[2], H. SUGIHARA[3], M. SUR[4]; [1]Picower Inst. For Learning & Memory, MIT & MGH, Cambridge, MA; [2]Brain and Cognitive Sciences, MIT, McGovern Inst. of Brain Res., Cambridge, MA; [3]Brain and Cognitive Sciences, MIT, Picower Inst. for Learning and Memory, Cambridge, MA; [4]Brain and Cognitive Sciences, MIT, Picower Inst. for Learning and Memory and Simons Ctr. for Social Brain, Cambridge, MA

Abstract: Two photon imaging using calcium sensors has provided important insights into neural circuit mechanisms with unprecedented detail, particularly in mice. With the advent of highly sensitive and targettable genetically encoded-calcium indicators (such as variants of GCaMP) riding on viral backbones of various vectors and aided by cell -specific promoters, the same population of neurons can be repeatedly imaged over several weeks or months to investigate neural circuit function (and dysfunction), and study neural mechanisms and plasticity

underlying perception, cognition, learning and memory. However, achieving a similar level of sophistication in imaging higher mammals, particularly primates with more complex and dense cerebral architecture, poses new challenges. From the published literature, it is already clear that a new set of tools needs to be developed for this purpose. These include hardware for animal stabilization, movement correction of the imaging data, the development of chronic optical windows for accessing population of neurons expressing florescent proteins over several weeks, and the optimization of viral vectors and promoters while keeping brain tissue physiologically viable and in good health. Here we present our experience in developing these tools for 2 photon calcium imaging with GCaMP6 variants for chronic imaging in anesthetized new world monkeys, from several striate and extrastriate areas of the visual cortex. We modified a 2 photon imaging system (Sutter Instruments) to couple with a custom-designed movable objective that affords maximal rotational degrees of freedom along X and Y axes. We also developed a flexible imaging platform that provides precise alignment with the imaging plane of the objective, and can be tailored to suit primates of various sizes. For head stabilization we designed a dual, low-profile head post system that provides excellent rigidity while allowing flexibility to orient the head in any position to target multiple areas on the cortical surface. To minimize body movement due to respiration, we designed a simple trampoline suspension. We have also developed and tested several versions of chronic imaging windows, including sealed, removable and replaceable optical windows that are easy to maintain, while minimizing risk of infection and with flexibility to re-inject viral vectors or remove interfering pial-tissue growth. Large field ex-vivo confocal imaging was used to confirm GCaMP expression in several layers of the cortex with hSynapsin and CaMKII promoters. Two photon imaging in new world primates provides exciting possibilities as the next generation of transgenic primates come online.

Disclosures: **J. Sharma:** None. **R. Landman:** None. **F. Yoshida:** None. **H. Sugihara:** None. **M. Sur:** None.

Poster

183. Optical Methods I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 183.22/CC10

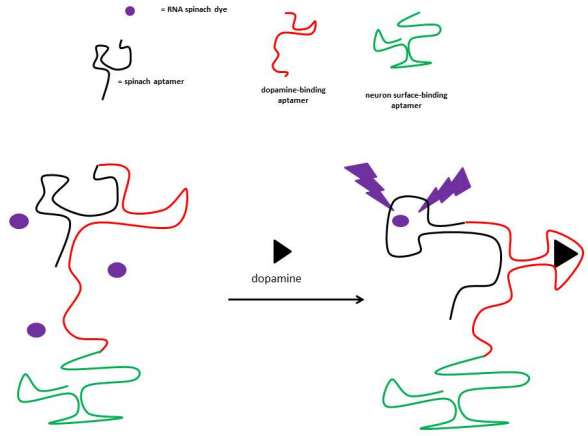
Topic: G.04. Physiological Methods

Title: Nucleic acid probes for neurochemicals optical tracking

Authors: ***J. L. CHAVEZ**[1], T. O'NEIL[2], R. CLARK[3], S. HARBAUGH[1], M. KADAKIA[3], J. A. HAGEN[1], N. KELLEY-LOUGHNANE[1];

[1]711 HPW/RHXBC, US Air Force Res. Lab., WPAFB, OH; [2]Biochem., Case Western Reserve Univ., Cleveland, OH; [3]Biochem. & Mol. Biol., Wright State Univ., Dayton, OH

Abstract: Recently, the White House Brain Research Initiative has highlighted the need for new tools to provide direct measurements of neurotransmitters like GABA, dopamine, serotonin and neuropeptides with improved temporal and spatial resolution. The information gathered with these tools will allow a deeper understanding of the flow of information across different brain circuits that affect arouse, emotion, motivation, and other processes. Our group is working on developing nucleic acid-based optical probes integrating multiple functional recognition elements to track secretion of different neurochemicals with spatial and temporal resolution during different cognitive processes in the brain. These tools will be used to monitor the release of neurochemicals that currently cannot be tracked directly or whose release is typically inferred by calcium ion activity, allowing a better understanding of neural communication. Our group has designed probes with nanoscale dimensions carrying multiple functionalities to allow precise probe placing on the surface of neurons, and biorecognition elements (BREs) for selective binding of neurotransmitters and neuropeptides. Importantly, these probes show high fluorescence in the presence of the target with minimal background signal in its absence, by utilizing RNA-based green fluorescence protein mimics. As a proof-of-concept, we designed a probe whose fluorescence is turned on by binding dopamine. The probe was optimized by testing different dopamine-binding aptamer clones, each with different sequences, lengths and affinities. Moreover, we supported our probe design by computational methods to optimize the linker section between the fluorescence probe and the analyte binding aptamer. In this presentation we will present the characterization of the probes' response linear range, limit of detection and binding kinetics *in vitro* and will present preliminary results in cell culture.



Disclosures: **J.L. Chavez:** None. **T. O'Neil:** None. **R. Clark:** None. **S. Harbaugh:** None. **M. Kadakia:** None. **J.A. Hagen:** None. **N. Kelley-Loughnane:** None.

Poster

183. Optical Methods I

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Topic: G.04. Physiological Methods

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NSFC Grant 81227901

NSFC Grant 61231004

NSFC Grant 81071205

NSFC Grant 81101095

NSFC Grant 81027002

NSFC Grant 81471739

Title: A multi-scale brain imaging strategy for small animals *in vivo*

Authors: *H. HUI[1,2], D. DONG[2], X. MA[2], X. YANG[2], J. TIAN[2];
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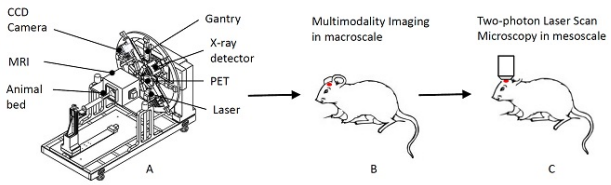
Abstract: The development of *in vivo* molecular imaging techniques has allowed the study of brain disease in intact brains of living animals. These imaging modalities can be used for performing the dynamic process both in macroscale and in mesoscale, namely, with resolution from millimeter to sub-micrometer. In this work, we propose a multi-scale strategy for *in vivo* imaging of mouse brain. We have developed a multimodality system for whole body imaging of small animals, in which Computed Tomography (CT), Fluorescence Molecular Tomography (FMT), Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) have been integrated. This hybrid molecular imaging system can be used to acquire anatomical, functional and metabolic information of brain disease in macroscale. To understand the mechanism of brain disease in its neuronal microenvironment, high-resolution imaging technology in mesoscale is required, such as two-photon laser scan microscopy (TP-LSM) because it can significantly reduce the background signal and allow imaging of thick living samples up to about 1mm depth with subcellular resolution. In this approach, a high-speed TP-LSM was used for acquiring imaging data in mesoscale. The setup of multimodality imaging system is shown in Figure 1A. Firstly, *in vivo* imaging is performed by multi-modality system. The segmentation, registration and visualization of the acquired images are conducted by our

developed software 3DMed and MITK to locate the three-dimensional position of brain disease. Then, a small craniotomy is made for the same mouse using a high-speed drill with a small-tip steel burr at the corresponding position. A craniotomy preparation process was carefully conducted for *in vivo* two-photon imaging. An appropriate region of the neural population was chosen. The region of interest (ROI) in the center of the FOV was placed to image neurovascular connections and monitor neural activities. The proposed approach has potential to be applied for monitoring diagnoses and therapy processes of brain disease both in macroscale and mesoscale.



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Poster

183. Optical Methods I

Location: Hall A

Time: Sunday, October 18, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 183.24/CC12

Topic: G.04. Physiological Methods

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Title: Image processing technique for an implantable image sensor with self-resetting function

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Abstract: An implantable image sensor deigned and fabricated with sophisticated complementary-metal-oxide-semiconductor (CMOS) technology is expected to be useful for neural activity imaging. The size and weight of the sensor are much smaller and lighter those of an adult mouse. By using a needle shape sensor, it is possible to observe deep brain area with some invasiveness. In order to observe weak intensity change signals, we have proposed to introduce a pixel array with self-reset function and realized approximately 60 dB of signal-to-noise ratio with intense illumination. In the sensor, the function to count the number of self-resetting is not equipped to reduce the pixel size and increase the spatial resolution. In this study, we developed image processing technique to generate intensity change images from images

obtained with the self-resetting image sensor. The pixel of the self-resetting sensor is reset by itself when the received optical energy becomes higher than its threshold. The number of self-resetting increases as the light intensity increases. Thus, there is the possibility that the number of self-resetting is different even if the sensor output is the same. It means that the number of self-resetting should be counted for general purposes. However, in many cases of living tissue observations, it is important to measure intensity changes. Thus, the it is not necessary to count the number of self-resetting. In this study, we performed the observation of blood flow through vessels on a rat brain. First, an image of intensity distribution was obtained with illumination as weak as no self-reset occurs. Next, the illumination was increased. Boundaries of self-reset number are observed as fringes. Here, the pixel intensities on the blood vessels change frame by frame due to blood flow. We enhanced the intensity changes by subtracting a reference image, which is an average image during the observation period as a reference image. The image was normalized by that with the low intensity illumination. On the boundary of the self-reset numbers, we defined a threshold and the output value was corrected if the output change is higher than the threshold. As a result of the image processing, we obtained images clearer than our previous sensors. By using this technique, the implantable self-resetting sensor can be applied for not only blood flow imaging but hemoglobin oxidation state or voltage sensitive dye imaging etc.

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