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Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.01/A1

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant EY027970
WMU FRACAA

Title: Characterizing retinal regeneration in a genetic mouse model of glaucoma

Authors: *J. R. PARIS[1], N. C. SKLAR[2], C. L. LINN[1];
[1]Biol. Sci., Western Michigan Univ., Kalamazoo, MI; [2]Homer Stryker MD Sch. of Med., Kalamazoo, MI

Abstract: Glaucoma is a degenerative retinal disease characterized by loss of vision due to progressive loss of retinal ganglion cells (RGCs). Previous studies in this lab have shown that the application of a specific α7 nicotinic acetylcholine receptor agonist, PNU-282987 (PNU), to the murine eye induces neurogenesis of numerous retinal cell types, including retinal ganglion cells (Webster et al., 2017). The aim of this study is to characterize the short-term and long-term effects of PNU in a glaucoma model. To address this issue, the effects of PNU were analyzed in a DBA/2J mouse model that auto-induces a glaucoma-like condition in adulthood. These mice manifest an elevated intraocular pressure (IOP) starting at 6 months, followed by loss of ganglion cells. We hypothesized that PNU would act to regenerate new retinal neurons in a genetic mouse model of glaucoma. PNU (1mM) and BrdU (1 mg/mL) were applied as eye drops for 2 weeks to male and female DBA/2J mice at various ages (3, 6, and 10 months) to determine the regenerative effects that occur at each of these time points. After two weeks of treatment, the retinas from both the control animals and from the treatment animals were removed, fixed, processed using IHC procedures, and RGC regeneration was assessed and compared. Antibodies against BrdU were used to identify new cells. Retinal ganglion cells were identified using antibodies against Thy-1.2. Cell counts were obtained using confocal images of flat mounted retinas. Four images were taken of the retinal ganglion cell layer of each retina, each image taken from a different quadrant of the retina and RGC counts were averaged. A one-way ANOVA was used to compare the number of cells between the different time points. IOP measurements were obtained under control and treated conditions. IOP measurements in untreated animals significantly changed from an average of 10.79 to an average of 19.79 mmHg ($p \leq 0.01$; $n=8$). Results showed a significant loss of retinal ganglion cells between the 3-month-old and the 10-month-old control mice ($p \leq 0.01$; $n=5$). The average number of RGCs dropped from 310($\pm$21) in 3 month DBA/2J animals to 221($\pm$12) in 10 month DBA/2J animals, a loss of about 30%. Similarly, the 10-month-old DBA mice had about 25% fewer cells than non-DBA control mice

at the same age ($p \leq 0.01$; n=5). The addition of PNU caused about a 15% increase in the number of retinal ganglion cells in all time points compared to the untreated DBA/2J retinas ($p \leq 0.01$, n=4). These were verified to be new cells using BrdU labeling. PNU treatment had no effect on IOP measurements. These results suggest the future clinical potential of PNU-282987 in the treatment of degenerative retinal diseases.

Disclosures: **J.R. Paris:** None. **N.C. Sklar:** None. **C.L. Linn:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.02/A2

Topic: A.04. Transplantation and Regeneration

Support: JSPS KAKENHI 26250019, 17H01392, JP16H06280, 25111727, 23680041, 17K07114, S2704
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NEXT LS104
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Title: New neurons migrate through the glial meshwork using Slit1 to approach the lesion for functional recovery

Authors: *N. KANEKO[1], V. HERRANZ-PÉREZ[2], T. OTSUKA[3], H. SANO[4], N. ONO[5], T. OMATA[1], H. NGUYEN[6], T. THAI[6], A. NAMBU[4], Y. KAWAGUCHI[3], J. GARCÍA-VERDUGO[2], K. SAWAMOTO[1,7];
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Abstract: Although new neurons continuously generated in the ventricular-subventricular zone (V-SVZ) migrate toward the lesion, the ability of the mammalian brain to regenerate neuronal circuits for functional recovery is quite limited. We investigated the mechanism regulating migration of the new neurons and their involvement in functional regeneration using a mouse

model for ischemic stroke. By time-lapse imaging and immunohistochemistry, we found that migration of immature new neurons referred to as neuroblasts was restricted by astrocytes activated in response to the tissue damage in and around the lesion. The neuroblasts secreted a diffusible protein Slit1 to disrupt the actin cytoskeleton in activated astrocytes at the site of contact via its receptor Robo2 for their migration through the meshwork of the astrocytic processes. By enhancing the Slit1 expression, V-SVZ-derived neuroblasts transplanted into the post-stroke brain could migrate closer to the lesion in the dorsolateral striatum. Some of the transplant-derived cells differentiate into mature neurons possessing morphological and electrophysiological properties of the striatal projection neurons. The proportion of the transplant-derived axons projecting into the lateral globus pallidus, where neurons in the lateral striatum preferentially project under physiological conditions, was significantly increased in the Slit1-overexpressing group compared with the control, suggesting that the new neurons that were positioned close to the injured area functionally replaced the neurons lost by stroke. Slit1-overexpression further caused long-lasting functional recovery in the post-stroke animals. Taken together, these results suggest that the positioning of new neurons is critical for functional neuronal regeneration in stem/progenitor cell-based therapies for brain injury.

Disclosures: **N. Kaneko:** None. **V. Herranz-Pérez:** None. **T. Otsuka:** None. **H. Sano:** None. **N. Ono:** None. **T. Omata:** None. **H. Nguyen:** None. **T. Thai:** None. **A. Nambu:** None. **Y. Kawaguchi:** None. **J. García-Verdugo:** None. **K. Sawamoto:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.03/A3

Topic: A.04. Transplantation and Regeneration

Support: NSERC Discovery Grant (2017-00008)
NSERC Discovery Grant (2015-003780)

Title: Endocannabinoid signalling during axolotl spinal cord regeneration

Authors: M. TOLENTINO, M. MORNEAULT, G. SPENCER, ***R. L. CARLONE**;
Brock Univ., St. Catharines, ON, Canada

Abstract: Research into the molecular mechanisms of the psychoactive effects of cannabis has led to the discovery of the endocannabinoid system (ECS), a neuromodulatory system conserved in many organisms. Although evidence for its modulatory role in normal CNS development is increasing, far fewer studies have focussed on its function in response to trauma in the CNS in mammals. Moreover, little is known regarding the role of endocannabinoids in CNS regeneration-competent amphibian species like the axolotl, one of the few vertebrates that can

regenerate a functioning spinal cord. We provide preliminary evidence that expression of the two main ECS receptors in the CNS (CB1 and CB2) are upregulated in the regenerating caudal spinal cord and tail tissues of larval axolotls at 7 and 14 days post amputation. In addition, bath application of the CB1 selective antagonist/reverse agonist, AM251, significantly inhibited caudal growth of the spinal cord and tail by 7 days post amputation. Preliminary immunofluorescence studies demonstrate expression of this receptor primarily in the ependymal cells of the spinal cord and dermal tissue of regenerates. The determination of CB1 and CB2 mRNA levels by qPCR and further immunofluorescent analyses to identify the tissue and cellular distribution of both receptors during specific stages of regeneration and after inhibition with AM251 (and AM630, a selective CB2 reverse agonist) are presently underway. Studies to assess the effects of CB1 and CB2 inhibition on proliferation and differentiation of ependymoglial cells as well as the production of transgenic animals expressing optogenetically regulated CB1 and CB2 receptor reporter constructs are in progress. These studies are the first to determine the role of the ECS during spinal cord regeneration in a regeneration-competent vertebrate, and may aid in developing novel therapies for human nervous system injuries or pathologies.

Disclosures: **M. Tolentino:** None. **M. Morneault:** None. **G. Spencer:** None. **R.L. Carlone:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.04/A4

Topic: A.04. Transplantation and Regeneration

Support: Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI17C2160)

Title: Glia-like cells from human adult stem cells protect against ischemic stroke through IGFBP-4

Authors: J. SON[1], J. PARK[2], Y. KIM[1], J. HA[2], D. PARK[1], S.-H. KOH[1], ***M.-S. CHANG**[2]; [1]Hanyang Univ. Col. of Med., Seoul, Korea, Republic of; [2]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Early-passage human mesenchymal stem cells (hMSCs) are typically used in clinical trials because of safety and efficacy issues. However, obtaining sufficient cells for treatment is difficult and expensive. Many more late-passage hMSCs can be obtained at lower cost, although efficacy is a large hurdle in clinical trials. The use of late-passage MSCs with better efficacy

would be a revolutionary solution for reducing cost and facilitating clinical trials. In the present study, glia-like cells (ghMSCs) were induced from hMSCs and used for both in vitro and in vivo models of ischemia, effectively protecting neurons from ischemia and restoring brain tissue damaged by cerebral infarction. These beneficial effects were significantly blocked by insulin-like growth factor-binding protein-4 (IGFBP-4) antibody. The current study demonstrated that late-passage hMSCs can be efficiently induced into ghMSCs with a better neuroprotective effect against ischemic stroke and that IGFBP-4 may be a key neuronal survival factor secreted by ghMSCs.

Disclosures: J. Son: None. **J. Park:** None. **Y. Kim:** None. **J. Ha:** None. **D. Park:** None. **S. Koh:** None. **M. Chang:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.05/A5

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R21NS108053

Title: Single cell transcriptional profiling of the corticospinal tract

Authors: *N. GOLAN[1], K. P. LUTTIK[1], W. B. CAFFERTY[2];
[2]Dept. of Neurol., [1]Yale Univ., New Haven, CT

Abstract: In addition to regeneration of axotomized CNS axons, localized sprouting or plasticity of intact axons plays an integral role in functional recovery after spinal cord injury (SCI). Therefore, defining and exploiting the molecular machinery that drives growth of intact axons post-injury could be a valuable approach to repair after SCI. The corticospinal tract (CST) presents a unique opportunity to explore the molecular mechanisms that are required to 'build' and 'rebuild' a spinal tract, as it transitions early postnatally from a growth competent mode (P0-10, build phase), to a circuit refinement mode (P14-P28, 'critical period'), to an adult growth incompetent mode (P56 and beyond), and critically, undergoes functional plasticity after partial SCI (re-build phase).
To explore the molecular mechanisms of these growth phases, we utilized the CST-specific *crym*-GFP mouse line to selectively isolate and transcriptionally characterize corticospinal motor neurons (CSMNs) in neonatal (P5), adolescent (P28) and adult (P56) mice using laser capture microdissection followed by bulk RNAseq. At P5, we identified differentially expressed genes associated with axon guidance, including *Robo1* and *sema6D*, consistent with CSMN pathfinding; at P28, genes associated with synaptic refinement, such as the *synaptotagmins*, consistent with CSMN synaptogenesis and pruning; and at P56, genes associated with synaptic

transmission, such as *Grik3* and *Grik4*, consistent with synaptic homeostasis. These temporal differential gene expression analyses reveal that a spectrum of cell autonomous growth machinery is engaged to build and sculpt a functional CST. However, while bulk sequencing of CSMNs broadly uncovers robust transcriptional events defining these growth epochs, it lacks the resolution to identify discrete anatomical and/or functional sub-populations.
To address this, we developed a method to identify and isolate single CSMNs at P5, P28, and P56. To gain functional specificity we used a layer V specific *cre* line (retinol binding protein-4, *rbp4-cre*) crossed with a Ai14 reporter line. To gain anatomical specificity we used retrograde viral tracing from the cervical spinal cord. We then leveraged fluorescent activated cell sorting (FACS) and single cell RNA sequencing (scRNA-seq) to comprehensively characterize CSMN heterogeneity and uncover the emergence of CSMN populations during these critical time points. These data will lay groundwork for identifying key intrinsic modulators of CST patterning to singularly or sequentially exploit after SCI.

Disclosures: **N. Golan:** None. **K.P. Luttik:** None. **W.B. Cafferty:** None.

Poster

363. Axon Regeneration

Location: Hall A

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Program #/Poster #: 363.06/A6

Topic: A.04. Transplantation and Regeneration

Support: R01EY018417
R01EY024519

Title: Sequential generation of neuronal cell types in the regenerating adult zebrafish retina

Authors: *M. LAHNE, D. R. HYDE;
Biol. Sciences, Ctr. for Stem Cells and Regenerative Med., Univ. of Notre Dame, Notre Dame, IN

Abstract: Müller glia in the damaged adult zebrafish retina reenter the cell cycle to produce neuronal progenitor cells (NPCs) that continue to proliferate and ultimately differentiate into both the lost neuronal cell types and those unaffected by the damage stimulus. Generating all neuronal cell types raises the questions, whether NPCs commit to different neuronal cell fates in a sequential order that is similar to retinal development and whether additionally-produced neuronal cell types integrate into the retinal circuit. To test whether retinal neurons in the regenerating retina are generated sequentially, *albino;Tg[atoh7:GFP]* or *albino;Tg[ptf1a:GFP]* zebrafish, which drive GFP expression under the control of either the ganglion or amacrine/horizontal cell commitment factors, respectively, were exposed to constant intense light for up to 96 hours (hLT). While Müller glia and NPCs proliferated at 36 and 48 hLT,

atoh7:GFP was only robustly expressed in a subset of NPCs in the inner (INL) and outer nuclear layers (ONL) starting at 60 hLT. In contrast, GFP expression under the *ptf1a* promoter began at 72 hLT in the INL and ONL, suggesting that amacrine/horizontal cell fates are induced subsequent to ganglion cell fates. Similarly, *atoh7:GFP*-positive cells expressed the postmitotic ganglion/amacrine cell marker, HuC/D, in the INL at 72 hLT prior to the presence of HuC/D in *ptf1a:GFP*-positive cells at 84 hLT. The *atoh7:GFP* and HuC/D double-positive ganglion cells began to accumulate in the ganglion cell layer (GCL) at 84 and 96 hLT. Retinal flatmounts revealed that these GCL-based *atoh7:GFP*-positive cells displayed neurite/axonal outgrowth at 84 hLT and subsequently at 96 hLT, *atoh7:GFP*-positive axons began fasciculating to form nerve tracks. A subset of INL-based *ptf1a:GFP*-positive amacrine cells also properly projected their axons to the inner plexiform layer at 96 hLT. In summary, temporal ganglion and amacrine/horizontal specification in the light-damaged retina mimics the sequential order of cell type determination in development and these newly produced ganglion/amacrine cells, which were not lost in response to light-damage, display properties of cells integrating into the neuronal circuit in the adult regenerating zebrafish retina.

Disclosures: **M. Lahne:** None. **D.R. Hyde:** None.

Poster

363. Axon Regeneration

Location: Hall A

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Program #/Poster #: 363.07/A7

Topic: A.04. Transplantation and Regeneration

Support: EY027970
WMU FRACAA

Title: Application of an alpha7 nicotinic acetylcholine receptor agonist in the adult mammalian retina causes retinal pigment epithelium mediated neurogenesis and regeneration in a murine glaucoma model

Authors: *S. E. WEBSTER[1], N. C. SKLAR[2], M. K. WEBSTER[1], D. M. LINN, Jr[3], C. L. LINN[1];
[1]Biol. Sci., Western Michigan Univ., Kalamazoo, MI; [2]Western Michigan Univ. Homer Stryker MD Sch. of Med., Kalamaoo, MI; [3]Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Neurodegenerative diseases of the eye cause blindness and are incurable. Application of PNU-282987 (PNU), an α7 nicotinic acetylcholine receptor agonist (nAChR), causes neurogenesis of the retina in the adult mammal (Webster et. al. 2017). These new neurons arise from the Müller glia (MG) which mirrors regeneration in other vertebrates. However, the MG lacks α7 nAChRs. Our objective was to better understand where PNU acts in the mammalian eye

to initiate neurogenesis and determine if PNU causes regeneration of new retinal ganglion cells (RGCs) in a glaucoma model. Our hypothesis was that PNU activates retinal pigment epithelium (RPE). To test this, RPE-J cell lines were treated with PNU for 24 hours before extensive washing. After 72hr in culture, the supernatant was collected and injected into both male and female adult mouse eyes. BrdU (1mg/mL) eyedrops were given for 7 – 14 days. IHC demonstrated incorporation of BrdU in all three nuclear layers after supernatant injection. In the INL, treated-RPE supernatant induced 50% (±10) BrdU-positive cells. To validate that PNU works through the α7 nAChRs in the RPE-J cell line, cells were pre-treated with an α7 antagonist, MLA, before PNU treatment. MLA decreased BrdU-positive cells to less than 5% (±1) which represents a significant reduction from those treated with PNU-modified supernatant ($n=6, p\leq 0.01$). To analyze potential genes involved in this process, RNAseq was performed on PNU-treated RPE-J cells. Cells were treated with PNU for 0.5, 1, 3, 8, and 12 hours before total RNA was collected (RIN ≥ 7.0). Controls were DMSO-treated, MLA-treated, and nicotine-treated. RNA was processed by GeneWiz; fold-change data was analyzed in Excel and pathway analysis through Reactome software. Resulting signaling molecules were mapped for fold-change expression over time. To determine if PNU can act in a regenerative capacity, we examined the effect of PNU in an induced mouse model of glaucoma. Control retinas with glaucoma show an average loss of 25% (±5) of Thy1.2-positive RGCs 28-days post induction surgery, which were correlated with an average increase of IOP to 14.38 mmHg (±1.7). Glaucomatous retinas treated with PNU for 7 days showed regeneration and RGC cell counts returned to control levels. This research demonstrates a novel mechanism for mammalian retinal neurogenesis mediated through RPE cells and indicates the influence of several important signaling molecules. Further, this work shows that PNU treatment caused regeneration of RGCs after damage has taken place. This is significant as it points to a potential therapeutic approach to the millions affected by neurodegenerative retinal diseases.

Disclosures: S.E. Webster: None. **N.C. Sklar:** None. **M.K. Webster:** None. **D.M. Linn:** None. **C.L. Linn:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.08/A8

Topic: A.04. Transplantation and Regeneration

Support: EY027970
WMU FRACAA

Title: Changes in gene expression associated with dedifferentiation of Müller glia in response to an alpha7 nicotinic acetylcholine receptor agonist

Authors: *M. L. STANCHFIELD, S. E. WEBSTER, M. K. WEBSTER, C. L. LINN;
Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: The adult mammalian retina does not typically regenerate. As a result, there is no cure for neurodegenerative diseases, such as glaucoma. We have found dedifferentiation of Müller glia (MG) occurs in the adult mammalian retina after application of an α7 nicotinic acetylcholine receptor (nAChR) agonist, PNU-282987 (PNU), to retinal pigment epithelial (RPE-J) cells in adult rodents (Webster, et al., 2019). RNAseq was performed in a MG cell line to determine changes in gene expression profiles following contact with RPE-J cells treated with PNU. RPE cultures were treated for 24 hours with 100 nM PNU and thoroughly washed to remove any residual PNU. MG were then exposed to treated RPE cells using a transwell system for 8, 12, 24 and 48 hours. A separate RPE culture was treated with DMSO for 48 hours as a vehicle control. Total RNA extracted from MG was sent to GeneWiz for Next Generation Sequencing (RIN>7) and basic bioinformatics was performed to determine significant changes in expression following transwell culture with treated RPE-J cells compared to control conditions. Up or down-regulated genes were compared with published literature of MG dedifferentiation that occurs in lower vertebrate regeneration or during early mammalian development. Between 8-12 hours, upregulation was observed in heparin-binding epidermal-like growth factor (HB-EGF) (log_2 fold change (FC) increase of 1.92). HB-EGF is rapidly induced in MG residing at an injury site in the neuronal retina of zebrafish and has been found to regulate the expression of Ascl1 (Goldman, et. al., 2012). After 48 hours, significant upregulation was found in genes Ascl1 (log_2 FC increase of 3.99) and Lin28a (log_2 FC increase of 4.77). Both Ascl1 and Lin28a are rapidly induced in dedifferentiating MG following injury in zebrafish. Ascl1 is a transcription factor essential for retinal regeneration in zebrafish (Goldman, et. al., 2008). Lin28a is an RNA binding protein whose expression has been found to share characteristics with embryonic stem cells (Nimmo & Slack, 2009). Importantly, downregulation was observed in BMP4 (log_2 FC decrease of -1.43) after 8 hours, which is a glial differentiation signal found to be necessary to fully repress glial gene expression for neurogenesis in early development in mice (Ueki, et al., 2015). These results suggest that MG are dedifferentiating in response to PNU-treated RPE-J cells. Analyzing these changes in gene expression could lead us to further our understanding of the barriers associated with adult mammalian neurogenesis and provide potential strategies to treat neurodegenerative diseases.

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Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.09/A9

Topic: A.04. Transplantation and Regeneration

Support: KAKENHI 17H05569, 16H06460

Title: The involvement of reactive glial cells in the formation of ectopic corticofugal projections after cortical ablation

Authors: L. CHANG, *N. SUGO, N. YAMAMOTO;
Osaka Univ, Grad Sch. Frontier Biosci, Suita, Osaka, Japan

Abstract: The brain function is known to recover after the injury by reorganizing neural circuits. After a unilateral cortical lesion, descending axons from the intact cortex are known to project ectopically to the contralateral midbrain nuclei, contributing to the functional recovery. However, the molecular mechanism for the lesion-induced axonal remodeling remains unknown. To address this issue, we investigated gene expression in the midbrain after unilateral cortical ablation, under the hypothesis that growth promoting factors are expressed in the denervated midbrain. For this, RNA-Seq analysis and the subsequent *in situ* hybridization were performed in the midbrain 2-4 days after hemispherectomy at P6 mice. The analyses demonstrated that a significant number of genes were upregulated in the denervated midbrain compared to the intact side and control midbrains, and were mostly found to be those expressed by astrocytes or microglia. Immunohistochemistry further revealed that GFAP-positive reactive astrocytes were distributed not only at the cerebral peduncle of the denervated midbrain but also at the area near the midline. Iba1-positive ramified microglia were broadly distributed in the midbrain after hemispherectomy, and Arg1-positive M2 microglia, a fraction of microglia, which are known to produce axon growth factors were also present in the denervated midbrain. Moreover, corticofugal axons labeled with EGFP by *in utero* electroporation at E12.5 were found to grow in the vicinity of the region where reactive glial cells emerged after hemispherectomy. Taken together, the present results demonstrate that reactive astrocytes and microglia cells emerge in the denervated midbrain and around the midline after hemispherectomy, suggesting that growth-promoting factors produced by reactive glial cells may contribute to the reorganization of axonal projections after the cortical injury.

Disclosures: L. Chang: None. **N. Sugo:** None. **N. Yamamoto:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.10/A10

Topic: A.04. Transplantation and Regeneration

Support: NIH R01 NS107039

Title: Enhanced stem cell therapy as a delayed treatment strategy for neonatal stroke

Authors: A. LARPTHAVEESARP[1], P. PATHIPATI[1], A. RAJAH[1], S. OSTRIN[1], D. M. FERRIERO[2], *F. GONZALEZ[1];
[1]UCSF, San Francisco, CA; [2]Pediatrics, Univ. of California San Francisco, San Francisco, CA

Abstract: Background: Stroke in the newborn period is a significant cause of mortality and life-long morbidity. In recent years, cell-based therapies have emerged as a potential treatment for different various CNS diseases. These improved outcomes occur even in the absence of survival of engrafted cells, suggesting transplanted cells may induce repair by stimulating secretion of growth and differentiation factors that provide an environment to enhance repair. It is possible that growth factors that have specific effects on stem cell function, including their role in the development of the neurovascular unit and cell fate, may provide additional benefit. **Objective**: To compare the efficacy of single-dose mesenchymal stem cells (MSC) pretreated with erythropoietin [EPO] (MSC-EPO) into the rat CNS versus MSC alone following transient neonatal focal ischemia-reperfusion on long-term histological and behavioral outcomes. **Design/Methods**: Focal cerebral ischemia-reperfusion was performed in P10 Sprague-Dawley rats by transient right middle cerebral artery occlusion (MCAO) for 3 hours, or sham surgery under isoflurane anesthesia. This age is considered equivalent to the full-term human newborn. Either vehicle, MSC ($1x10^6$ cells) or MSC pre-treated with EPO (1 IU/mL x 24 hours) were administered intranasally 72 hours following reperfusion. N=8 per group. Animals underwent sensorimotor (cylinder rearing) and cognitive (novel object recognition) testing at 8 weeks of age, after which brains were harvested for histological analysis. **Results**: MSC alone and MSC-EPO groups had a significant improvement in calculated hemispheric brain volumes (ipsi/contra ratio) at 2 months of age. While both treatment groups had improved sensorimotor function and cognitive memory retention testing compared to vehicle-treated MCAO animals, MSC-EPO animals had a significant improvement in both functional measures compared to MSC alone animals. **Conclusions**: A delayed, single-dose combination strategy to enhance cell type-specific effects using MSC and EPO provides the most long-term benefit following early stroke, lasting until young adulthood. The specific effects of this strategy on vasculogenesis, cell proliferation and fate remains to be determined.

Disclosures: A. Larpthaveesarp: None. **P. Pathipati:** None. **A. Rajah:** None. **S. Ostrin:** None. **D.M. Ferriero:** None. **F. Gonzalez:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.11/A11

Topic: A.04. Transplantation and Regeneration

Support: Korea Brain Research Institute funded by Ministry of Science and ICT(19-BR-02-07)
Brain Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry and ICT (2015M3C7A1029037)

Title: Development of clarified tissue scaffold using oil-based decellularized method for tissue engineering

Authors: *Y.-J. JANG, B. HA, S.-J. JEONG;
Korea Brain Res. Inst., Daegu, Korea, Republic of

Abstract: Tissue engineering is a field of regenerative medicine ranging from cells to artificial organs. It is based on a discipline that deals with biological and engineering techniques from biomaterials to materials that can help restore tissues and organs. Various methods have been studied to understand the relationship between structures and function of living tissue, and further to make a living body substitute and implant, thereby restoring, maintaining and improving the functions of the body. Particularly, one of the key technologies of tissue engineering is to produce scaffolds that support cells to grow, and the scaffolds are usuful for the biomedical engineering. The scaffolds can be made of a variety of materials, and the related studies are being actively conducted to develop a scaffold for tissue regeneration using natural materials, synthetic polymers, bioceramics, and polymer-ceramic composite materials. Among various tissues in the human body, brain tissue has higher cell density and weaker mechanical strength of tissues than other body tissues. When cells are removed by applying a general decellularization method for the production of scaffolds, the various problems have to be considered because the tissue is easily to be damaged and the shape is not completely preserved occurring the low reproducibility. In order to develop the suitable decellularization method for brain tissue, several attempts have been made to solve these problems by changing various parameters such as concentration of several chemical components, appropriate temperature and time. In this study, we confirmed that it is possible to preserve the intact form of brain and microarchitecture when the fixed brain tissues were decellularized with 4% hydrogel followed by the immersion in oil. Moreover, the protein and extracellular matrix present in the tissue are observed as an intact form without any damaging. Therefore, we suggest that this method can be widely used in various fields requiring tissue regeneration.

Disclosures: Y. Jang: None. **B. Ha:** None. **S. Jeong:** None.

Poster

363. Axon Regeneration

Location: Hall A

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Topic: A.04. Transplantation and Regeneration

Support: Deutsches Zentrum für Neurodegenerative Erkrankungen
International Foundation for Research in Paraplegia
Wings for Life
ERANET RATER SCI
ERANET AXON REPAIR
European Molecular Biology Organization (EMBO) ALTF 28-2017
Craig H. Neilsen Foundation (Neilsen Foundation)

Title: High resolution 3D imaging and analysis of axon regeneration in unsectioned spinal cord with or without tissue clearing

Authors: *B. J. HILTON[1], O. BLANQUIE[1], A. TEDESCHI[2], F. BRADKE[1];
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Abstract: Here we present a protocol for analyses of axon regeneration and density in unsectioned adult mouse spinal cord. This includes methods to injure and trace dorsal column sensory and corticospinal axons; clear and stain unsectioned spinal cord; visualize axon degeneration and regeneration in cleared and uncleared specimens using 2-photon microscopy; and analyze axon density and regeneration in 3-dimensional space either manually or semi-automatically using Imaris and ImageJ software. This protocol can be used to understand the molecular and cellular mechanisms underlying nervous system degeneration and regeneration and to establish the therapeutic efficacy of candidate neuroregenerative treatments. By obviating tissue sectioning, this protocol enables unambiguous evaluation of regeneration and greatly accelerates the speed at which analyses can be conducted. Surgical procedures take <30 min per mouse, with a wait period of 2 weeks between axonal injury and tracing and 2-8 w between tracing and tissue processing. Clearing and immunolabelling take ~1-2 w depending on the size of the sample. Imaging and analysis can be performed in 1 d. All these procedures can be accomplished by a competent graduate student or experienced technician.

Disclosures: B.J. Hilton: None. **O. Blanquie:** None. **A. Tedeschi:** None. **F. Bradke:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.13/A13

Topic: A.04. Transplantation and Regeneration

Support: Indiana Spinal Cord and Brain Injury Research with the Indiana State Department of Health
Morton Cure Paralysis Fund

Wings for Life

Title: RGDS and IKVAV peptide-modified capillary alginate hydrogels to improve axon regeneration after spinal cord injury

Authors: *P. KUMAR[1], R. MÜLLER[2], A. BLESCH[1];
[1]Dept. of Neurolog. Surgery, Indiana Univ. Sch. of Med., indianapolis, IN; [2]Dept. of Physical and Theoretical Chem., Univ. of Regensburg, Regensburg, Germany

Abstract: Biomaterials in combination with other experimental approaches such as cell grafts have shown some promise in promoting tissue sparing, increasing remyelination and enhancing axonal regeneration in the injured spinal cord by providing physical and chemical guidance cues. Frequently, biomaterials with a random spongy microstructure, which only promote randomly oriented axonal growth, have been examined limiting the lesion distance that can be bridged. To overcome these problems, we have used alginate-based hydrogels with linear anisotropic capillaries.
Previously, we have shown that these alginate hydrogels are stable, biocompatible and support linear host axon growth into and beyond a lesion site. In the current study, we modified the surface of the hydrogels with RGDS and IKVAV peptides, the active fragment of fibronectin and laminin, respectively. An N-terminal positively charged poly-L-lysine tail was included in active peptides (K12-QAAGRGDS and K12-QAASIKVAV) for electrostatic binding to -at physiological pH- negatively charged hydrogels. Uncoated and poly-D-lysine (PDL) coated gels served as controls. ELISA depletion assays with hydrogel blocks (2×2×1.3 mm) showed that the blocks were saturated with 50-100 µg of each peptide after incubation at 37°C for 16h. *In vitro*, hydrogel slices (7×7×0.2 mm) modified with RGDS peptide resulted in a 2-fold increase in the attachment of postnatal day 4 astrocytes compared to PDL-coated hydrogels after 16h. The same effect was also observed when coated hydrogels were first incubated for one week at 37°C in PBS with daily washing. Furthermore, surface modification using IKVAV peptide showed a 27% increase in neurite length from differentiated E14 neural stem cells (NSCs) compared to PDL-coated hydrogels 48h post cell seeding. To evaluate whether peptide modification enhances *in vivo* bridging of neural stem cell transplants across a lesion site, adult female F344 rats underwent a C4 unilateral hemisection (removing 2 mm spinal tissue) followed by implantation of hydrogel blocks and grafting of E14 NSCs rostral to the lesion site. Ongoing tissue analyses examine whether peptide-modified hydrogels improve bridging of grafted axons into the distal spinal cord, and/or promote host axon regeneration.

Disclosures: **P. Kumar:** None. **R. Müller:** None. **A. Blesch:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.14/A14

Topic: A.04. Transplantation and Regeneration

Title: Effects of neuronal loss and regeneration on learning and memory in adult zebrafish

Authors: *K. SKAGGS;
Biol., Univ. of Findlay, Findlay, OH

Abstract: Zebrafish (*Danio rerio)* have become a popular model vertebrate organism for analyzing disorders of the nervous system because, unlike mammals, they have the ability to regenerate neurons following brain injury or degenerative neuronal loss. The genomic and cellular mechanisms of neuronal regeneration following brain injury have been investigated by many groups; however, the functional implications of neuronal loss and regeneration have received relatively less attention. Recent studies using learning and memory tasks have provided evidence that zebrafish can also be useful models for investigating cognitive function, such as investigation of the effects of drugs of abuse on learning. The purpose of these experiments was to examine the effect of brain injury and regeneration on learning and memory. We examined several established tasks to determine their sensitivity of neuronal loss and recovery following telencephalic injury in adult zebrafish. The tasks selected use natural behaviors, such as shoaling and exploration, to assess learning and memory: light-dark preference (LDT), associative learning (AL), and novel object recognition (NO). Groups of adult zebrafish (equal numbers of males and females) were randomly assigned to task, condition within the task (if appropriate) and injury condition. Results indicated that LDT, AL, and NO were potentially sensitive to the effects of injury on behavior and recovery but that intra-individual differences evident on all tasks confounded attribution of results to the effects of injury and neuronal regeneration. Modifications to test conditions and exclusion criteria were employed to minimize inter- and intra-individual variability in behavior on all tasks both before and after injury.

Disclosures: K. Skaggs: None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.15/A15

Topic: A.04. Transplantation and Regeneration

Support: Morton Cure Paralysis Fund

Title: Fluorescent screen for identifying axon regeneration genes

Authors: *N. W. F. GROOMS, S. E. URENA, M. Q. FITZGERALD, S. H. CHUNG; Bioengineering, Northeastern Univ., Boston, MA

Abstract: Central nervous system (CNS) injuries are prevalent and currently lack effective therapies. A "conditioning" lesion of a peripheral sensory axon triggers robust central axon regeneration in mammals. Lesion conditioning could drive powerful therapies for CNS injuries. We established a model for lesion-conditioned regeneration in the invertebrate roundworm *C. elegans* in their ASJ neuron. This approach allows for a more comprehensive and faster search for neuronal regeneration genes compared to mammalian models. The *C. elegans* model permits unbiased screening. We used ethyl methanesulfonate to stochastically introduce mutations and disrupt the regeneration pathway. In our screen, we used the intensity of green fluorescence protein (GFP) as a proxy for neuronal regeneration. We selected candidate worms based on reduced GFP intensity by eye. The mutations in these worms, however, may underlie fluorescence but not regeneration. Under physiological stress, disruptions to sensory pathways in *C. elegans* produce ectopic axon outgrowths, which are mediated by the same genetic pathway as lesion conditioning. Therefore, we measure ectopic outgrowth to determine if candidate genes are involved in regeneration pathways. Ectopic outgrowth of dim strains is assessed via fluorescent microscopy by counting axonal outgrowths after culturing at 25°C. Following mutagenesis, we isolated twelve strains, originating from six distinct F1s. These mutations have varying penetrance. In each selected strain, around 30-50% of the worms have reduced fluorescence. Ectopic outgrowth studies in some mutagenized strains show decreased axonal outgrowths (~59%) compared to the original strain (~72%). After identifying strains with reduced outgrowth, laser surgery will be performed to assess regeneration. To identify the afflicted gene, we will perform one-step whole genome sequencing. We are also measuring the fluorescent intensity of each strain's ASJ neuron. Maximum intensity projections of 3D images were taken of worms. The quantification of fluorescent intensities allows us to correlate intensity with regeneration on an individual and an overall population basis. The identified genes will provide a roadmap for accelerating vertebrate lesion conditioning research and help guide future studies. This study permits future translation into CNS regeneration research to drive the development of innovative treatments.

Disclosures: **N.W.F. Grooms:** None. **S.E. Urena:** None. **M.Q. Fitzgerald:** None. **S.H. Chung:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.16/A16

Topic: A.04. Transplantation and Regeneration

Support: NJ Commission on Spinal Cord Research-CSCR16ERG014
NJ Commission on Spinal Cord Research-CSCR18FEL006

Title: Aligned fibrous scaffolds containing glycosaminoglycan mimetics promotes neurite extension and myelination

Authors: *S. HASHEMI[1], P. MAUREL[2], M. OUDEGA[3], T. L. ARINZEH[4];
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Abstract: Spinal cord injury (SCI) causes axon damage resulting in functional impairments for which there is no effective treatment available at the present time. Tissue engineered scaffolds mimicking the native extracellular matrix may be a promising strategy to promote growth of the damaged axons. Glycosaminoglycans (GAGs), specifically chondroitin sulfate (CS), can elicit axon growth depending upon the degree and pattern of sulfation. We have developed a GAG mimetic derived from cellulose that can be tailored to have varying degrees of sulfation similar to native GAGs. Schwann cells (SCs) are of interest to be used in combination with this scaffold since they secrete neurotrophic factors stimulating neuron survival and axon growth.
The aligned fibrous scaffolds were prepared by electrospinning a solution of gelatin (bovine skin) with GAG mimetics. Partially sulfated cellulose (pCelS) mimicking chondroitin sulfate-C (CS-C) has ~0.5 sulfates per glucose monomer unit. Fully sulfated cellulose (fCelS) has ~3 sulfates per glucose unit. The scaffolds were crosslinked using EDC/NHS. Fiber morphology was evaluated using SEM. SCs were cultured for up to 14 days to evaluate cell survival. Purified dorsal root ganglion (DRG), from the spinal cord of rat embryos (E-16), were seeded onto scaffolds with or without SCs. Neurite extension was evaluated by confocal microscopy and the length was measured using ImageJ. SC myelination was investigated by purifying dissociated DRGs on scaffolds for 2 weeks followed by seeding SCs and maintaining the culture for 3 more weeks.
The scaffolds had a uniform morphology with 90% alignment. Significantly longer neurite outgrowth was determined for DRGs cultured with or without SCs on fCelS scaffolds in comparison to the other groups. More myelination was observed on both pCelS and fCelS scaffolds in comparison to Gel alone. The fCelS group appeared to have the greatest myelination.

A combination of SCs and GAG-mimetic scaffolds can be used as a novel approach to promote and direct axon growth. The significant neurite growth and myelination on fCelS scaffolds suggest that neurite outgrowth can be promoted, and it may depend on the degree of sulfation. A combination of SCs and GAG-mimetic scaffolds may be a novel approach to effectively promote and direct axon growth after SCI.

Disclosures: **S. Hashemi:** None. **P. Maurel:** None. **M. Oudega:** None. **T.L. Arinzeh:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.17/A17

Topic: A.04. Transplantation and Regeneration

Support: 465656/2014-5 - Instituto Nacional de Ciência e Tecnologia em Medicina Regenerativa
Institutos Nacionais de Ciência e Tecnologia
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Title: Neuroprotective and proregenerative effects of human Wharton's jelly-derived mesenchymal stem cell in a optic nerve lesion model

Authors: *A. J. SILVA, Jr[1], L. A. MESENTIER-LOURO[2], G. NASCIMENTO DOS SANTOS[3], J. VASQUES[7], L. CHIMELI-ORMONDE[4], L. COELHO TEIXEIRA PINHEIRO[8], V. BODART-SANTOS, SR[5], L. R. P. CARVALHO[9], R. L. LOUZADA[10], M. F. SANTIAGO[6], R. MENDEZ-OTERO[11];
[1]Biophisics Inst. Carlos Chagas Filho, Federal Univ. of Rio De Janeiro, Rio de Janeiro, Brazil; [2]Dept. of Ophthalmology, Stanford Univ., Palo Alto, CA; [3]Univ. Federal Do Rio De Janeiro, Rio De Janeiro, Brazil; [4]Inst. de Biofísica Carlos Chagas Filho, Univ. Federal Do Rio De Janeiro, RIO DE JANEIRO, Brazil; [5]Inst. de Bioquímica Médica, [6]Univ. Federal Do Rio De Janeiro, Rio de Janeiro, Brazil; [7]UFRJ, Rio de Janeiro, Brazil; [8]Laboratório De Neurobiologia Celular E Mol., Rio De Janeiro, Brazil; [9]Federal Univ. of Rio de Janeiro, Biophysics Institute of Rio de Janeiro, Brazil; [10]Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil; [11]Univ. Federal do Rio de Janeiro, Rio De Janeiro, Brazil

Abstract: Optic nerve lesions affect retinal ganglion cells (RGCs), which can lead to irreversible visual loss. The potential of allogeneic therapy with mesenchymal cells in a model of optic nerve injury in animals has been demonstrated by our group to increase the RGC survival and regeneration. It is necessary to investigate the potential of mesenchymal cells of human origin for future clinical studies. In the present study, the efficacy of human umbilical cord-derived mesenchymal stromal cells (hWJ-MSCs), stimulated or not by serum deprivation, and its

extracellular vesicles, was investigated in a optic nerve crush model. The hWJ-MSCs, subjected or not to serum deprivation, demonstrated a neuroprotective effect on RGCs 14 days after optic nerve lesion, evidenced by Tuj1 immunostaining in flat mounted retinas. However, the dose used for administration of vesicles did not result in neuroprotection. The neuroprotective effect of hWJ-MSC persists for at least 120 days after lesion, especially for larger soma RGCs, which include the αRGC population.Treatment with hWJ-MSCs promoted axonal regeneration observed by a great number of $GAP43^{+}$ axons at 14 days after lesion, and cholera toxin staining along the entire extension of the optic nerve, and in the lateral geniculate nucleus and superior colliculus 120 days after lesion, resulting in active synapse formation evidenced by the enhancement of NGFI-A-expressing cells. The electrophysiological analysis showed a decrease in the bioelectrical response of the external and inner layers of the retina 90 days after injury to the optic nerve, that were not recovered by administration of hWJ-MSCs. Visual tests for optomotor responses, looming response and light/dark behavior, did not show any improvement of visual responses of treated animals. We conclude that administration of hWJ-MSCs protects and promotes complete regeneration of a fraction of RGCs after injury to the optic nerve, but not results in visual recovery by the evaluated tests.

Disclosures: **A.J. Silva:** A. Employment/Salary (full or part-time):; Instituto Nacional de Ciência e Tecnologia em Medicina Regenerativa, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. **L.A. Mesentier-Louro:** None. **G. Nascimento Dos Santos:** None. **J. Vasques:** None. **L. Chimeli-ormonde:** None. **L. Coelho Teixeira Pinheiro:** None. **V. Bodart-Santos:** None. **L.R.P. Carvalho:** None. **M.F. Santiago:** None. **R. Mendez-Otero:** A. Employment/Salary (full or part-time):; Instituto Nacional de Ciência e Tecnologia em Medicina Regenerativa, Federal University of Rio de Janeiro.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.18/A18

Topic: A.04. Transplantation and Regeneration

Title: The combinatorial effect of constituatively active Rheb and Taxol on axon outgrowth *in vitro* on inhibitory and growth-promoting substrates

Authors: *L. R. BENITEZ-RAMOS[1], L. MARTIN[1], C. LE[1], N. ALEGER[1], V. VELEZ[1], A. L. HAWTHORNE[2];
[2]Burnett Sch. of Biomed. Sci., [1]Univ. of Central Florida, Orlando, FL

Abstract: Central nervous system (CNS) injury can lead to irreversible paralysis and other clinical manifestations due to the inability of axons to regenerate. One approach to promote axon growth is by enhancing the intrinsic growth capacity of the neuron. We aimed to promote axon

regeneration *in vitro* by targeting two separate pathways: microtubule stabilization and the mTOR pathway. Taxol has both chemotherapeutic and neuroregenerative properties and has been shown to increase axon outgrowth. In addition, constitutively active Rheb (cRheb) has been shown to enhance axonal regeneration by upregulating the mTOR pathway. We hypothesized that by activating two different pathways in the cell, the combinatorial treatment of applying Taxol and transfecting cRheb would lead to further enhanced axon outgrowth than either single treatment alone. We used two cell lines: mouse/rat hybrid sensory neuronal cell line F11 and neuroblastoma cell line Neuro2a (N2a). The neurons were differentiated by switching to a serum-free media and applying forskolin. The neurons were grown on either a growth-promoting substrate of low levels of laminin or an inhibitory substrate of low levels of laminin plus inhibitory aggrecan to simulate the high chondroitin sulfate proteoglycan (CSPG) environment of the glial scar. Neurons were transfected with pcRheb-GFP, treated with a continuous or discontinuous application Taxol, or a combination of transfection and Taxol treatment. Transfection without DNA and DMSO were used as controls. After 3 days *in vitro*, neurons were immunostained and imaged. Neurites were traced using NeuronJ/ImageJ (NIH). For N2a-derived neurons, the continuous application of Taxol produced significantly longer neurites than the discontinuous application on both substrates. The combination treatment with discontinuous Taxol resulted in significantly longer neurites than either single treatment alone on the inhibitory substrate. cRheb transfection did not provide an added benefit to the continuous application of Taxol, however. It is possible that the neurons were already at their maximal growth state. For F11 neurons, cRheb alone had significantly longer neurites than the combination treatments on either substrate. The combination treatment did provide an added benefit to the continuous Taxol condition on aggrecan and laminin and to the discontinuous Taxol condition on laminin. Further experiments will compare differing behavior of the two cell types and the possibility of favoring different levels of Taxol. By testing combinations of treatments, we may find an optimal therapy for axon regeneration *in vivo*.

Disclosures: L.R. Benitez-Ramos: None. **L. Martin:** None. **C. Le:** None. **N. Aleger:** None. **V. Velez:** None. **A.L. Hawthorne:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.19/A19

Topic: A.04. Transplantation and Regeneration

Support: Saneron CCEL Therapeutics

Title: A gutsy move for Parkinson’s disease: Targeting the gut microbiome with human umbilical cord blood plasma to arrest neuroinflammation and gut motility deficits

Authors: J.-Y. LEE[1], J. P. TUAZON[2], J. EHRHART[2], P. R. SANBERG[3], ***C. V. BORLONGAN**[4];
[1]Neurosurg. Brain Repair, [2]Neurosurg., [3]Dept. of Neurosurg. & Brain Repair, [4]Univ. of South Florida, Tampa, FL

Abstract: Current therapies for Parkinson's disease (PD) have generated mixed results with the eventual induction of dyskinesia during chronic treatment. Cell therapy has been introduced as an experimental treatment for PD. Despite lack or minimal capacity to differentiate into mature dopaminergic neurons, transplantation of human umbilical cord blood (hUCB) stem/progenitor cells has been shown to significantly attenuate parkinsonian symptoms likely via bystander effects, specifically stem cell graft-mediated secretion of growth and anti-inflammatory factors, altogether promoting brain repair. Recognizing this non-cell replacement mechanism, we examined here the effects of intravenously delivered hUCB-derived plasma into the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced rodent model of PD. Animals received repeated dosing of either hUCB-derived plasma or vehicle at 3, 5, and 10 days after induction of MPTP lesion, then behaviorally and immunohistochemically evaluated over 56 days post-lesion. Compared to vehicle treatment, transplantation with hUCB-derived plasma significantly improved gut motility and nigral dopaminergic neuronal survival, which coincided with reduced pro-inflammatory cytokines in both the SNpc and the intestinal mucosa and dampened inflammation-associated gut microbiota. These novel findings implicate a key pathological crosstalk between gut and brain harnessing a new therapy that targets the gut microbiome with hUCB-derived plasma to retard PD-associated gut motility dysfunction and dopaminergic depletion.

Disclosures: J. Lee: None. **J.P. Tuazon:** None. **J. Ehrhart:** A. Employment/Salary (full or part-time):; University of South Florida. 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.; Saneron CCEL. **P.R. Sanberg:** A. Employment/Salary (full or part-time):; University of South Florida. 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.; Saneron CCEL. **C.V. Borlongan:** A. Employment/Salary (full or part-time):; University of South Florida. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Astellas, Asterias, Athersys, ISCO, KMPHC, SanBio. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athersys, Sanbio.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.20/A20

Topic: A.04. Transplantation and Regeneration

Support: NIA Grant 2T32AG029796-11

Title: Characterizing astrocytes as a cell source for reprogramming and transplantation to repair the brain

Authors: *A. R. STEEVENS[1], S. RADHA[1], E. F. MARRON[2], W. C. LOW[1];
[1]Neurosurg., Univ. of Minnesota Twin Cities, Minneapolis, MN; [2]Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: The human brain is an exquisite example of nature's engineering; however, its major design flaw is the inability of self-repair. Increasing evidence suggests the potential of using glial cells as a therapy for brain injury and neurodegenerative diseases. For instance, using viruses to reprogram endogenous astrocytes to neurons is a method with demonstrated functional benefit in a variety of neurological disease models. Additionally, transplantation of astrocyte progenitor cells has demonstrated improvements in models of brain disease such as Huntington's disease. While both of these approaches hold great therapeutic promise, they have not been extensively characterized. We set out to establish a baseline for astrocyte reprogramming and transplantation in non-diseased *in vitro* and *in vivo* models. To do this, we developed an *in vitro* culture system derived from mouse primary cortical astrocytes to test reprogramming by adeno-associated viral vectors (AAVs) and to generate cells for transplantation. Astrocytes were harvested from post-natal day 1 mouse pups, expanded in culture, and their purity was characterized using immunocytochemistry (ICC). Our culture system efficiently generated astrocytes that expressed glial fibrillary acidic protein (GFAP) and excitatory amino acid transporter 1 (EAAT1) astrocyte markers and displayed characteristic astrocyte-like morphology. *In vitro* reprogramming experiments were performed in astrocytes transduced with the following AAV9-based system: an astrocyte specific Cre recombinase (GFAP::Cre-WPRE) co-administered with either a reporter (CAG::DIO-mRUBY) or reprogramming virus (CAG::DIO-Neurod1-P2A-mRuby). Upon analysis we discovered that, in some cases, astrocytes transduced with the reprogramming AAV migrated to assemble into neuronal-like arrangements on a global level and formed large aggregates that were organized to have the appearance of a soma and multiple extending processes. Moreover, cells in these arrangements expressed the neuronal marker TUJ1, indicating that they were being reprogrammed, but still also expressed the astrocyte marker GFAP, indicating that they were in an intermediate reprogramming state. Next, the reprogramming viruses were tested *in vivo* as was astrocyte transplantation. Stereotactic

injections were made into mouse striata using a Hamilton syringe containing either reprogramming or control AAV viral constructs or astrocytes. Our study makes great strides in characterizing novel glial cell based approaches in non-disease models both *in vitro* and *in vivo*.

Disclosures: A.R. Steevens: None. **S. Radha:** None. **E.F. Marron:** None. **W.C. Low:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.21/A21

Topic: A.04. Transplantation and Regeneration

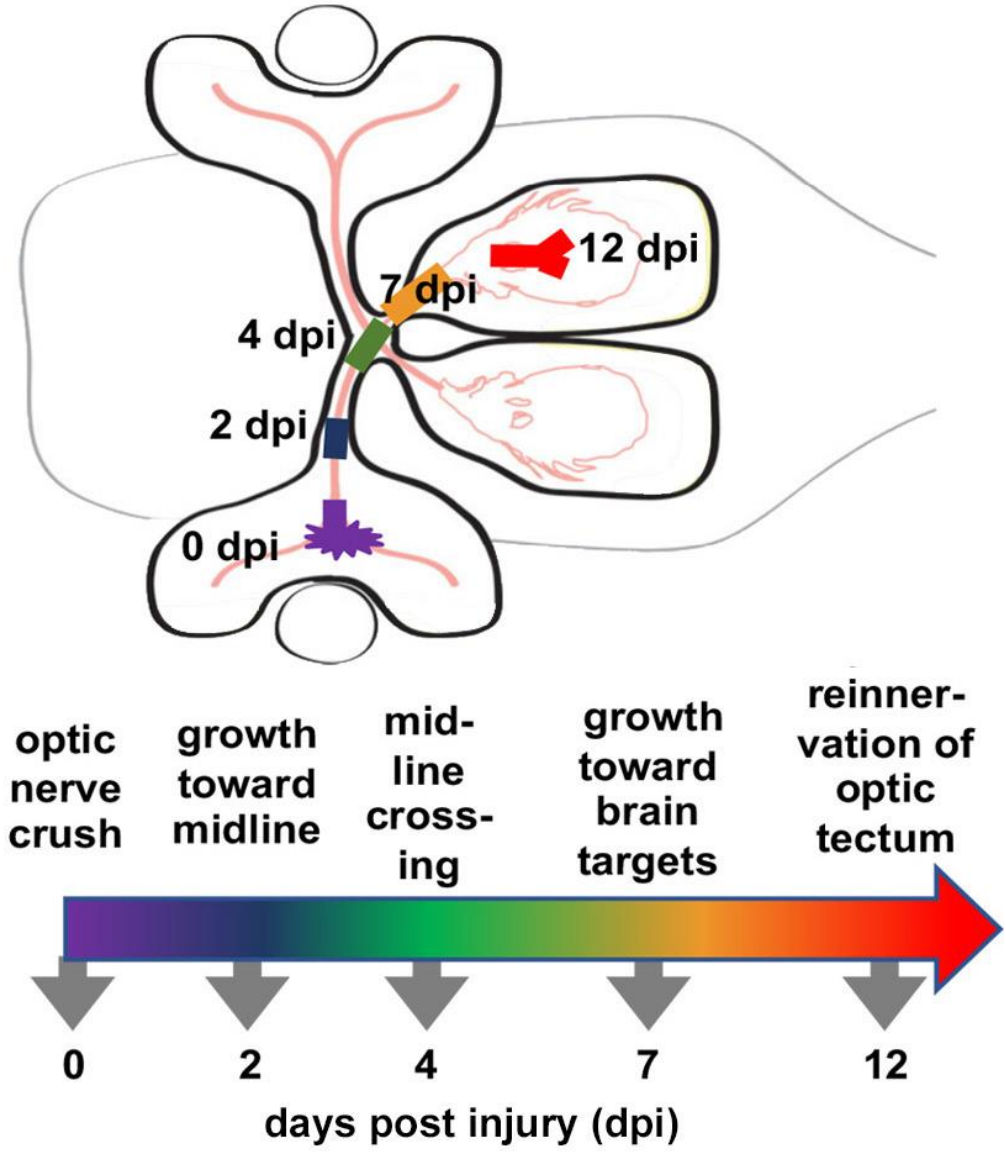
Support: UWM Research Growth Initiative

Title: Successful zebrafish optic nerve regeneration is dependent upon dynamic regulation of protein-DNA and protein-protein interactions

Authors: *A. J. UDVADIA[1], **S. P. DHARA**[1], H. L. LESKINEN[1], A. RAU[3], P. L. AUER[2]; [1]Biol. Sci., [2]Joseph J Zilber Sch. of Publ. Hlth., Univ. of Wisconsin Milwaukee, Milwaukee, WI; [3]Animal Genet. and Integrative Biol., Inst. Natl. de la Recherche Agronomique, Jouy-en-Josas, France

Abstract: Injuries or diseases that damage CNS axons typically result in permanent loss of function in human patients. The functional loss is due, in part, to an inability of adult CNS neurons to undergo regenerative axon growth. A major challenge to mammalian CNS axon regeneration lies in initiating and sustaining gene expression programming that promotes axon growth and guidance to re-establish functional connections with appropriate targets after injury. Unlike mammals, zebrafish respond to optic nerve injury by reinitiating programs of axon growth and guidance, leading to restoration of the visual circuitry. We have recently completed a comprehensive analysis of gene expression changes (RNA-seq) and DNA regulatory element accessibility (ATAC-seq) that accompany distinct stages of optic nerve regeneration in zebrafish (see figure). We find over 7,000 genes that are temporally regulated over the course of optic nerve regeneration, from which we identified distinct signaling pathways that characterize each phase of the regenerative process. We also identified over 40,000 potential DNA regulatory elements, many of which are found within 100 kb of differentially expressed genes and are enriched in binding sites for regeneration-associated transcription factors. Surprisingly, of the over 40,000 accessible elements, we discovered only 233 whose accessibility changed in response to optic nerve injury. Furthermore, we found that the accessibility changes were mostly restricted to two timepoints corresponding to re-initiation of axon growth and re-establishment of connections with brain targets. We hypothesize that differentially accessible elements may play roles in (1) higher order chromatin organization, and (2) regulating the expression of key

transcription factors responsible for triggering a regeneration-associated gene regulatory cascade. Our long-term goal is to identify DNA and protein interactions essential for successful CNS axon regeneration that may serve as targets for therapeutic design.



Disclosures: **A.J. Udvadia:** None. **S.P. Dhara:** None. **H.L. Leskinen:** None. **A. Rau:** None. **P.L. Auer:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.22/A22

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant NS093985

Title: Integrin facilitates optimal growth of primary rat cortical endothelial cells *in vitro*

Authors: R. KUWAR, X. WEN, N. ZHANG, *D. SUN;
Virginia Commonwealth Univ., Richmond, VA

Abstract: Neovascularization and angiogenesis in the Central Nerve System (CNS) are important physiological processes for CNS normal development and tissue repair/regeneration following insults. Integrins are tissue adhesion molecules that play important roles in survival, growth and development of cells during tissue organization, differentiation and organogenesis. In this study we examined the effect of different integrins in the growth and development of primary brain microvascular endothelial cells (ECs). The primary microvascular endothelial cells were isolated from rat brain at post-natal day 7. Cells were seeded in a custom-designed integrin array, an integrin-coated 48-well plate containing 16 types of integrins. BrdU was added into the culture at 48 hrs prior to fixation to assess cell proliferation. After 7 days culture, the cells were fixed and processed for immunostaining with endothelial markers including VWF and PECAM-1. Among 16 integrins tested, we found that $\alpha_5\beta_1$, $\alpha_v\beta_5$ and $\alpha_v\beta_8$ greatly promoted proliferation of the culture ECs. To examine the effect of integrins in promoting neovascularization and angiogenesis, these three integrins were selected for 3-D cultures using our tailored hydrogel with addition of VEGF. Following a 7-day 3-D culturing, the culture was fixed and processed for double labeling of phalloidin with VWF or PECAM-1. The staining pattern particularly the blood vessel formation was assessed using confocal microscopy. We found that EC cell rearrangement forming clumps and the capillary like network structures with lumens inside were clearly identifiable with the 3-D reconstructed imaging in the 3-D hydrogel system. Our study optimized the growth condition of ECs in the 3-D culture system using the most appropriate integrin in hydrogel system that are ideal for primary brain EC cell tubule formation in vitro. This information is important for designing organoids cultures and for in vivo cell transplantation study for the injured brain following neurological insults.

Disclosures: R. Kuwar: None. **X. Wen:** None. **N. Zhang:** None. **D. Sun:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.23/A23

Topic: A.04. Transplantation and Regeneration

Support: MBL Institutional Funding; Eugene Bell Center for Regenerative Biology and Tissue Engineering
NIH R03NS078519-01A1 Transcriptome Analysis of a Regenerating Vertebrate Spinal Cord After Injury (PI: OB) 09/01/2012-07/31/2014

Title: Cross-species comparison of gene expression following spinal cord injury supports a role for ATF3 in central nervous system regeneration

Authors: *H. R. KATZ[1], A. A. ARCESE[2], O. BLOOM[2], J. R. MORGAN[1];
[1]Eugene Bell Ctr. for Regenerative Biol. and Tissue Engin., Marine Biol. Lab., Woods Hole, MA; [2]The Feinstein Inst. For Med. Res., Manhasset, NY

Abstract: Unlike mammals, some non-mammalian vertebrate species are able to spontaneously regenerate and achieve functional recovery after traumatic spinal cord injury (SCI). Comparing physiological responses across organisms can provide important insights into the conserved mechanisms of successful recovery from SCI. Here, we compared the molecular responses after SCI in regenerative and non-regenerative species in order to begin identifying the molecular profiles that are shared between highly regenerative species. We compared published functional transcriptomic data (RNA-Seq, microarray) from spinal cord of two regenerative species, lamprey and zebrafish, (Herman et al., 2018; Hui et al. 2014) and two non-regenerative species, rat and mouse (Chamankhah et al., 2013; Wu et al., 2013) after SCI. We found that 432 transcripts were differentially expressed exclusively in both rat and mouse at 3 days post injury (dpi) compared to uninjured animals. As expected, gene ontology analysis revealed that immune pathways were enriched among genes that were upregulated in rodents. At 3dpi in lampreys and zebrafish, 36 transcripts were identified as differentially expressed in both highly regenerative species. These included transcripts for regeneration-associated genes: Plexin D (downregulated), SLIT3 and activating transcription factor 3 (ATF3) (upregulated). Of these, ATF3 was one of the most highly induced genes after SCI (3.57 and 5.11 Log2 Fold Change in zebrafish and lamprey, respectively). A protein BLAST analysis revealed that lamprey ATF3 is 51% identical and 67% similar to human ATF3, with even greater homology in the DNA binding domain (62% identical and 83% similar). Immunofluorescence labeling of lamprey spinal cord sections revealed that ATF3 protein levels were increased in both motor and sensory neurons at 3wpi and returned to control levels by 11wpi, when functional recovery is typically achieved. This expression profile and comparative species analysis is consistent with the hypothesis that ATF3 promotes regeneration in the lamprey. Moving forward, we will determine the role for ATF3 in regeneration in lampreys by manipulating its expression and examining the impact on neuronal regeneration and functional recovery after SCI.

Chamankhah, M., et al. (2013). BMC genomics 14(1), 583.
Herman, P. E., et al. (2018). Scientific Reports 8(1), 742.
Hui, S. P., et al. (2014) PLoS One 9(1), e84212.
Wu, J., et al. (2013). Journal of Neuroscience 33(30), 12447-12463.

Disclosures: H.R. Katz: None. **A.A. Arcese:** None. **O. Bloom:** None. **J.R. Morgan:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.24/A24

Topic: A.04. Transplantation and Regeneration

Title: Cellular reprogramming in experimental Parkinson's disease

Authors: *S. RADHA[1], A. R. STEEVENS[2], E. MARRON[3], A. CRANE[4], A. W. GRANDE[5], W. C. LOW[5];
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Abstract: Direct cellular reprogramming to drive lineage switching from one differentiated cell type to another can be exploited to develop cell-based therapies for neurodegenerative diseases. *In-vivo* reprogramming provides an attractive therapeutic strategy to circumvent the hurdles of immune rejection and ethical constraints associated with transplant-based therapy. Supporting cells of the CNS can be reprogrammed to neurons by targeted viral delivery of transcription factors and small molecules. Previous studies have demonstrated the effectiveness of a single proneural transcription factor NeuroD1 to drive reprogramming in the reactive glial lesions of Alzheimer's and stroke. However, the ability of NeuroD1 to promote a similar benefit in models of Parkinson's disease has yet to be demonstrated. Here we tested the hypothesis that NeuroD1 delivered via an Adeno-Associated Virus (AAV) can promote reprogramming in striatal astrocytes to neurons in an *in vivo* PD model. To do this, we designed a dual plasmid approach: one expressing the Cre recombinase, driven by an astrocyte-specific GFAP promoter and the second reporter construct with CAG::DIO-mRuby2 as control or a CAG::DIO-NeuroD1-P2A-mRuby2 reprogramming virus. Aiming to transduce non-dividing cells with minimal off-target effects, our approach employs an AAV9 delivery system, which is FDA approved for human clinical trials. To gain mechanistic insight into the process, we additionally set out to characterize NeuroD1-mediated reprogramming in an *in vitro* model. Cortical astrocytes isolated from P0 mice were cultured and characterized for astrocyte maturity, culture purity. Reprogramming was examined in homeostatic and reactive glial conditions, induced by transforming growth factor-β (TGF-β) treatment. IHC analysis of the NeuroD1-transduced astrocytes revealed neuronal marker expressions and intriguing patterns of aggregated $mRuby^+$ cell clusters connected by multiple processes. The dynamics and trajectory of the fate conversion was traced using IHC and time-lapse imaging. Collectively the *in vitro* results demonstrate the ability of NeuroD1 to reprogram astrocytes. To evaluate NeuroD1-mediated reprogramming in a murine Parkinson's model, glial lesions were induced by unilateral intracranial injections of 6-OHDA into the striatum, followed by the transduction of dual viral vectors. IHC was performed to assess the

efficiency of viral transduction and reprogramming. Our study establishes a novel AAV9-based approach to NeuroD1-mediated astrocyte reprogramming in the context of Parkinson's disease.

Disclosures: S. Radha: None. **A.R. Steevens:** None. **E. Marron:** None. **A. Crane:** None. **A.W. Grande:** None. **W.C. Low:** None.

Poster

363. Axon Regeneration

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 363.25/A25

Topic: A.04. Transplantation and Regeneration

Support: MBL Institutional Funding; Eugene Bell Center for Regenerative Biology and Tissue Engineering

Title: Spinal cord injury in lampreys induces microglia/macrophage interactions with regenerating axons

Authors: *E. GUADARRAMA, J. R. MORGAN;
Eugene Bell Ctr. for Regenerative Biol. and Tissue Engin., Marine Biol. Lab., Woods Hole, MA

Abstract: A growing number of neuro-immune interactions have been observed after spinal cord injury (SCI) in both mammals and non-mammalian vertebrates, but the full impacts remain unclear. Both positive and negative immune responses have been reported in commonly used SCI models, including in highly regenerative animals like lampreys, fishes and amphibians, as well as mammals with limited regeneration. The post-SCI immune response often involves infiltration and/or proliferation of microglia/macrophages in and around the lesioned area, leading to an interest in understanding how microglial responses influence central nervous system (CNS) regeneration. Here, we address this question in sea lampreys (*Petromyzon marinus*), which are basally diverging vertebrates that undergo functional recovery after SCI supported by robust axon and synapse regeneration. We previously showed that elevated cAMP increased microglia/macrophage density in the regenerating lamprey spinal cord, correlating with enhanced lesion repair and axon regeneration. This raises the possibility that microglia/macrophages in lampreys promote axon regeneration after SCI. To test this, we examined the distribution of microglia/macrophages in relation to regenerating axons. After spinal cord transection, lampreys were allowed to recover for 3-11 weeks before harvesting the spinal cords for sectioning and immunofluorescence. Microglia/macrophages within control and transected spinal cords were labeled with fluorescein-conjugated Isolectin B4, and axons were labeled with a lamprey-specific NF-180 antibody. As expected, SCI significantly increased microglia/macrophage density, compared to uninjured controls. Isolectin-labeled cells exhibited both amoeboid and stellate morphologies and were in similar density 500 μm rostral and caudal

to the lesion site. Furthermore, the microglia/macrophages made physical contacts with regenerating axons and extended elongated projections along or around them. These microglia-axon contacts were observed within the immediate vicinity of the lesion site within transected spinal cords, but were rarely, if ever, observed within control spinal cords. Preliminary data suggest these contacts may occur at newly-formed synapses within regenerated axons. Thus, the working hypothesis is that microglia/macrophages facilitate axon regeneration by remodeling axons and synapses. Future experiments will test this hypothesis through ablation of microglia/macrophages. Understanding the importance of these neuro-immune interactions could lend insight into cellular and molecular mechanisms that promote regeneration in the CNS.

Disclosures: E. Guadarrama: None. **J.R. Morgan:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.01/A26

Topic: A.05. Axon and Dendrite Development

Support: JSPS KAKENHI Grant 24390271
JSPS KAKENHI Grant 23390275
JSPS KAKENHI Grant 26893252
JSPS KAKENHI Grant 17k16315

Title: Role of Per3, a circadian clock gene, in corticogenesis

Authors: *K.-I. NAGATA, M. NODA, I. IWAMOTO, H. TABATA, H. ITO;
Inst. for Developmental Research, Aichi Developmental Disability Ctr., Kasugai, Japan

Abstract: Per3 is one of the primary components of circadian clock system. While circadian dysregulation is known to be involved in the pathogenesis of several neuropsychiatric diseases. It remains largely unknown whether they participate in embryonic brain development. Here, we examined the role of clock gene *Per3* in the development of mouse cerebral cortex. *In situ* hybridization analysis revealed that *Per3* is expressed in the developing mouse cortex. Acute knockdown of *Per3* with *in utero* electroporation caused abnormal positioning of cortical neurons, which was rescued by RNAi-resistant Per3. Per3-deficient cells showed abnormal migration phenotypes, impaired axon extension and dendritic arbor formation. Taken together, Per3 was found to play a pivotal role in corticogenesis via regulation of excitatory neuron migration and synaptic network formation.

Disclosures: K. Nagata: None. **M. Noda:** None. **I. Iwamoto:** None. **H. Tabata:** None. **H. Ito:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.02/A27

Topic: A.05. Axon and Dendrite Development

Support: 16K08478

Title: PlexinA1 is crucial for the midline crossing of cingulate axons in the developing corpus callosum under BALB/cAJ genetic background

Authors: *M. HOSSAIN[1,2,3], T. TSUZUKI[1], K. SAKAKIBARA[1], F. IMAIZUMI[1], A. IKEGAYA[1], M. INAGAKI[1], I. TAKAHASHI[1], T. ITO[2], H. TAKAMATSU[3], A. KUMANOGOH[3], T. NEGISHI[1], K. YUKAWA[1];
[1]Grad. Sch. of Pharm., Meijo Univ., Nagoya, Japan; [2]Dept. of Neurol., Aichi Med. Univ., Nagakute, Japan; [3]Dept. of Immunopathology, Osaka Univ., Suita, Japan

Abstract: The corpus callosum (CC) is the biggest commissure that links cerebral hemispheres. In the cortical midline during CC development, the guidepost structures emerge and express axon guidance molecules to instruct neurons about the proper direction of axonal elongation toward and across the midline. Neuropilin-1 (Npn1), a high-affinity receptor for class 3 semaphorins (Sema3s), localized on the cingulate pioneering axons has a crucial role for the axon guidance to the midline through the interactions with Sema3s. However, it remains unclear which type of Plexin acts as a component of Sema3 holoreceptors with Npn1 during the axon guidance of the cingulate pioneering axons. To address the role of PlexinA1 in CC development, we examined the localization of PlexinA1, Npn1 and Sema3s by immunohistochemistry (IHC) with embryonic brains from wild-type (WT) and the PlexinA1-deficinet (PlexinA1 KO) mice under BALB/cAJ background. The IHC confirmed the expression of PlexinA1 in callosal axons derived from cingulate and neocortex of WT mice at embryonic day 17.5 (E17.5) but not in PlexinA1 KO mice. To examine the role of PlexinA1 in the navigation of callosal axons, the extension of callosal axons toward and across the midline was traced in WT and PlexinA1 KO brains at E17.5. In the absence of any misguidance of callosal axons to ectopic areas other than the midline, callosal axons of PlexinA1 KO brains at E17.5 showed significantly lower incidence in the midline crossing of callosal axons as compared with WT. To examine further the role of PlexinA1 in CC development, CC phenotype was examined in PlexinA1 KO mice at postnatal day 0.5 (P0.5). Agenesis of corpus callosum in the anterior half of CC was observed in 76.9% of the PlexinA1 KO mice at P0.5. These results indicate the crucial involvement of PlexinA1 in the midline crossing of callosal axons during the CC development under BALB/cAJ background.

Disclosures: **M. Hossain:** None. **T. Tsuzuki:** None. **K. Sakakibara:** None. **F. Imaizumi:** None. **A. Ikegaya:** None. **M. Inagaki:** None. **I. Takahashi:** None. **T. Ito:** None. **H. Takamatsu:** None. **A. Kumanogoh:** None. **T. Negishi:** None. **K. Yukawa:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.03/A28

Topic: A.05. Axon and Dendrite Development

Support: the Research Grants Council Hong Kong

Title: Phosphorylation of FE65 serine 459 by protein kinase C zeta potentiates neurite outgrowth

Authors: ***W. LI**[1,2], D. CHAU[2], K. LAU[2];
[1]Shenzhen Inst. of Advanced Technol., Shenzhen, China; [2]The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Neurite outgrowth is an essential process for establishing connections between developing neurons. FE65 is a brain-enriched phosphor-adaptor protein that has been shown to stimulate neurite extension by recruiting and activating the small GTPase ARF6. However, the mechanism(s) that regulates FE65-ARF6 interaction is still not known. In this study, we show that phosphorylation of FE65 Serine 495 (S459) enhances FE65-ARF6 interaction and FE65-mediated ARF6 activation. Moreover, the synergistic effect of FE65 and ARF6 on neurite outgrowth is further potentiated by FE65 S459 phosphorylation. Additionally, we demonstrate that FE65 S459 is phosphorylated by protein kinase C zeta (PKCζ). Knockdown of PKCζ suppresses the stimulatory effects of FE65 on ARF6 activation. Furthermore, overexpression of PKCζ promotes whereas siRNA knockdown or pharmacological inhibition of PKCζ attenuates FE65 stimulation of neurite outgrowth. Hence, we reveal a novel mechanism that FE65 is phosphorylated by PKCζ at S459 to potentiate the stimulatory effect of FE65 on ARF6 activation and in turn neurite outgrowth.

Disclosures: **W. Li:** A. Employment/Salary (full or part-time):; The Chinese University of Hong Kong. **D. Chau:** None. **K. Lau:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.04/A29

Topic: A.05. Axon and Dendrite Development

Title: Exploring the unexpected - How a glycan biosynthesis inhibitor changes cell morphology and mitochondria

Authors: *C. MENCIO[1], S. TILVE[1], H. KATAGIRI[1], H. M. GELLER[2];
[1]NIH, Bethesda, MD; [2]Office of Educ., Natl. Heart, Lung, and Blood Institute, NIH, Bethesda, MD

Abstract: Proteoglycans (PGs) are important to a variety of neurological functions. From neural development to memory to regeneration and disease, both heparan sulfate (HS) and chondroitin sulfate proteoglycans (CSPGs) play several promoting and inhibitory roles. While research is clear that these macromolecules are involved in neural development and repair, their actual role of action is difficult to understand. This is in part due to the lack of available research tools and a limited understanding of existing tools, such as xylosides. Consisting of an aglycone attached to a xylose residue, xylosides are known to affect glycosaminoglycan (GAG) biosynthesis both *in vitro* and *in vivo.* Used in high concentrations (≥1mM) in research since the 1970s, treatment has been shown to lead to the inhibition of endogenous PGs and the production of primed GAGs, or GAG chains built on the xyloside and as such lack a core protein. Primed GAGs are pushed out of the cell and can be found in the extracellular space. However, it is only recently that application of lower, more therapeutically relevant concentrations has been explored. The presented research offers evidence that acute exposure with nanomolar, but not millimolar, concentrations of xyloside leads to altered cytoskeleton *in vitro* evidenced by enlarged growth cones and lamellipodia. Both microtubules (MTs) and actin are misregulated and their dynamics altered after xyloside treatment. Examining two of the major players in actin turnover and MT stabilization, we found changes in phosphorylation in both cofilin and GSK3β signaling pathways. The speed of MT, measured by EB3 comets, is significantly increased and lamellipodia appear to show increased formation and persistence. Additionally, assessment of mitochondria has shown increased capacity and altered trafficking in xyloside treated neurons indicating xyloside treatment may play a role in energy production. Both the changes in cytoskeleton and mitochondria may factor into the morphological changes observed in neurites and could lead to an improved ability of xyloside-treated neurons to overcome injury and degenerative stress. This research indicates that xylosides may serve as more than just GAG biosynthesis inhibitors. Exploring nanomolar xyloside treatment may provide new insights into these other functions, and provide novel insights into neuron development, survival and repair.

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

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.05/A30

Topic: A.05. Axon and Dendrite Development

Support: FONDECYT 11180536
FONDECYT 1181814
USA1899-Vridei 021943LE-PAP Universidad de Santiago

Title: Ca^{2+} -activated non-selective cation channel (TRPM4) modulates neurite growth in cortical neurons

Authors: N. JUANCHUTO-VIERTEL, C. ALAMOS, E. LEIVA-SALCEDO, *D. RIQUELME;
Dept. of Biol., Univ. de Santiago, Santiago, Chile

Abstract: Neurite growth and extension is a critical process to determine neuronal function; changes in the actin cytoskeleton and Ca^{2+}_i dynamics underlie this process. In this regard, filopodia formation experiments performed in absence of Ca^{2+}_i showed that neurite progression is arrested at initial stages. Moreover, it has been demonstrated that neurite elongation and neuronal development is affected by the activation of ion channels that regulates Ca^{2+}_i levels. Among the channels which participate in Ca^{2+}_i dynamics, TRPM4, a non-selective monovalent cation conducting channel activated by Ca^{2+}_i, could be having a role in this process by promoting changes in the cytoskeleton dynamics through the modulation of intracellular levels of Ca^{2+} as it has been described in other cellular models. In this work, we assess the participation of TRPM4 in the neuritogenesis and Ca^{2+}_i dynamics during early stages of neurite development. Using a combination of pharmacological modulation and genetic suppression of TRPM4 in a model of cortical neurons in culture, we performed multiplex immunofluorescence using cytoskeleton markers such as MAP2 (microtubules), phalloidin (actin) and then, we characterized the neurite initiation and measure the neurite number and length during neuritogenesis. Additionally, we assessed the Ca^{2+} dynamics during the first stages of neuritogenesis using Fura-2 and Fluo-4 calcium probes. We found that pharmacological inhibition of TRPM4 change neurite length and number, thus affecting the stage progression. These results were similar to the effects observed with TRPM4 silencing using an shRNA. Furthermore, experiments using electrical stimulation and K^+ induce-depolarization showed that TRPM4 inhibition affects Ca^{2+} dynamics during neurite growth. Our current efforts are focused in the understanding of the actin dynamics and its relationship with changes in Ca^{2+}_i. These results bring insight in the role of TRPM4 as an

indirect modulator of the cytoskeleton dynamics through the regulation of the intracellular calcium.

Disclosures: **N. Juanchuto-Viertel:** None. **C. Alamos:** None. **E. Leiva-Salcedo:** None. **D. Riquelme:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.06/A31

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01 NS099405
Hilldale Undergraduate/Faculty Research Fellowship

Title: Dysregulation of adhesion dynamics in TSC2 deficient human forebrain neurons

Authors: *M. M. ONESTO, T. S. CATLETT, T. M. GOMEZ;
Dept. of Neurosci., Univ. of Wisconsin-Madison, Madison, WI

Abstract: Growing evidence suggest that patients with Tuberous Sclerosis Complex (TSC), like other neurodevelopmental disorders, have mis-wiring of neuronal connections that form during development. These defects in neuronal connectivity likely contribute to symptoms of TSC, such as cognitive deficits, autism and epilepsy. Precise connectivity depends on proper regulation of growth cone motility downstream of axon guidance cues. Directed leading edge protrusion/retraction and adhesion is mediated in part through coordinated F-actin polymerization the coupling of the F-actin cytoskeleton to nascent focal adhesions, or “point contacts”. This process slows retrograde flow to support actin polymerization at the leading edge of growth cones. Chemorepulsive factors slow axon extension or induce retraction by activating signaling pathways that result in endocytosis, adhesion destabilization and rapid actin depolymerization. To examine whether these processes also function in human neurons and test if they are defective in TSC2 mutant neurons, we used induced pluripotent stem cells to generate neurons from corrected control and TSC2+/- mutant forebrain neurons. Utilizing this human model system we have demonstrated that TSC2+/- mutant forebrain neurons exhibit increased axon outgrowth and have reduced sensitivity to several chemorepellants known to signal through RhoA. Further, our data show that TSC2+/- neurites have lower basal active RhoA and fail to activate RhoA normally in response to inhibitory cues. Here I show using live cell fluorescence imaging that TSC2+/- growth cones also have reduced focal adhesions, but lower retraction of new protrusions. I hypothesize that TSC2+/- neurons have reduced myosin II-mediated contractility and heightened adhesion turnover rate, which is due to dysregulated RhoA activity leading to rapid axon extension rates. Further, I hypothesize that inability to TSC2+/- neurons to

activate RhoA prevents adhesion site destabilization and F-actin contraction, making these mutant neurons less sensitive to chemorepellants. This research will further our basic understanding of the role of F-actin and adhesion dynamics downstream of chemorepulsion in human neurons with autism spectrum disorder mutation.

Disclosures: **M.M. Onesto:** None. **T.S. Catlett:** None. **T.M. Gomez:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.07/A32

Topic: A.05. Axon and Dendrite Development

Support: NSF Grant 1822517
NIH Grant MH117488
CNSI Challenge Grant

Title: Serotonergic fibers as random walks - A spatial analysis

Authors: *K. C. MAYS, K. L. THOMAS, C. A. LOUIE, A. ZHANG, J. H. HAIMAN, S. JANUSONIS;
Psychological and Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Serotonergic densities in brain regions may form as a result of random walks performed by individual serotonergic axons (fibers). We have recently shown that these walks can be described by the directional von Mises-Fisher (vMF) distribution, parametrized by the "concentration parameter" κ (Janusonis, Mays, Hingorani (2019) *ACS Chemical Neuroscience*, in press). This parameter controls the rigidity of the walk, which can potentially affect the equilibrium density of fibers in the adult brain.
In the present study, we visualized individual serotonergic fibers in the adult mouse brain using three methods: immunohistochemistry with confocal microscopy, (ii) tissue clearing (CUBIC) with immunohistochemistry and high-resolution light sheet fluorescence microscopy, and (iii) imaging in an inducible Brainbow transgenic model (with the Cre-recombinase under the promoter of tryptophan hydroxylase 2). The trajectories of serotonergic fibers were converted into numerical arrays using semi-manual and automated tracing algorithms, and the optimal step-length of the random walk was determined based on modeling and data analysis. Estimates of κ values were obtained in a number of forebrain regions (cortical and subcortical) using published methods. Our results indicate that the vMF-random walk can closely approximate the behavior of individual serotonergic fibers. The analysis shows that κ can be used as a robust parameter to assess the regional tortuosity of serotonergic fibers in health and disease, and that this approach

may support studies of Autism Spectrum Disorder, Major Depressive Disorder, and other mental disorders.

Disclosures: **K.C. Mays:** None. **K.L. Thomas:** None. **C.A. Louie:** None. **A. Zhang:** None. **J.H. Haiman:** None. **S. Janusonis:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.08/DP01/A33

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: A.05. Axon and Dendrite Development

Support: MOST 105-2320-B-009-005-MY3

Title: GTP-bound ran regulates non-centrosomal microtubule formation and is transported by actin waves towards the neurite tip

Authors: C.-H. HSU[1], Y.-A. HUANG[2], H.-C. CHIU[1], C. T. HO[1], W.-L. LO[2], ***E. HWANG**[1,2,3]; [1]Inst. of Mol. Med. and Bioengineering, [2]Dept. of Biol. Sci. and Technol., [3]Inst. of Bioinformatics and Systems Biol., Natl. Chiao Tung Univ., Hsinchu, Taiwan

Abstract: Microtubule is the most abundant and arguably the most important cytoskeleton in neurons. While the organizing center of microtubules in mitotic cells is typically located at the centrosome, microtubule nucleation in post-mitotic neurons switch to non-centrosomal sites. A handful of proteins and organelle have been shown to promote non-centrosomal microtubule formation in neurons, yet the regulation mechanism remains unknown. Here we demonstrate that the small GTPase Ran is a key regulator of non-centrosomal microtubule nucleation in neurons. The GTP-bound Ran (RanGTP) localizes to the neurite tips and around the soma. Using the RanGTP- and RanGDP-mimic mutants, we show that RanGTP promotes microtubule nucleation at the tip of the neurite. To demonstrate that RanGTP can promote microtubule nucleation in region along the neurite other than the tip, an optogenetic reagent (RanTRAP) was constructed to enable local production RanGTP in the neuronal cytoplasm. An increase of non-centrosomal microtubule nucleation can be observed by elevating the RanGTP level along the neurite using RanTRAP, establishing a new role for Ran in regulating neuronal microtubules. Additionally, the mechanism of RanGTP localization at the neurite tip was examined. We discovered that the actin wave drives the anterograde movement of RanGTP to the neurite tip. Pharmacological disruption of the actin wave abolishes the localization of RanGTP and reduces the non-centrosomal microtubule nucleation at the neurite tip. These observations provide a novel indirect connection between the actin and microtubule cytoskeletons in neurons.

Disclosures: **C. Hsu:** None. **Y. Huang:** None. **H. Chiu:** None. **C.T. Ho:** None. **W. Lo:** None. **E. Hwang:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.09/A34

Topic: A.05. Axon and Dendrite Development

Support: NIMH Grant R01 MH104491
NIMH Grant F31 MH115541
NIMH Grant R01 MH103455

Title: Interrogating functional interactions between Cadherin 8 and Cadherin 11

Authors: *C. R. MERCEDES, R. E. MESIAS, Y. ZAKI, G. W. HUNTLEY, D. L. BENSON; Nash Family Dept. of Neurosci. and Friedman Brain Inst., Grad. Sch. of Biomed. Sciences, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Genome wide association studies highlight involvement of cadherin adhesion proteins in many neuropsychiatric and neurobehavioral disorders. In particular, rare microdeletions in Cadherin 8 (Cdh8), a type II classic cadherin, are associated with Autism Spectrum Disorders (ASD), while another type II classic cadherin, Cadherin 11 (Cdh11), is associated with non-syndromic ASD. Classical cadherins are cell adhesion molecules involved in synaptic plasticity, cell to cell communication, and circuit formation. Cadherins have selective binding preferences and specific expression patterns enabling them to work in a combinatorial, complementary or collaborative fashion to delineate cortical regions and circuits. For example, in retina, several type II cadherins, including Cdh8, act both collaboratively and independently to generate appropriate functional circuits (Duan et al., 2014; 2018). In cell-based adhesion assays, Cdh8 and Cdh11 can bind heterophilically (Brasch et al., 2018; Shimaoyama et al. Biochem. J. 2000), suggesting these two cadherins may work together. The distribution of Cdh8 and Cdh11 mRNAs is partially overlapping. For example, both are expressed in medial prefrontal cortex, but in striatum, Cdh8 mRNA expression shows a dorsal-to-ventral gradient, while Cadh11 mRNA expression shows the reverse (Friedman et al., 2015; Suzuki et al., 1997). Despite these patterns, it is not known if these two cadherins functionally interact in vivo, nor is it clear where Cdh11 protein is localized. By western blot, our data show that Cdh11 is expressed in different regions of the brain with significant levels detectable by postnatal day 10, during the peak period of synaptogenesis. By immunofluorescence, we find that Cdh11 is localized to discrete puncta along postsynaptic dendrites similar to Cdh8. In addition, both Cdh8 and Cdh11 are found in sub-populations of cortical cells where they form clusters on postsynaptic membranes. Functional interactions between these two cadherins are being tested using target selection and

cell-substrate based assays. Together these patterns may reveal combinatorial interactions important for the establishment of corticostriatal circuits that are impaired in autism.

Disclosures: C.R. Mercedes: None. **R.E. Mesias:** None. **Y. Zaki:** None. **G.W. Huntley:** None. **D.L. Benson:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.10/DP02/A35

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: A.05. Axon and Dendrite Development

Support: NIH U01 EY027266-01
NIH T32 EY027721
NIH R01 NS099405

Title: Human stem cell-derived photoreceptors generate processes by non-cell autonomous pulling

Authors: *S. K. REMPEL[1], Y. KANCHERLA[1], D. J. ZACK[3], D. M. GAMM[2], T. M. GOMEZ[1];
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Abstract: Photoreceptors (PR) are our primary visual sensory cells, and their loss causes irreversible vision loss. Cell replacement therapy provides a potential treatment approach to those who have lost PRs through damage or disease. While PR transplant research is ongoing in animal models, success is hindered by our limited understanding of PR axonogenesis and synaptogenesis in both developing and regenerating conditions. Using a human pluripotent stem cell line that labels PRs ($Crx^{+/tdTomato}$), we generated PRs within retinal optic vesicles to study their development within this human organoid system and address the mechanisms of process extension of these potential transplant candidate neurons *in vitro*. We made the unexpected finding that while PRs do form terminal specializations that resemble classical growth cones *in vitro*, they do not extend axons by cell autonomous terminal extension. Instead, we observed that, on a variety of substrata tested, PR neurite extension is primarily achieved through interactions with motile non-PR cells in these mixed neuroretinal cultures. Close examination of PR terminals by high-resolution live cell imaging and super-resolution microscopy showed that PR terminals are highly adherent and have a disorganized actin cytoskeleton. Our observation that human PR axons elongate primarily in association with motile support cells *in vitro* suggests

that PR axon development and regeneration may also occur through interactions with motile neuroretinal support cells *in vivo*. To begin to test this possibility, we are imaging PR axon elongation within organoids using 2-photon microscopy and find that PRs undergo apical-basal migration that may be related to their axon development. These findings will be vital for understanding human PR development and for informing our methods to promote PR connectivity in cell replacement therapies to cure blindness.

Disclosures: **S.K. Rempel:** None. **Y. Kancherla:** None. **D.J. Zack:** None. **D.M. Gamm:** 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.; Opsis Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Opsis Therapeutics. **T.M. Gomez:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.11/A36

Topic: A.05. Axon and Dendrite Development

Support: NSF 1154394
NSF 0806963

Title: Decreasing geranylgeranylation of RhoA and Rac1 increases cortical actin content by increasing activation of WAVE/ARP and JNK pathways

Authors: *N. G. R. RAUT[1], J. M. REDDY[2], D. L. HYNDS[1];
[2]Biol., [1]Texas Woman's Univ., Denton, TX

Abstract: Abnormal cytoskeletal organization of actin is one of the common features of many neurodegenerative diseases like Alzheimer’s disease (AD), Parkinson diseases (PD) and Huntington’s disease (HD). Actin polymerization and depolymerization are controlled in large part by Rho guanosine triphosphatases (GTPases). These proteins are active when bound to guanosine triphosphate (GTP) and inactive when bound to guanosine diphosphate (GDP). Rho GTPases are targeted to the plasma membrane by the addition of a 20-carbon lipophilic geranylgeranyl isoprene. It is not known how Rho and RhoA geranylgeranylation affects the location and activity of downstream effectors to facilitate either polymerization or depolymerization of actin. We used non-geranygeranylatable RhoA and Rac1 constructs to test how inhibiting geranygeranylation affects morphology, localization of activation of RhoA and Rac1 cell signaling pathways. Western blotting, Co-immunoprecipitation and confocal microscopy analyses show that expressing non-geranylgeranylatable constructs increases cortical

actin filament content in growth cones, but have differential effects on process outgrowth from neuroblastoma cells and rat primary cortical neurons. Expressing non-geranylgeranylatable Rac1 decreased neurite initiation and elongation, while expressing non- geranylgeranylatable RhoA increased neurite elongation. Furthermore, expressing non-geranylgeranylatable RhoA or Rac1 significantly altered formation of the actin nucleation complex of WAVE with the Arp2/3 complex and activation of some mitogen activated protein MAP kinase pathways, including JNK. Elucidating the signaling cascades of the aberrantly-localized active Rho GTPases and the effect on actin may also identify the distinct functions of these GTPases or their downstream effectors, which may identify novel targets to facilitate axon regeneration in traumatic or degenerative neurological conditions.
This research was supported by the TWU Department of Biology, a TWU biology summer research award (to NGRR) and grants from the National Science Foundation NSF 1154394 & NSF 0806963 , the TWU Center for Student Research (to NGRR), the TWU Research Enhancement and Proposal Development Programs (to DLH). We would like to thank Dr. DiAnna Hynds and laboratory associates for their guidance and collaboration.

Disclosures: **N.G.R. Raut:** None. **J.M. Reddy:** None. **D.L. Hynds:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.12/A37

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS101786
NIH Grant GM083889
NIH Grant MH108025

Title: LIM and SH3 protein 1 promotes actin-based axon development

Authors: *S. L. POLLITT[1], K. R. MYERS[1], J. YOO[2], G. ARORA[3], J. Q. ZHENG[1];
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Abstract: During brain development, neurons extend axons rapidly and precisely towards their specific targets to form synaptic connections. Axonal growth is primarily driven by a highly motile, actin-rich structure found at the tips of axons, called the axon growth cone. LIM and SH3 Protein 1 (LASP1) is a unique member of the nebulin family of actin binding proteins and contains several protein interaction motifs for mediating signal transduction and actin regulation, and thus may facilitate complex signaling pathways to regulate actin dynamics. *LASP1* has been implicated in autism and schizophrenia, but its role in neuronal development is unknown. We

found that LASP1 is highly expressed in both developing and mature rat brains. Interestingly, the LASP1 protein is concentrated at the leading edge of motile growth cones where actin-based membrane protrusion occurs. Using live microscopy of motile CAD neuroblastoma cells, we found that LASP1 accumulates at the leading edge specifically during membrane protrusion, but is then lost or diminished from the cell edge during membrane retraction. Furthermore, we found that the leading edge enrichment of LASP1 depends upon its LIM and nebulin domains. Finally, our work shows that the loss of LASP1 in mammalian hippocampal neurons reduces axonal growth. Together these data identify LASP1 as a novel actin-binding protein that regulates membrane protrusion and growth cone motility. The modular structure of LASP1 may enable it to link multiple signaling cascades to the actin-based events underlying brain development.

Disclosures: **S.L. Pollitt:** None. **K.R. Myers:** None. **J. Yoo:** None. **G. Arora:** None. **J.Q. Zheng:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.13/A38

Topic: A.05. Axon and Dendrite Development

Support: NIH grant NINDS R01NS051709-14

Title: TNFR2 activation enhances neurite outgrowth in cortical neurons

Authors: M. GERALD[1], *J. RICARD[1], ***V. BRACCHI-RICARD**[1], R. FISCHER[1], R. KONTERMANN[2], K. PFIZENMAIER[2], D. RICCI[3], Y. ARGON[3], J. BETHEA[1]; [1]Drexel Univ., Philadelphia, PA; [2]Univ. of Stuttgart, Stuttgart, Germany; [3]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Promoting axonal regeneration or neuroplasticity have long been pursued to improve functionality after spinal cord injury. In a previous study we have shown that continuous infusion in the spinal cord of EHD2-sc-mTNF$_{R2}$, an agonist of tumor necrosis factor receptor 2 (TNFR2) improved recovery after contusion and enhanced cortical response following hindlimb stimulation (Gerald *et al.*, 2019) leading us to examine the effect of TNFR2 activation on neurite outgrowth. We report that treatment of cortical neurons *in vitro* with the TNFR2 agonist EHD2-sc-mTNF$_{R2}$ enhanced the growth and branching of neurites. The outgrowth correlated with increased BDNF release and JNK activation that led to the phosphorylation of Superior Cervical Ganglion-10 protein (SCG10), a microtubule destabilizing factor. The actin-binding protein filamin A was also up-regulated as a result of TNFR2 engagement. Futhermore, we showed that inhibiting the RNase activity of IRE1α abrogated the TNFR2-mediated neurite outgrowth. Our

study identifies a new role for the TNFR2 receptor on neurite outgrowth that could be harnessed to enhance regeneration following spinal cord injury.

Disclosures: **M. Gerald:** None. **J. Ricard:** None. **V. Bracchi-Ricard:** None. **R. Fischer:** None. **R. Kontermann:** None. **K. Pfizenmaier:** None. **D. Ricci:** None. **Y. Argon:** None. **J. Bethea:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.14/A39

Topic: A.05. Axon and Dendrite Development

Support: NIH R01NS099405

Title: Growth cone invadosome formation and function in developing spinal axon growth and guidance

Authors: ***C. A. SHORT**[1], G. D. FRANCIS, Jr.[2], T. M. GOMEZ[3];
[1]Neurosci. Dept., Univ. of Wisconsin, Madison, WI; [2]Univ. of Wisconsin, Madison, Madison, WI; [3]Dept of Neurosci., Univ. of Wisconsin Madison Sch. of Med. and Publ. Hlth., Madison, WI

Abstract: Invadosomes are specialized F-actin rich adhesions, which protrude from cells to promote remodeling of the extracellular matrix through the targeted release of matrix metalloproteases (MMPs). Invadosomes are formed by many types of migrating cells including metastatsic cancer and immune cells, where they have been extensively studied. Recent work from our lab suggests that developing nerve growth cones also form invadosome-like protrusions and that disruption of invadosome formation significantly inhibits motor neuron (MN) exit from the spinal cord into the periphery in *Xenopus laevis* embryos. We hypothesize that pioneering sensory Rohon-Beard (RB) and MN axon growth cones utilize invadosomes to breech the dense ECM basement membrane around the spinal cord to facilitate exit from the central nervous system and that the formation of these protrusions is regulated by signaling proteins in the local environment. Preliminary data suggests that growth factors, including epidermal growth factor (EGF), promote the formation of invadosome precursors in developing neurons *in vitro*. EGF is expressed in the muscle and skin of developing embryos around the time RB and MN axons exit to the periphery. EGF is known to promote invadosome formation in other cell types through the activation of Src and PI3K and we determined that inhibition of either of these kinases disrupts invadosome formation in nerve growth cones. Kinase activity is also increased in response to EGF in nerve growth cones. Local EGF receptor activation, downstream actin assembly, and targeted MMP delivery are being examined in response to EGF in growth cones with a combination of immunochemical and molecular biosensors. In addition, the targeting of MMPs

in invadosome-like protrusions is being examined *in vivo*. Future work will continue to elucidate the signaling mechanisms controlling the formation of invadosomes and downstream delivery of MMPs in developing nerve growth cones *in vivo*.

Disclosures: C.A. Short: None. **G.D. Francis:** None. **T.M. Gomez:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.15/A40

Topic: A.05. Axon and Dendrite Development

Title: Systematic analysis of effects of glucose in CNS neurons-implications to diabetes related neurodegeneration

Authors: *V. DEVANATHAN[1], H. CHAKRAVARTHY[2], G. SURESH[2], S. SHARMA[2], A. MARIMUTHU[1];
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Abstract: Neurodegeneration due to diabetes is gaining importance as our understanding towards this complex phenomenon is increasing. Diabetes is a complex, heterogeneous metabolic disorder that affects millions of population worldwide. India ranks highest in harbouring diabetes patients leading to multiple organ dysfunctions including brain damage. Diabetes in CNS results in neuronal dysfunction, neurodegeneration both in neurons of the brain and eye (retinal neurons). Altered signalling mechanisms that regulate neuronal differentiation are one of the main causes of neuronal dysfunction and degeneration. Neuronal proteins abundantly expressed in the brain, eye and spinal cord are crucial players in orchestrating signalling for neuritogenesis which is essential for regeneration of neurons. Secondary signalling pathways required to maintain cellular homeostasis, such as signalling mediated by Gai proteins are key regulators of diabetes. Previously G proteins has been hypothesised to be involved in neuritogenesis and "may be" neuronal regeneration. Role of G protein signalling pathway along with neuronal transmembrane proteins in neuronal regeneration after the onset of diabetes is unexplored. Our research focusses in understanding the role of neuronal transmembrane proteins and also other molecules such as Neuronal cell adhesion molecule (NCAM), Prion and L1 that might be involved in neuritogenesis. Our preliminary data towards this strongly shows that modulating glucose concentrations regulate expression of an abundantly expressed neuronal protein. Currently we are understanding this observation further and hope to have an interesting science finding to share in SfN, Chicago. From our findings it is also interesting to see the same protein is differentially expressed in retinal neurons treated with different concentrations of glucose. Such systematic studies will allow us to explore other modes of managing diabetes associated neuronal complications in CNS (brain and eye).

Disclosures: **V. Devanathan:** None. **H. Chakravarthy:** None. **G. Suresh:** None. **S. Sharma:** None. **A. Marimuthu:** None.

Poster

364. Neuronal Morphogenesis

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.16/A41

Topic: A.05. Axon and Dendrite Development

Title: Nox-derived hyrogen peroxide is required for neural development in zebrafish embryos

Authors: *A. TERZI[1], C. J. WEAVER[2], H. S. ROEDER[1], T. M. GUROL[1], Q. DENG[1], Y. F. LEUNG[1], D. M. SUTER[1];
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Abstract: Reactive oxygen species (ROS) are derivatives of oxygen molecule, often highly reactive due to the free electrons. Excess production of ROS is associated with oxidative stress in several pathologies including cancer, Alzheimer's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis. In homeostatic conditions, optimal ROS levels are required for cellular signaling. In the nervous system, physiological levels of ROS mediate neural progenitor cell maintenance and differentiation, cerebellar development, axon growth, neuronal polarity, and axon regeneration. NADPH oxidases (Nox enzymes) are transmembrane proteins, catalyzing electron transfer from NADPH to O_2 to generate superoxide and hydrogen peroxide (H_2O_2). Based on previous observations, we hypothesized that Nox-derived ROS mediate axonal growth and guidance. To test this, we adopted CRISPR-Cas9 genome editing strategy to establish *nox* mutants in zebrafish embryos. We found that *nox2*$^{-/-}$ mutants exhibit defects in ganglion cell layer (GCL) formation in embryonic zebrafish retina and misguided axons of retinal ganglion cells (RGCs) in the optic tecta. Besides retinal development, anterior commissure in the forebrain and longitudinal spinal cord axons exhibited aberrant projections, suggesting a role for Nox-derived ROS in axonal growth and guidance. The general brain development was not affected, since well-established CNS markers *pax6a* (forebrain), *otx2* (midbrain), and *fgf8* (midbrain hindbrain boundary) showed normal expressions in *nox2*$^{-/-}$ mutants. *In vitro*, cultured RGC neurons from *nox2*$^{-/-}$ mutants exhibited loss of proper response to guidance cues slit-2 and netrin-1, implying a cell-autonomous relationship between Nox2 and cues. To test this, we are now mutating *nox2* specifically in RGCs, along with cell transplantation experiments between mutant and wildtype embryos.

Disclosures: **A. Terzi:** None. **C.J. Weaver:** None. **H.S. Roeder:** None. **T.M. Gurol:** None. **Q. Deng:** None. **Y.F. Leung:** None. **D.M. Suter:** None.

Poster

364. Neuronal Morphogenesis

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 364.17/A42

Topic: A.05. Axon and Dendrite Development

Support: NRF-2015M3C7A1028790
NRF-2018R1A2A2A05023615
NRF-2017R1D1A3B03030324
HRF-201902-010

Title: Regulation of axonal length and 4EBP1, independent of mTORC1 activity, by a farnesylation-defective Rheb in embryonic primary neurons

Authors: S. CHOI[1], A. SADRA[1], J.-E. KANG[1], J. RYU[2], J. KIM[2], W. SUN[2], ***S.-O. HUH**[1]; [1]Hallym Univ. Med. Sch., Chuncheon, Korea, Republic of; [2]Brain Korea 21, Korea Univ., Seoul, Korea, Republic of

Abstract: Rheb (Ras homolog enriched in the brain) is a small GTPase protein that plays an important role in cell signaling for development of the neocortex through modulation of mTORC1 (mammalian-target-of-rapamycin-complex-1) activity. mTORC1 is known to control various biological processes including axonal growth in forming complexes at the lysosomal membrane compartment. As such, anchoring of Rheb on the lysosomal membrane via the farnesylation of Rheb at its cysteine residue (C180) is required for its promotion of mTOR activity. To test the significance of Rheb farnesylation, we overexpressed a farnesylation mutant form of Rheb, Rheb C180S, in primary rat hippocampal neurons and also in mouse embryonic neurons using *in utero* electroporation. Interestingly, we found that Rheb C180S maintained promotional effect of axonal elongation similar to the wild-type Rheb in both test systems. On the other hand, Rheb C180S failed to exhibit the multiple axon-promoting effect which is found in wild-type Rheb. The levels of phospho-4EBP1, a downstream target of mTORC1, were surprisingly increased in Rheb C180S transfected neurons, despite the levels of phosphorylated mTOR being significantly decreased compared to control vector transfectants. A specific mTORC1 inhibitor, rapamycin, also could not completely abolish axon elongation characteristics of Rheb C180S in transfected cells. Our data suggests that Rheb in a non-membrane compartment can promote the axonal elongation via phosphorylation of 4EBP1 and through an mTORC1-independent pathway

Disclosures: S. Choi: None. **A. Sadra:** None. **J. Kang:** None. **J. Ryu:** None. **J. Kim:** None. **W. Sun:** None. **S. Huh:** None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.01/A43

Topic: A.05. Axon and Dendrite Development

Title: Distribution regularity of mitochondria and en passant presynaptic sites along axon in the cerebellar granule neurons

Authors: *I. HORI[1], N. MATSUMOTO[1], S. MIYAKE[2,3], Y. KONISHI[1,3];
[1]Fac. of Engineering, Univ. of Fukui, Fukui, Japan; [2]Fac. of Med. Sciences, Univ. of Fukui, Fukui, Japan; [3]Life Sci. Innovation Center, Univ. of Fukui, Fukui, Japan

Abstract: In axons, mitochondria are transported via microtubules and motor proteins. On the other hand, stationary mitochondria stopping movement are also widely distributed and are considered to be involved in axon branching and elongation. The systems regulating the distribution of stationary mitochondria remain to be elucidated. In this study, we investigated the distribution patterns of stationary mitochondria. The probability distribution did not follow the Poisson distribution, suggesting that it is not a random distribution. Furthermore, by calculating the distribution concentration index called $I\delta$ index, it was suggested that there is a tendency of equal distribution. Next, in order to investigate the relevance of the distribution of stationary mitochondria and presynaptic factors, we analyzed the distribution of presynaptic factors using a same method. We found that there is a tendency of random distribution at the early stage of development, and it became close to equal distribution as maturation progresses. From the above, we performed time lapse imaging to test the possibility that the distribution of mitochondria controls the distribution of presynaptic factors. Notably, we observed that part of presynaptic factors and mitochondria were cotransported. These results suggested that mitochondria could regulate the distribution of presynapses.

Disclosures: I. Hori: None. **N. Matsumoto:** None. **S. Miyake:** None. **Y. Konishi:** None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.02/A44

Topic: A.05. Axon and Dendrite Development

Support: PICT 2014-2119 PRESTAMO BID
PROYECTO INVEST. UNVM

Title: Sara protein participation as a negative regulator of the transforming growth factor β signaling pathway during neuronal development

Authors: *V. ROZES-SALVADOR[1,2], S. SIRI[1], C. GONZALEZ-BILLAULT[3], C. B. CONDE[4];
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Abstract: Several events are necessary for proper neuronal development, such as differential protein expression, cytoskeletal dynamics, and endosomal trafficking. Smad Anchor for Receptor Activation (SARA) is a protein that binds to early endosomes; carrying out specific functions related to traffic but also participating in signaling such as in Transforming Growth Factor β (TGFβ) pathway. In this regard, it has been described that SARA recruits Smad2/3 and, therefore, favors the activation of this pathway but also can modulate the TβRI dephosphorylation by PP1c, and inactivate the pathway both in epithelial cells and cell lines. Moreover, it has been shown that TGFβ signaling specifies axon during neuronal development; however, SARA participation in this signaling pathway during the developmental process remains unknown. For this reason, we proposed to analyze the participation of SARA in TGFβ signaling during neuronal development. Results obtained in cultures of hippocampal neurons, through FRET Acceptor Photobleaching showed physical interaction between SARA and the TGFβ receptor I (TβRI). Also, performing loss and gain of function experiments, we found that SARA suppression (through shRNA expression) generates both greater axonal growth and loss of axonal specification since neurons have more than one axon compared with the control condition. In addition, the transition of polarity stage is accelerated in these neurons. Interestingly, this same phenotype is repeated when we use a mutant form of SARA (SARA-F728A) that alters its binding to PP1c protein (involved in TGFβ inactivation) and therefore, the TβRI remains hyperphosphorylated, keeping the pathway activated. Also, by FRET we found that SARA-F728A has more interaction with PP1c than control conditions, suggesting that SARA arrests to PP1c. Taking together; these results suggest that SARA participates in the TGFβ pathway in neurons through the negative regulation, which seems to be a necessary requirement for the correct neuronal development.

Disclosures: V. Rozes-Salvador: A. Employment/Salary (full or part-time):; National University of Villa María UNVM, University of Chile. **S. Siri:** None. **C. Gonzalez-Billault:** None. **C.B. Conde:** None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.03/A45

Topic: A.05. Axon and Dendrite Development

Support: NIGMS P30 GM110767
NIH R01 EY025205
NIGMS COBRE P30 GM103650

Title: Precise removal of Calm1 long 3′UTR isoform by CRISPR-Cas9 genome editing impairs dorsal root ganglion development in mice

Authors: H. N. GRUNER[1], ***B. BAE**[1], M. LYNCH[1], D. OLIVER[2], K. SO[1], G. S. MASTICK[1], W. YAN[2], P. MIURA[1];
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Abstract: Alternative polyadenylation often produces different length 3′UTR isoforms with the same protein-coding sequences. In mice and humans, thousands of alternative long 3′UTR isoforms are preferentially expressed in neural tissues. However, few studies have evaluated the function of alternative 3′UTR isoforms *in vivo*. In mice, the Calmodulin1 gene (*Calm1*) transcribes two mRNA isoforms, *Calm1-S* (0.9 kb 3′UTR) and *Calm1-L* (2.5 kb 3′UTR) in the nervous system. *Calm1* is one of three genes that encode calmodulin (CaM), a calcium signaling modulator important for the proper development of precerebellar and dorsal root ganglia (DRG) neurons. Our characterization of the expression patterns of *Calm1-S* and *Calm1-L* showed neural-enriched expression of *Calm1-L*, with particularly strong expression in DRG. To understand the functional impact of the *Calm1-L* loss in vivo, we performed CRISPR-mediated long 3′UTR-specific deletions. Our novel approach successfully abolished the expression of the *Calm1-L* specifically without impairing the expression of the short 3'UTR isoform and allowed us to directly assess the long 3′UTR isoform-specific phenotypes. Mice embryos lacking *Calm1-L* displayed abnormal positioning of cell bodies and aberrant axon extension of DRG neurons. Furthermore, *ex vivo* explant culture of mutant DRG showed abnormal axon fasciculation. We performed low-input capillary western of dissected DRGs and found that overall CaM protein levels were not changed between wild-type and mutants. Fluorescence *in situ* hybridization (FISH) and RNA-seq analysis of DRG revealed that *Calm1-S* but not *Calm1-L* was localized to axons. Consistent with this finding, 3′UTR isoform-specific stability assays showed that *Calm1-L* is less stable than *Calm1-S*. FISH analysis using 3′UTR-specific probes suggested that the *Calm1* 3′UTR is cleaved to generate isolated 3′UTR fragments. Further investigation is required

to determine the mechanism by which these isolated *Calm1* 3′UTR fragments are produced, and whether they contribute to the DRG phenotypes observed upon *Calm1-L* loss.

Disclosures: H.N. Gruner: None. **B. Bae:** None. **M. Lynch:** None. **D. Oliver:** None. **K. So:** None. **G.S. Mastick:** None. **W. Yan:** None. **P. Miura:** None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.04/A46

Topic: A.05. Axon and Dendrite Development

Support: NIH F30 DA047775

Title: Subcellular regulation of protein synthesis in dopaminergic neurons

Authors: *B. D. HOBSON[1,2], L. KONG[1], O. LIEBERMAN[2], D. SULZER[2], P. A. SIMS[1];
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Abstract: Subcellular localization and translation of mRNA is involved in neuronal development and synaptic plasticity, but most studies of local translation have focused on excitatory and inhibitory neurons. Midbrain dopaminergic neurons provide critical input to circuits involved in reward processing, movement control, and cognitive function. Dopamine neurons produce extensive, unmyelinated axons that travel via the medial forebrain bundle to innervate basal ganglia and cortical targets. In addition to axonal release in the forebrain, dopamine neurons also exhibit somatodendritic dopamine release in the midbrain, including from dendrites projecting ventrally into the substantia nigra pars reticulata. The molecular mechanisms by which dopamine neurons regulate neurotransmission across expansive subcellular compartments remain unclear. Therefore, detailed studies of subcellular translation in DA neurons may shed light on molecular mechanisms of dopaminergic function in health and disease.

Given the dynamic regulation of dopamine release across an elaborate cytoarchitecture, we suspected that dopamine neurons might employ local protein synthesis to enable rapid changes in the dendritic and axonal proteome. Here, we employed a combination of metabolic labeling, fluorescence *in situ* hybridization, and dopamine neuron-specific ribosome-bound RNA-sequencing to investigate local protein synthesis in midbrain dopamine neurons. In DA neuronal cultures, protein synthesis is particularly prominent in migrating DA axons (growth cones). We identified localization of mRNAs encoding for dopamine transmission machinery in both axons and dendrites of dopamine neurons *in vitro*. Fluorescence *in situ* hybridization revealed extensive mRNA in dopaminergic dendrites *in vivo*. Finally, ribosome-bound RNA sequencing revealed translating mRNAs in striatal axons and dendrites of the substantia nigra pars reticulata. These

results demonstrate that similar to excitatory and inhibitory neurons, dopamine neurons employ local translation in both axons and dendrites. Ongoing studies will address the relationship between local translation, regulation of dopamine release, and exposure to drugs of abuse.

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Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.05/A47

Topic: A.05. Axon and Dendrite Development

Support: R01NS089633

Title: Molecular, cellular, electrophysiological and behavioral correlates of neuronal dysfunction in KSRP knockout mice

Authors: *M. DELL'ORCO[1], P. PATEL[3], S. L. OLGUIN[1], A. S. GARDINER[2], R. D. COLE[5], P. I. ORTINSKI[4], A. M. ALLAN[1], J. L. BRIGMAN[1], J. L. TWISS[3], N. I. PERRONE-BIZZOZERO[1];
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Abstract: The KH-type splicing regulatory protein (KSRP) is an RNA-binding protein (RBP) associated with the decay of short-lived mRNAs containing AU-rich elements in the 3' untranslated region. Our previous studies demonstrated that KSRP is expressed in neurons during late stages of differentiation, where it destabilizes GAP-43 and other growth-associated mRNAs, halting axonal outgrowth. In contrast, loss of KSRP results in increased GAP-43 mRNA stability and axonal outgrowth in hippocampal neurons (Bird et al, PLoS One 2013). To understand the role of KSRP in neuronal differentiation and function in vivo, we first analyzed gene expression in adult mice that were either heterozygous (Het) or knockout (KO) to the *Khsrp* gene, along wild type (WT) littermates. Using microarrays, we found upregulation of 1902 transcripts in the neocortex, 532 of which are high affinity KSRP interacting mRNAs. This set was further compared to a set of neuron-specific mRNAs to identify neuronal targets of this RBP. Biological pathway analyses revealed that these targets are involved in dendritic and axonal formation and neurotransmission. Supporting these results, we found that neurons in the neocortex of *Khsrp* Het and KO mice exhibit increased dendritic spine density and axon growth. *Khsrp* KO mice also show increased frequency of miniature excitatory postsynaptic currents (mEPSC) in the hippocampus, while prefrontal cortex mEPSC frequency is increased in both KO

and Het mice. Primary neuron cultures from these mice point to a neuron-intrinsic alteration in axonal and dendritic growth. *Khsrp* KO mice showed significant trace conditioning deficits compared to age- and sex-matched WT, indicating impaired hippocampal-dependent learning. *Khsrp* KO mice also had significant deficits in behavioral flexibility as shown by their impaired performance in the reversal and set-shifting of species-specific stimulus domains, which require intact working memory. In addition, *Khsrp* KO mice display novelty-induced hyperactivity that was observed in several tests including open field and zero maze. Overall, our results indicate that prenatal deletion of KSRP impairs neuronal differentiation resulting in alterations in neuronal morphology and activity as well as prefrontal- and hippocampal-dependent behavior.

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Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.06/A48

Topic: A.05. Axon and Dendrite Development

Support: NIH R01NS082283
NIH P20GM103620

Title: A CLN6-CRMP2-KLC4 complex regulates anterograde, ER-derived vesicle trafficking in cortical neurites

Authors: *K. A. WHITE[1], J. T. CAIN[1], S. KOH[1], T. B. JOHNSON[1], D. TIMM[1], D. STURDEVANT[1], K. HENSLEY[2], J. M. WEIMER[1];
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Abstract: As neurons establish vast connections throughout the central nervous system, the transport of cargo along the microtubule network of the axon is a crucial step in neuronal differentiation, maintenance, and health. Specifically, building blocks such as membrane components, organelles, transmembrane receptors, adhesion molecules, and neurotransmitters all require proper transport to the distal end of the axon. Here, we identify a novel complex regulating anterograde vesicular transport in axons, composed of CLN6: an ER-associated protein of unknown function implicated in Batten disease, CRMP2: a tubulin binding protein important in regulating neurite microtubule dynamics, and KLC4: a classic transport motor protein. We find that this CCK complex allows ER-tagged vesicles to properly migrate to the distal end of the axon, aiding in proper neurite outgrowth and arborization. In the absence of CLN6, the CCK complex is unable to form, leading to deficits in CRMP2 binding to a host of

other protein partners, as well as reduced vesicular transport and stunted neurite outgrowth. Interestingly, treatment with a CRMP2 modulating compound, lanthionine ketamine ester, partially restored these deficits in a mouse model of CLN6 deficiency, indicating that stabilization of CRMP2 interacting partners may prove beneficial in lieu of restoring the CCK complex. Taken together, these findings boast a novel mechanism of ER vesicle transport in the axon, and provide new insights into therapeutic targets for neurodegenerative disease.

Disclosures: **K.A. White:** None. **J.T. Cain:** None. **S. Koh:** None. **T.B. Johnson:** None. **D. Timm:** None. **D. Sturdevant:** None. **K. Hensley:** None. **J.M. Weimer:** None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.07/A49

Topic: A.05. Axon and Dendrite Development

Support: the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
R01-NS089633
R01-NS041596
Wings for Life-WFL-US-09/18-185

Title: Selective axonal translation of prenylated Cdc42 mRNA isoform supports axon growth

Authors: *M. D. ZDRADZINSKI, S. J. LEE, P. PATEL, A. N. KAR, P. SAHOO, K. D. LANTZ, J. L. TWISS;
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Abstract: Cdc42 is a member of the Rho family of GTPases that plays a key regulatory role in actin dynamics. Actin polymerization is integral to neuronal growth, regeneration, axon pathfinding and circuit formation. The *CDC42* gene gives rise to two splice variants that have mRNAs with different 3'UTRs and encode proteins with distinct C-termini containing a CCAX motif for palmitoylation and a CAAX motif for prenylation (Palm-Cdc42 and Prenyl-Cdc42, respectively). Palm-Cdc42 has been shown to regulate dendritic spine maturation, while Prenyl-Cdc42 acts in axon specification (Yap et al, 2016). We show that knockdown of the Prenyl-Cdc42 and not Palm-Cdc42 reduces axon length in cultured Dorsal Root Ganglion (DRG) neurons. Despite that both proteins are seen in axons, Prenyl-Cdc42 mRNA and not Palm-Cdc42 mRNA localizes into axons. The 3'UTR of Prenyl-Cdc42 mRNA drives its localization into axons of both PNS and CNS neurons in culture. Full axon growth promotion by Prenyl-Cdc42 requires that both the mRNA localize into axons and the encoded protein has an intact CAAX motif for post-translational modification in axons. Interestingly, the axonally translated Prenyl-Cdc42 protein localizes to the distal membrane of axonal growth cones, which is distinct from

the patterns seen for Palm-Cdc42 when its mRNA is targeted into axons with a heterologous 3'UTR and for cell body-translated Prenyl-Cdc42 protein. On the other hand, the Palm-Cdc42 mRNA localizes into dendrites raising the possibility that localized translation of Prenyl-Cdc42 is needed for dendrite growth. Taken together, these data show that alternative splicing of the CDC42 gene product generates unique subcellular localizing mRNAs that likely underlie its role in neuronal growth and maturation.

Disclosures: M.D. Zdradzinski: None. **S.J. Lee:** None. **P. Patel:** None. **A.N. Kar:** None. **P. Sahoo:** None. **K.D. Lantz:** None. **J.L. Twiss:** None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.08/A50

Topic: A.05. Axon and Dendrite Development

Support: ERC StG 2015, grant number 677844

Title: Investigating the role of Miro 1-dependent mitochondrial trafficking in GABAergic interneurons

Authors: *K. NDOCI[1], P. PELZER[1], V. SAKTHIVELU[1], G. WANI[1], J. M. SHAW[2], M. BERGAMI[1,3];
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Abstract: GABAergic interneurons are generally characterized by fast firing rates and a higher energetic demand as compared to most excitatory neurons. By supplying ATP and several biosynthetic precursors, mitochondria critically contribute to energy metabolism in neurons, particularly at synaptic sites, suggesting a key role for mitochondrial trafficking in interneurons. Recent evidence indicates that interfering with mitochondrial ATP supply at early stages of neuronal development specifically impairs the migration of interneurons, but not excitatory neurons, through the neocortex. However, whether and to which extent mitochondrial motility per se is required for interneuronal migration during development or for synaptic transmission and circuit activity later on is incompletely understood. Mitochondrial transport relies on microtubule-based motors: kinesin proteins carry the organelles toward the plus end of the microtubules, whereas dynein proteins move them in the opposite direction. The attachment of mitochondria to these motors is mediated by an adaptor complex which comprises the Ca^{2+}-dependent mitochondrial GTPases Miro1 and 2 as well as their binding partners (Traks). Here, we examined the role of Miro1 in GABAergic interneurons *in vivo* and tested the hypothesis that

mitochondrial trafficking is important to sculpt the connectivity of these cells. We conditionally deleted Miro1 from the two most represented interneuronal subtypes, i.e. parvalbumin-positive (PV+) and somatostatin-positive (SST+) interneurons. Loss of Miro1 disrupted mitochondrial trafficking in both classes of interneurons, leading to altered mitochondrial distribution and morphology along neurites, despite negligible neuronal death. Intriguingly, we found that mice lacking Miro1 in SST+ neurons, but not in PV+ neurons, developed epileptic seizures during their first months of life, indicating a key role of Miro1 in maintaining the inhibitory tone of specifically this class of interneurons. Our finding suggests that a differential susceptibility of distinct classes of GABAergic interneurons to defective mitochondrial dynamics may underlie the emergence of pro-epileptogenic symptoms.

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Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.09/DP03/A51

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: A.05. Axon and Dendrite Development

Support: Project no. LQ1605 from the national program of sustainability II (MEYS CR)

Title: Identifying regulators of neuronal transport

Authors: *M. FEOLE[1], V. M. POZO DEVOTO[2], M. ČARNA[3], G. B. STOKIN[3];
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Abstract: Neurons are highly polarized cells continuously relying on intracellular transport, which allows them to send information outside trough the axon and receive them through the dendrites. Movement along different regions is a key feature of neuronal transport, allowing many proteins with different roles in neuron homeostasis to reach long distances. Several neurodegenerative diseases, such as Alzheimer's, Parkinson's or Amyotrophic Lateral Sclerosis, exhibit significant axonal pathology, where transport impairment can have a crucial influence. However, the mechanism underlying the disruption of neuronal transport remains unclear. In this study we aim to characterize neuronal transport dynamics of specific proteins and the molecular machinery that is regulating this complex process. Human Neural Stem Cells derived neurons were terminally differentiated in a multichannel device (Ibidi) for 30 DIV. Plasmids encoding for

different GFP coupled proteins were designed to express those who are involved in important neuronal functions (development, synaptic activity, disease signaling) and transfected in neurons. Time-lapse movies of the cargos were acquired to detect possible changes in movement. Data analysis was performed in Imaris software, using specific object segmentation and tracking algorithms. Raw data from automated analysis were processed to describe the main motion parameters: velocity, directionality, track length, and others. Post-imaging Immunocytochemistry experiments were performed to assess the localization of our cargos, and discriminate between axonal or dendritic transport. Preliminary results showed different cargo's behavior during movement: Amyloid Precursor Protein (APP) cargos with higher velocity in anterograde movement were observed compared with the retrograde one; meanwhile for Synaptophysin a major population of stationary particles was found. Moreover, sorted data for axonal and dendritic transport description shown faster APP particles in axonal anterograde movement. Relying on the description of movement dynamics in neurons, we decided to screen for protein-protein interactions of our cargos, using GFP trap and CoIP approaches followed by Mass Spectrometry analysis, which will shed light on possible partners involved in the regulation of transport. Our work describes an *in vitro* model for neuronal transport study to highlight the different behavior of proteins involved in crucial physiological processes. The complete view will elucidate details about neuronal transport regulation and will open a way to understand the role of this process in the pathophysiology of neurodegenerative diseases.

Disclosures: M. Feole: None. **V.M. Pozo Devoto:** None. **M. Čarna:** None. **G.B. Stokin:** None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.10/A52

Topic: A.05. Axon and Dendrite Development

Support: NS062047

Title: Cooperation of MAP7 and tau controls regional regulation of axonal transport in sensory neurons

Authors: *S. TYMANSKYJ, L. MA;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: Different regions of a neuron have varying functional needs that require differential regulation of axonal transport driven by microtubule motors, but the underlying mechanisms of motor regulation remains unclear. *In vitro* studies have shown that binding of motors to microtubules can be regulated by microtubule associated proteins (MAPs). For example, tau inhibits the binding of the conventional kinesin (kinesin-1) to microtubules, whereas MAP7

recruits kinesin-1 to microtubules. Here, we examined the function and regulation of these two MAPs in sensory neurons from the dorsal root ganglion (DRG). We show that MAP7 is enriched at branch junctions, whereas tau has an opposing localization, concentrated distally towards the growth cone. Interestingly, transport of mitochondria in these regions is correlated with MAP7 and tau localization, with increased anterograde transport at branch junctions but reduced anterograde transport in the distal axons. We further examined the mechanisms mediating MAP7 and tau localization. In COS cells where single microtubules can be resolved, we show MAP7 and tau occupy different regions along individual microtubules, with MAP7 preferring stable regions and tau binding to more dynamic regions. This binding property of MAP7 creates a boundary that prevents microtubule depolymerization and rescues microtubule polymerization. In DRG neurons, this property allows MAP7 to promote branching by reducing branch retraction. Further domain analysis shows that this MAP7 function is mediated by the middle 'P'-domain, which is responsible for competing against tau for microtubule binding. In addition to harboring a second microtubule binding site, the P-domain also contains multiple phosphorylation sites that influences the microtubule binding kinetics of MAP7 and hence impacts microtubule stability and kinesin-mediated transport. Taken together, our studies demonstrate that a cooperative mechanism mediated by differential microtubule binding by MAP7 and tau that impacts kinesin mediated delivery of cargos into different compartments of the neuron.

Disclosures: S. Tymanskyj: None. **L. Ma:** None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

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Topic: A.05. Axon and Dendrite Development

Support: NIH NRSA 1F31-NS103262
NIH Pioneer Award DPI OD02461
Allen Frontiers Group
Brain Research Foundation

Title: Growth cone molecular machinery locally implements the development of subtype-specific neocortical circuitry

Authors: *J. HATCH[1], A. POULOPOULOS[2], A. ENGMANN[1], J. MACKLIS[1];
[1]Stem Cell & Regenerative Biology, Ctr. for Brain Sci., Harvard Univ., Cambridge, MA;
[2]Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: During development, growth cones (GCs) of diverse projection neuron (PN) subtypes navigate complex extracellular environments to reach distant, subtype-specific targets. These axon-terminal structures must respond to substrate-bound and diffusible signals in a subtype- and context-specific fashion to construct functional circuitry. Recent studies strongly indicate that subcellular localization of specific molecular machinery to GCs might underlie the precise behaviors of these structures during circuit "wiring." While great progress has been made toward identifying diffusible and substrate-bound signals that guide axon growth, it is becoming increasingly clear that intracellular, local growth cone biology underlies the distinct behaviors of specific neuronal subtypes at specific developmental stages. Molecular determinants of these critical processes remain largely unstudied with respect to distinct neuronal subtypes under physiological conditions. Because most current knowledge of growth cone biology was identified *in vitro,* often with heterogeneous populations, access to subtype-specific growth cones in their native environments during normal development will substantially elucidate molecular bases of cortical and other neural circuit formation.
Our laboratory has recently developed an integrated approach that enables high-throughput, high-depth proteomic and transcriptomic investigation of purified GCs from fluorescently labeled subtype-specific cortical projection neurons. This approach has already revealed unanticipated depth of GC molecular machinery, subtype-specificity, and GC enrichment of hundreds of transcripts and proteins. Building on this foundational work, GCs have been isolated from closely-related PN subtypes with distinct axonal trajectories at critical developmental stages to investigate whether and how subtype-specific GC molecular machinery might functionally enable specific subtypes to build and maintain specific circuitry. In particular, we investigate dynamic regulation of GC transcriptomes before and after midline crossing in interhemispheric callosal PN (CPN), and investigate whether and how local molecular machinery might implement a subcortical vs. intracortical trajectory in closely related subtypes, including CPN, corticospinal neurons (CSN), and corticothalamic PN (CThPN). Together, this work has revealed a number of exciting new candidate regulators of cell-autonomous, subtype-specific axon guidance, as well as generated conceptually novel hypotheses about how regulation of growth cone transcriptomes might contribute to the formation of specific circuitry.

Disclosures: J. Hatch: None. **A. Poulopoulos:** None. **A. Engmann:** None. **J. Macklis:** None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.12/A54

Topic: A.05. Axon and Dendrite Development

Support: NIH K01 NS110449-02
NIH R01 NS050674

Title: Defects in transport of SFPQ RNA granules by KIF5A/KLC1 motors contribute to axon degeneration in CMT

Authors: *Y. FUKUDA, M. F. PAZYRA-MURPHY, O. E. TASDEMIR-YILMAZ, H.-S. SEO, S. DHE-PAGANON, *R. A. SEGAL;
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Abstract: Neuronal axons and dendrites can span enormous distances, and so rapid intracellular transport is needed for sustained activity of these extensive projections. RNA binding proteins (RBPs) are needed for transport of RNA granules and local translation, and thus are critical regulators of the axonal transcriptome. Multiple mutations that disturb functions of RBPs can cause neurological disorders. However, whether disrupted axonal transport of RBPs directly compromises axon integrity in neurological diseases is not yet known. Splicing factor proline-glutamine rich (SFPQ) is a RBP that packages NGF-regulated transcripts, such as *Bclw*, into RNA granules and enables axonal translation of these transcripts to prevent axonal degeneration. To identify the mechanisms that transport SFPQ to axons**,** we first asked which kinesins can interact with SFPQ using co-precipitation studies. In lysates of primary rat dorsal root ganglion neurons (DRG), SFPQ selectively binds to KIF5A, one of the three conventional kinesins, and not to the other, highly related conventional kinesins, KIF5B and KIF5C, or to other KIF motors. Further specificity was evident as SFPQ selectively interacts with tetrameric motors, containing KIF5A motors and the cargo adaptor KLC1, and not those containing KIF5A and KLC2. To define the mechanism for this dual specificity, we identified the domains responsible for the interactions. We find that the highly divergent carboxy-terminal regions of KIF5A and KLC1 are required for binding to SFPQ. Recently, a Y-acidic motif residing in the cargos of kinesin-1 motor has been demonstrated to provide specificity for binding to KLC1 over KLC2. Interestingly, SFPQ contains the Y-acidic motif within its coiled coil domain. Using Isothermal titration calorimetry, we demonstrate that the peptide region spanning the Y-acidic motif of SFPQ is sufficient to directly bind to KLC1. Mutations in KIF5A are a frequent cause of axon degeneration and neuropathy in patients with CMT2 We find that expression of human, CMT2-associated mutations of KIF5A in DRGs lead to degeneration of axons. Similarly, specific disruption of kinesin-driven transport of SFPQ by expression of the Y-acidic mutant of SFPQ , which does not bind to KIF5A/KLC1 motor complex, also causes axon degeneration. Together these data demonstrate that disrupted transport of a KIF5A/KLC1-specific cargo, SFPQ, may be one of the key mechanisms underlying axon degeneration in patients harbouring KIF5A CMT2 mutations.

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Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.13/A55

Topic: A.05. Axon and Dendrite Development

Support: DP1 NS106665

Title: 3'Utr RNA sequence motifs that direct subtype-specific axonal RNA trafficking during development of cerebral cortex projection neurons: Identification and functional analysis via computationally-enabled, massively parallel reporter assay (mprau) *in vivo*

Authors: *P. VEERARAGHAVAN, J. J. HATCH, J. D. MACKLIS;
Dept. of Stem Cell and Regenerative Biol. & Ctr. for Brain Sci., Harvard Univ., Cambridge, MA

Abstract: Neurons are by far the most polarized cell type, and they need to isolate cellular functions from their dendrites at one end to their distal axons at the other, with cell body functions in between. Projection neurons (PN) of the cerebral cortex, in particular, are among the longest of any neuron classes, and I focus on these. Cortical PN exemplify the extreme of spatially distributed functionality; they underlie sensory-motor integration, cognition, and behavior, and are thus critically important to understand. Advances in molecular understanding of their subtype-specific development enables their functional molecular interrogation. During development, PN extend axons led by semi-autonomous growth cones (GCs) over great distances (10^3 -10^5 soma diameters), through varied extracellular environments, to reach defined, circuit-appropriate targets. The mechanisms by which GCs sense and respond to the many environments through which they move are not fully understood, nor is the extent to which the nucleus communicates or controls the transcriptomic and proteomic makeup of subtype-specific GCs *in vivo*. RNA trafficking and stability are major determinants of GC transcriptomic makeup; while these general mechanisms have been well studied in other cell types, subtype-specific RNA trafficking and stability in PN are not deeply understood.
Our lab has recently developed an approach that enables deep transcriptomic and proteomic investigation of purified GCs from fluorescently-labeled subtype-specific projection neurons. Our lab has already identified an early GC-localized motif, but this alone is insufficient to explain the hundreds of transcripts we find localized to GCs. I extend this work through a combined computational and experimental approach to identify (potentially minimal) *cis*-acting sequence elements within the GC-localized transcriptome that might direct axonal RNA trafficking, and therefore control circuit development and later synaptic function. In particular, I adapt novel tools, notably a massively parallel reporter assay (MPRA), to perform a screen of *cis*-acting RNA sequences that drive axonal localization in PN *in vivo,* computationally informed by our existing subcellular transcriptomic data. I functionally test perturbations of RNA

sequences that drive GC localization, in order to discover minimal, potentially combinatorial, subelements responsible for GC localization. These investigations aim to reveal novel *in vivo* biology that underlies the spatial distribution of function in developing neurons, and to provide new knowledge about the mechanisms by which neurons generate precise, subtype-specific circuitry.

Disclosures: P. Veeraraghavan: None.

Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.14/A56

Topic: A.02. Postnatal Neurogenesis

Support: Core Research for Evolutional Science and Technology (CREST) from Japan Science and Technology Agency
Grant-in-Aid for Scientific Research(C) Grant 19K06502

Title: DNA-methyltranseferase 1 participates in methylation of neuronal enhancer in post-mitotic neuron of mammalian brain

Authors: *M. NAMIHIRA[1], A. KIMURA[2], Y. SAITO[3], T. MATSUDA[2], T. IMAMURA[2], H. NOGUCHI[2], M. I. OTSUKA[4], K. IGARASHI[4], F. MIURA[5], T. ITO[5], S. KATADA[2], K. NAKASHIMA[2];
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Abstract: Maintenance methyltransferase, DNA methyltransferase 1 (DNMT1) is essential to copy the methylation pattern on newly replicated genomic DNA in proliferating cells. Although the abundant expression of DNMT1 in post-mitotic neurons at developmental stage and in the adult mammalian brain has been observed, its function in these cells still remains elusive. To investigate the function of DNMT1 in post-mitotic neurons, we induced DNMT1 deficiency in these cells and examined the phenotype in vitro and in vivo using neuron-specific Dnmt1 conditional knock-out (cKO) mice. We found that the dendritic complexity in DNMT1-deficient cultured neurons and in dentate gyrus (DG) neurons of Dnmt1 cKO mice was increased compared to that in control neurons. Moreover, Dnmt1 cKO mice exhibited a symptom of hyperactivity in behavioral analysis, especially the open field test and light/dark transition test. DNA methylome analysis shows preferential hypomethylation of neuron-specific enhancers together with the slight genome-wide hypomethylation in DNMT1-deficient neurons directly

isolated from DG of Dnmt1 cKO mice. Correlating with this result, the upregulated expression of neuronal genes that are controlled by these enhancers was observed in DNMT1-deficient cultured neuron. Collectively, these results suggest that DNMT1 in post-mitotic neuron play a crucial role in proper neuronal development and function via DNA methylation on neuronal enhancers. Further analysis of the DNMT1 function in post-mitotic neurons is currently underway.

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Poster

365. Axon Growth and Guidance: Axonal Transport and Trafficking

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 365.15/A57

Topic: A.05. Axon and Dendrite Development

Title: LKE maintains tubulin polymerization and CRMP2 function in cortical neurons and oligodendrocyte precursor cells during inflammation *in vitro*

Authors: *V. L. SAVCHENKO, D. L. FEINSTEIN;
Anesthesiol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Neuroinflammation includes the release of pro-inflammatory cytokines from astrocytes, microglia or other immune cells and it may trigger axonal degeneration. Collapsin response mediator protein-2 (CRMP2), a tubulin-binding protein, has been shown to be cleaved by calpain1 during neurodegeneration and considered as a potential target for therapeutic approaches. Lanthionine ketimine ester (LKE) binds to CRMP2 and prevents its cleavage during protease activation. The effect of LKE was examined in cortical cultured neurons at 12 days in vitro (DIV) in normal and under inflammatory conditions induced by glutamate, interferon gamma (IFNγ) or conditioned medium from microglial cells treated with IFN (mIFNγ). The same effects were assessed in purified cultured oligodendrocytes (OLGs) and their precursor cells (OPCs) *in vitro.* The morphological features of neurons and glia and the distribution of cytoskeletal proteins were analyzed by immunofluorescent method and image analysis. CRMP2 and β3 tubulin were distributed in neuronal soma and neurites uniformly under normal conditions. LKE increased CRMP2 expression in cortical neurons and their neurites become thicker and longer. The overexcitation of cortical neurons induced by glutamate evoked reduction of β3 tubulin expression and the neurites became swollen and shortened, whereas LKE partially maintained the expression of β3 tubulin and CRMP2. IFNγ induced down-regulation of β3 tubulin in CRMP2 expressed neurons. However, mIFNγ was found to be most harmful and induced damage in cortical neurons. Quantitative analysis shows that CRMP2 expression was

increased by incubation with LKE, or glutamate. The expression of β3 tubulin was increased by LKE and decreased by glutamate, IFNγ or mIFNγ. The ratio of CRMP2 and β3 tubulin expression was increased due to LKE, but decreased during inflammation. The highly branched OPCs expressed PDGFRα and were regulated by PDGF and glutamate. The purified cultured OPCs were less branched and CRMP2 was expressed in the growth cones and protrusions connected to ECMs; CRMP2 was highly expressed in branched OLGs that were labeled with myelin basic protein. The purified OPCs were vulnerable and CRMP2 expression was suppressed during inflammation. The response of purified cultured microglia to the cytokines was stronger than in the mixed cultured neuronal cells suggesting the contribution with factors released from neurons and astrocytes. The results suggest that cleaved CRMP2 protein does not stabilize microtubules during neuroinflammation and LKE maintains CRMP2-mediated microtubule polymerization in neurons and the differentiation of OPCs into OLGs.

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Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.01/A58

Topic: A.07. Developmental Disorders

Support: Start-up Fund from the University of Illinois at Urbana-Champaign
NARSAD Young Investigator Award (336605)
National Institutes of Health (R01NS105615)

Title: Dysregulation and restoration of homeostatic network plasticity in fragile X syndrome mice

Authors: *D. E. EAGLEMAN[1], K. A. JEWETT[1], K. LEE[1], S. SORIANO[2], D.-C. LIU[2], N.-P. TSAI[1,2];
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Abstract: Chronic activity perturbations in neurons induce homeostatic plasticity through modulation of synaptic strength or other intrinsic properties to maintain the correct physiological range of excitability. Although similar plasticity can also occur at the population level, what molecular mechanisms are involved remain unclear. In the current study, we utilized a

multielectrode array (MEA) recording system to evaluate homeostatic neural network activity of primary mouse cortical neuron cultures. We demonstrated that chronic elevation of neuronal activity through the inhibition of GABA(A) receptors elicits synchronization of neural network activity and homeostatic reduction of the amplitude of spontaneous neural network spikes. We subsequently showed that this phenomenon is mediated by the ubiquitination of tumor suppressor p53, which is triggered by murine double minute-2 (Mdm2). Using a mouse model of fragile X syndrome, in which fragile X mental retardation protein (FMRP) is absent (*Fmr1* knockout), we found that Mdm2-p53 signaling, network synchronization, and the reduction of network spike amplitude upon chronic activity stimulation were all impaired. Pharmacologically inhibiting p53 with Pifithrin-α or genetically employing *p53* heterozygous mice to enforce the inactivation of p53 in *Fmr1* knockout cultures restored the synchronization of neural network activity after chronic activity stimulation and partially corrects the homeostatic reduction of neural network spike amplitude. Together, our findings reveal the roles of both *Fmr1*and Mdm2-p53 signaling in the homeostatic regulation of neural network activity and provide insight into the deficits of excitability homeostasis seen when *Fmr1* is compromised, such as occurs with fragile X syndrome.

Disclosures: **D.E. Eagleman:** None. **K.A. Jewett:** None. **K. Lee:** None. **S. Soriano:** None. **D. Liu:** None. **N. Tsai:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.02/A59

Topic: A.07. Developmental Disorders

Support: DBT core grant
TIFR core grant
NaMoR

Title: Impaired reliability and precision of spiking in adults but not juveniles in a mouse model of Fragile X syndrome

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Abstract: Fragile X Syndrome (FXS) is the most common source of intellectual disability, affecting 1 in 100 people. Previous studies have implicated altered functioning of ion channels in

multiple symptoms manifested in FXS. Our knowledge about intrinsic conductance changes, at the cellular level in disease models is still limited, despite extensive network level studies in mouse models. Studies in the past few years have shown reduced synchrony of spike discharge in CA1 hippocampus in FXS, which might be due to alterations in synaptic connections between neurons or changes in intrinsic conductances of the neuron. The present study examines intrinsic conductances which might underlie synchrony changes in neurons of FXS mice. The objective of the study was to see if one can establish single-cell correlates of the network incoherence observed in vivo. Firing variability at the single cell level was measured using whole cell patch clamp in CA1 pyramidal cells of hippocampal slices in FXS mice, in both juvenile and adult animals. We used a step current pulse protocol repeated over multiple trials and probed for variability in spike number and spike timing precision across different trials. We found that both spike numbers and spike timing were more variable for adult KO animals as compared to their WT littermates, but the same phenotype was not observed in juveniles. Spike number variability was increased at both within and between cell levels across multiple trials, in FXS neurons. Increased variability had a high correlation with reduced spike number, only for adult but not for juvenile CA1 cells. In contrast, juvenile CA3 cells were found to be hyperexcitable in FXS mice, indicating differential effects of FMRP KO in different regions. The reduction in spike numbers in CA1 was due to increased mAHP which we attributed to elevated SK currents. Blocking the SK current led to a partial rescue of the phenotypes of cell variability. We did not find differences in levels of I_h current and M currents between WT and KO, which were other putative candidates affecting mAHPs. We probed levels of SK channels at the soma and dendrites using immunfluorescence experiments. In agreement with previously published results we did not observe a significant change in the levels of SK channel at either soma or dendrites. We interpret this to mean that FMRP has an alternate mechanism, such as some previous studies have shown that it can interact via protein - protein interaction and modulate ion channels functioning. Thus, the present study has established a single cell level correlate of increased spike incoherence observed in FXS mice where SK currents are found to be partially responsible for the observed phenotype.

Disclosures: **D. Dwivedi:** None. **S. Chattarji:** None. **U.S. Bhalla:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

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Topic: A.07. Developmental Disorders

Support: NIH Grant R01MH078972
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NIH Grant R01NS105200

NIH Grant R01MH116582
NIH Grant P30HD03352
NIH Grant U54HD090256

Title: Mitochondrial deficits contribute to impaired dendritic maturation in FXS human and mouse neurons

Authors: *M. SHEN, X. ZHAO;
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Abstract: Fragile X syndrome results from a loss of the RNA-binding protein fragile X mental retardation protein (FMRP). How FMRP regulates neuronal development and function remains unclear. During embryonic development, mitochondria are important for neural progenitor proliferation and neuronal survival. Alterations in mitochondrial morphology and function directly impact morphological development of neurons. Here, we report that FMRP has a critical role in dendritic maturation of adult new neurons, neonatal hippocampal neurons, and human neurons developed in transplanted mouse brains. We discover that FMRP-deficient immature neurons exhibited altered expression of mitochondrial genes, fragmented mitochondria, impaired mitochondrial function, and increased oxidative stress. Enhancing mitochondria fusion by either a chemical activator or exogenous expression of mitochondrial fusion genes rescued both mitochondrial morphology and dendritic maturation deficits of FMRP-deficient neurons. We discovered that FMRP deficient neurons had reduced HTT levels and acute knockdown of HTT recapitulates both mitochondrial fusion and neuronal maturation deficits seen in Fmr1 KO neurons. We used guide RNAs to target modified CRISPR/Cas9 (dCas9VP64-SAM) to selectively activate the endogenous Htt gene in neurons and show that increased Htt transcription rescued both mitochondrial fusion and dendritic maturation deficits of Fmr1 KO neurons. Finally, we show that mice with HTT knockdown in the hippocampus exhibit several behavioral deficits similar to Fmr1 mutant mice and treatment with a mitochondrial fusion compound rescued behavioral deficits of both Fmr1 KO mice and mice with hippocampal knockdown of HTT. Our data demonstrate that mitochondrial dysfunction contributes to the impaired maturation of FMRP-deficient developing neurons and present a crosstalk between FMRP and HTT in pathogenesis of human diseases.

Disclosures: M. Shen: None. **X. Zhao:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

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Program #/Poster #: 366.04/A61

Topic: A.07. Developmental Disorders

Support: SFARI #336605
NARSAD Young Investigator Grant
NIH Grant R01NS105615

Title: Loss of fragile x protein fmrp impairs homeostatic synaptic downscaling through tumor suppressor p53 and ubiquitin E3 ligase Nedd4-2

Authors: *K. LEE[1], K. A. JEWETT[1], H. CHUNG[1,2], N.-P. TSAI[1,2];
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Abstract: Synaptic scaling allows neurons to homeostatically readjust synaptic strength upon chronic neural activity perturbations. Although altered synaptic scaling has been implicated to underlie imbalanced brain excitability in neurological disorders such as autism spectrum disorders and epilepsy, the molecular dysregulation and restoration of synaptic scaling in those diseases have not been demonstrated. Here, we showed that the homeostatic synaptic downscaling is absent in the hippocampal neurons of Fmr1 KO mice, the mouse model of the most common inherited autism, fragile X syndrome (FXS). We found that the impaired homeostatic synaptic downscaling in Fmr1 KO neurons is caused by loss-of-function dephosphorylation of an epilepsy-associated ubiquitin E3 ligase, neural precursor cell expressed developmentally down-regulated gene 4-2, Nedd4-2. Such dephosphorylation of Nedd4-2 is surprisingly caused by abnormally stable tumor suppressor p53 and subsequently destabilized kinase Akt. Dephosphorylated Nedd4-2 fails to elicit 14-3-3-dependent ubiquitination and down-regulation of the GluA1 subunit of AMPA receptor, and therefore impairs synaptic downscaling. Most importantly, using a pharmacological inhibitor of p53, Nedd4-2 phosphorylation, GluA1 ubiquitination and synaptic downscaling are all restored in Fmr1 KO neurons. Together, our results discover a novel cellular mechanism underlying synaptic downscaling, and demonstrate the dysregulation and successful restoration of this mechanism in the FXS mouse model.

Disclosures: K. Lee: None. **K.A. Jewett:** None. **H. Chung:** None. **N. Tsai:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

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Program #/Poster #: 366.05/A62

Topic: A.07. Developmental Disorders

Support: 5R01MH106490
FRAXA research foundation Postdoctoral fellowship

Title: FMRP deficiency up-regulates miR-128 and contributes to GLT1 dysregulation in Fmr1 KO astrocytes

Authors: *Y. MEN, V. PROMES, R. JARVIS, J. YELICK, E. BROWN, Y. YANG;
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Abstract: Fragile X syndrome (FXS) is a neurodevelopmental disorder which is caused by the loss-of-function of fragile X mental retardation protein (FMRP). We previously showed that astroglial glutamate transporter subtype GLT1 is significantly reduced, resulting from the decreased mGluR5 signaling in cortical astrocytes of Fmr1 KO mice. The downregulation of GLT1 contributes to dysregulated extracellular glutamate homeostasis and enhanced neuronal excitability in Fmr1 KO mice. In our current study, we investigated the miRNA (miR)-mediated mechanisms for mGluR5 and GLT1 dysregulation in Fmr1 KO astrocytes. We first compared miR expression levels from both wild type (WT) and Fmr1 KO primary astrocytes and neurons using miR microarrays. The loss of FMRP has little effect on the overall miR expression in neurons. In contrast, a large percentage (29.1%) of miRs are found up- (but not down-) regulated in Fmr1 KO astrocytes compared to WT astrocytes, implicating a primarily inhibitory role of FMRP in regulating miR expression in astrocytes, but not in neurons. Re-expression of FMRP in Fmr1 KO astrocytes sufficiently reverses increased miR expression. Consistently to the elevated miR levels in Fmr1 KO astrocytes, the primary transcripts of representative miRs (pri-miRs) are also increased in Fmr1 KO astrocytes, implicating that FMRP likely suppress the transcription of miRs in astrocytes. We further analyzed up-regulated miRs and found that one such miR, miR-128 (15-fold increase in Fmr1 KO vs. WT astrocytes) has a predicted binding site on mGluR5 3'UTR. Subsequent transfection of miR-128 abolished 36% mGluR5 protein level by directly binding to mGluR5 mRNA transcript in astrocytes. MiR-128 transfection also reduces 33% of neuron-induced GLT1 in neuron and astrocyte co-cultures. Our results suggest that miR-128 has an inhibitory effect on GLT1 by directly inhibiting mGluR5 expression through binding to its 3'UTR in astrocytes. Thus, the substantial upregulation of miR-128 reveal a potential new role of miR-mediated pathological mechanisms in mGluR5 and GLT1 dysregulation in mouse models of FXS, providing potential new targets to modulate FXS symptoms.

Disclosures: Y. Men: None. **V. Promes:** None. **R. Jarvis:** None. **J. Yelick:** None. **E. Brown:** None. **Y. Yang:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

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Program #/Poster #: 366.06/A63

Topic: A.07. Developmental Disorders

Support: NIH R01NS08056501A1

NIH F31DC016192

Title: Altered ionotropic receptor maturation underlies network dysregulation and impaired auditory critical periods of Fragile X knockout mice

Authors: *Y. J. SONG, F. E. JENSEN;
Neurol., Univ. of Pennsylvania, Perelman Sch. of Med., Philadelphia, PA

Abstract: Fragile X syndrome (FXS) is the leading genetic cause of intellectual disability and autism. Patients have deficits in auditory processing, communication and language development, making the auditory cortex of interest. *Fmr1* KO mice exhibit impaired tonotopic plasticity during the auditory critical period (CP) (Kim et al, 2013), impaired parvalbumin cell development in auditory cortex (Wen et al, 2017), and altered auditory processing (Rotschafer et al, 2013). Such plasticity and excitatory-inhibitory (E-I) imbalance phenotypes suggest a dysregulated auditory circuitry that causes FXS symptoms.
AMPA subunits of the glutamate receptor and $GABA_A$ receptor subunits undergo dynamic regulation during development to mediate E-I levels and optimize plasticity. We hypothesized that altered maturation of AMPA and $GABA_A$ receptor subunits in the auditory cortex contributes to the impaired CP in *Fmr1* KOs. Compared to WTs, *Fmr1* KOs exhibit an earlier developmental switch in AMPAR subunits with the relative GluA2:GluA1 expression significantly increased in KOs at P9 ($p=0.011$; prior to ear canal opening) and P12 ($p=0.015$; onset of WT auditory CP) that normalizes at older ages, as measured by Western blot. $GABA_A$ $\alpha3$ and $\alpha1$ subunits also have an accelerated developmental switch: $\alpha3$ is reduced at P12 ($p=0.021$) (trends at P9, $p=0.0695$), and $\alpha1$ is increased at P24 ($p=0.0056$) (pattern appears at P15, closure of CP). Consistent with the pattern of precocious maturation of AMPAR and $GABA_A$Rs, extracellular recordings in L2/3 of auditory cortex slices with L4 stimulation revealed that KOs are significantly more excitable at P12-13 ($p<0.0001$) and less excitable at P21-25 ($p<0.0001$) compared to WT. Furthermore, *Fmr1* KOs further exhibit an earlier and enhanced age-dependent sensitivity to $GABA_A$ antagonist picrotoxin. In P12-13 KOs I-O curves are significantly attenuated after picrotoxin ($p=0.0009$), indicating depolarizing GABA, and increased at P21-25 ($p=0.0005$), indicating hyperpolarizing GABA. However, WTs do not exhibit such responses until P15-16 and P40-45, respectively. Additionally, preliminary patch-clamp electrophysiology experiments are suggestive of functional differences in $GABA_A$R kinetics and zolpidem sensitivity in KOs across development. Our results highlight dysregulation of normal maturation patterns, a phenomenon where a precocious functional maturation of such receptors precedes ear canal opening to limit CP plasticity and contribute to functional deficits.

Disclosures: Y.J. Song: None. **F.E. Jensen:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.07/A64

Topic: A.07. Developmental Disorders

Title: The role of potassium channels as pharmacological targets for Fragile X syndrome

Authors: *W. E. CRUSIO[1], S. MIDDEI[2], J. GAUDISSARD[1], M. PREMOLI[1,3], V. LEMAIRE-MAYO[1], S. PIETROPAOLO[1];
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Abstract: Fragile X Syndrome (FXS) is a pervasive developmental disorder caused by expansion of a CGG repeat in the promoter region of the *Fmr1* gene, located on the X chromosome, resulting in hypermethylation of a CpG island, silencing of the gene transcription, and subsequent deficiency of the encoded protein known as FMRP (fragile X mental retardation protein). In the last decade, several pharmacological therapies for FXS have been proposed targeting specific synaptic components, but none of the tested therapeutic agents demonstrated a full effect on FXS symptoms. Recent studies have highlighted the implication of potassium channels in FXS pathology, and on large-conductance Ca2+-activated K+ channels (BKCa channels) in particular, as a reduced expression and functionality of these channels have been observed in FX patients and mice. Hence, we have investigated the role of BKCa channel as a new pharmacological target in FXS therapy. Here we evaluated the neurobehavioral therapeutic effects of a BKCa channel opener molecule (BMS-204352) in the *Fmr1*-KO mouse model of FXS. The focus of our work was on the long-term effects of BMS and on their age-dependency. We demonstrated that BMS chronic administration during a critical period of neurobehavioral development (i.e., at adolescence), but not later at adulthood, was able to correct FXS-like behavioral deficits, an effect that was accompanied by the rescue of the dendritic spine morphological abnormalities.

Disclosures: W.E. Crusio: None. **S. Middei:** None. **J. Gaudissard:** None. **M. Premoli:** None. **V. Lemaire-Mayo:** None. **S. Pietropaolo:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

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Program #/Poster #: 366.08/A65

Topic: A.07. Developmental Disorders

Support: 1U54HD082013 (To G.J.B.)

Title: Cell type-specific profiling of molecular defects in a human induced pluripotent stem cell model of fragile X syndrome

Authors: *N. RAJ[1], Z. T. MCEACHIN[1], M. J. TALIAFERRO[4], F. ZHANG[5], Y. ZHOU[1], M. E. MERRITT-GARZA[6], C. M. HALES[7], E. M. BERRY-KRAVIS[8], S. T. WARREN[2], P. JIN[5], Z. WEN[3], G. J. BASSELL[1];
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Abstract: Aberrant translation and disrupted signaling are molecular defects common to several neurodevelopmental disorders, including Fragile X syndrome (FXS), the most common monogenic cause of autism and inherited intellectual disability. Studies in animal models of FXS have shown that in the absence of the RNA-binding protein FMRP, there is an increase in global translation and dysregulation of key signaling pathways. However, the molecular pathogenesis of FXS in humans has remained understudied. Here we use induced pluripotent stem cell (iPSC)-derived neural precursor cells (NPCs) from multiple control and FXS patients to characterize molecular defects in a human disease-relevant model. We found that a major subset of FXS patient derived NPCs show increased protein synthesis, increased cell proliferation and altered differentiation profiles. FXS patient derived cerebral organoids also express more KI67+/SOX2+ proliferating cells. Furthermore, genome-wide splicing analysis of patient cerebral organoids revealed alternative splicing of a key cell-cycle regulated kinase in FXS. We developed a multi-parametric flow cytometry based assay to quantify protein synthesis and proliferation within specific neural subpopulations in our patient cells. Our results suggest that increased cell proliferation is a core molecular phenotype in FXS, and that this may be the result of dysregulated protein synthesis. The loss of FMRP also affects the differentiation of neural progenitor cells during development and results in increased protein synthesis in specific neural subtypes. We anticipate that this study using a human disease-relevant cellular model will shed new light on phenotypes in FXS, as well as provide a platform to be used in the development of therapeutic strategies. Our results provide strong support for an approach that involves patient

stratification based on cellular and molecular phenotypes, facilitating pre-clinical testing of potential treatments for precision medicine.

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Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.09/A66

Topic: A.07. Developmental Disorders

Support: MH060163

Title: Hypoactivity and increased variability of neural dynamics in fragile X circuits

Authors: ***H. MOTANIS**[1], D. V. BUONOMANO[2];
[1]Dept. of Neurobio., [2]Dept Neurobiol, UCLA, Los Angeles, CA

Abstract: A variety of network-level alterations have been reported in *Fmr1* knockout (*Fmr1*-KO) mice. One of the most well-studied network-level properties within cortical circuits are Up-states, which reflect transitions from a quiescent state to a stable network-wide increases in depolarization and firing rates. Up-states are network-level emergent phenomenon, and require the tuning of many cellular and synaptic properties. Here we examined whether spontaneous and evoked neural dynamics in isolated Fmr1-KO circuits exhibit developmental abnormalities. We used two-photon Ca^{2+} imaging to examine spontaneous network activity in $Fmr1^{-/y}$ KO slices. The mean $\Delta F/F$ of $Fmr1^{-/y}$ circuits (0.04 ± 0.004, n=10) was significantly reduced compared to $Fmr1^{+/y}$ circuits (0.11 ± 0.019, n=9; $p < 0.001$) at 11-16 days in vitro (DIV). Quantification of Up-state frequency revealed that $Fmr1^{-/y}$ circuits also exhibited a significant decrease in Up-state frequency (0.006 ± 0.004 Hz) compared to $Fmr1^{+/y}$ circuits (0.034 ± 0.009 Hz; p=0.01). Interestingly, at DIV 25-30 there was no difference in mean $\Delta F/F$ and Up-state frequency between $Fmr1^{+/y}$ and $Fmr1^{-/y}$ circuits. While Up-state frequency normalized over the course of *in vitro* development, it is important to determine whether the spatiotemporal patterns of Up-states is equivalent. Therefore, we calculated the mean pairwise correlation of the spatiotemporal patterns of activity during Up-states. Group analysis revealed that $Fmr1^{-/y}$ circuits exhibited significantly reduced correlation coefficient of Up-states (0.68 ± 0.01; n=7) compared to WT circuits (0.83 ± 0.03; n=6; $p<0.001$), indicating that the variability of the Up-states in KO circuits was higher. Thus, even though the levels of spontaneous activity in KO and WT circuits were similar at DIV 25-30, the stability of this activity was different. Since we observed a sharp decrease in Up-states in $Fmr1^{-/y}$ circuits at 11-16 DIV, we asked whether this delay might reflect

weaker excitatory connections. To characterize the net excitatory drive, input-output (IO) curves were derived using evoked electrical stimulation (40-140 μA). Surprisingly, even though KO circuits exhibited fewer Up-states at DIV 11-16, the excitatory drive between WT and KO circuits was not different. At DIV 25-30, however, EPSPs were significantly weaker in $Fmr1^{-/y}$ circuits as measured by the asymptote magnitude (5.78 + 0.54, n= 13) compared to WT circuits (8.55 + 0.86, n=16; $p<0.01$). Our results establish a sequence of in vitro developmental delays, and importantly show that even when overall activity is equivalent between WT and FX circuits, the spatiotemporal structure of this underlying activity is increased.

Disclosures: H. Motanis: None. **D.V. Buonomano:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

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Topic: A.07. Developmental Disorders

Support: KBRI Grant 19-BR-02-07
NRF Grant 2015M3C7A1029037

Title: Alteration in the intracellular cross-talk between mitochondria and exosome in the developmental disorder

Authors: *B. HA, J. HEO, Y.-J. JANG, T.-S. PARK, K.-H. LIM, S.-J. JEONG;
Korea Brain Res. Inst., DAEGU, Korea, Republic of

Abstract: Extracellular vesicles (EV) including exosomes are considered as emerging tools for biomarkers screening and drug/gene delivery in diagnostic and therapeutic strategies, respectively, because they include tissue-specific and disease-related molecules such as lipids, proteins and RNAs. *Exosomes,* 50-150 nm nano-sized vesicles, are secreted by cells and important mediators of intercellular communications. In central nervous system, various evidences show that exosomes can transfer pathogens such as prion protein (PrP), α-synuclein, amyloid β (Aβ) and phosphorylated tau. Recent findings reveal that mitochondrial component including mtDNA can be packaged in exosome and thus be horizontally transferred between cells. It has been observed that intact mitochondria or their components can be transferred between cells in disease conditions such as cancer, stroke, and lung injury. However, until now, it is not elucidated the intracellular transferring mechanism between mitochondria and exosome in the developmental disorders yet. In this study, we performed the protein profiling in exosome derived from either the brain or primary neuron/astrocyte of the developmental disorders. Various mitochondrial components were detected in exosomes isolated from the brain and primary neuron/astrocyte prepared from the mouse model. Our findings show that mitochondrial

proteins were remarkably decreased in disease mouse models which was confirmed by analysis with mitotracker and qRT-PCR for mtDNA/nDNA ratio as well as gene expression related to mitochondrial biogenesis. In conclusion, these results suggest that the intracellular trafficking system between mitochondria and exosome is altered under the developmental dysfunction and exosomes-derived mitochondrial components have a possibility as potential diagnostic/prognostic/therapeutic targets.

Disclosures: **B. Ha:** None. **J. Heo:** None. **Y. Jang:** None. **T. Park:** None. **K. Lim:** None. **S. Jeong:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.11/A68

Topic: A.07. Developmental Disorders

Support: HD082013

Title: Cell type-specific deletion of FMR1 in somatostatin and parvalbumin interneurons results in distinct behavioral phenotypes

Authors: *M. KALINOWSKA, M. VAN DER LEI, E. KLANN;
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Abstract: Fragile X syndrome (FXS) is the most common genetic cause of autism spectrum disorder (ASD) and intellectual disability that results from abnormal trinucleotide CGG repeat expansion in the *FMR1* gene, which encodes fragile X mental retardation protein (FMRP), leading to its silencing and lack of FMRP expression. FMRP is an mRNA binding protein with roles in mRNA transport, localization and translation. Thus, mRNA translation is dysregulated, which has been linked to pathophysiology, including abnormal synaptic function and dendritic morphology, and autistic-like behavioral phenotypes in mice lacking *Fmr1*. The role of FMRP in excitatory neuronal morphology and function has been well studied in *Fmr1* KO mice; however, the impact of on inhibitory synapses remains less characterized. Altered expression of GABA synthesizing enzymes and receptor subunits have been observed previously in multiple brain regions of *Fmr1* KO mice, indicating both pre- and post-synaptic dysregulation of the GABAergic system. Further, inhibitory neurotransmission is altered in a number of brain regions in *Fmr1* KO mice including amygdala, hippocampus and neocortex. However, the contribution of deleting *Fmr1* in specific subtypes of interneurons to the behavioral deficits observed in *Fmr1* KO mice has yet to be explored. Using Cre -lox recombinase technology, we generated mice with cell type-specific deletion of *Fmr1* in either parvalbumin (PV) or somatostatin (SOM) expressing interneurons, two of the major interneuron subtypes in the central nervous system. To

elucidate the complex interaction between GABAergic dysfunction and behavioral phenotypes in FXS, we assessed anxiety-like behaviors, motor function, memory, repetitive, and social behaviors in *PV-Fmr1*$^{-/y}$ and *SOM-Fmr1*$^{-/y}$ mice. Our findings indicate a cell type-specific role for FMRP in regulating distinct behavioral features associated with FXS.

Disclosures: M. Kalinowska: None. **M. van der Lei:** None. **E. Klann:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

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Program #/Poster #: 366.12/A69

Topic: A.07. Developmental Disorders

Support: Simons Research Foundation

Title: Mitochondrial inefficiency linked to abnormal metabolism, synaptic immaturity and behavioral deficits in fragile X syndrome

Authors: *P. LICZNERSKI[1], H.-A. PARK[1,3], P. MIRANDA[1], R. CHEN[1], M. GRAHAM[2], N. MNATSAKANYAN[1], J. WU[1], N. CRUZ-REYES[4], N. MEHTA[4], S. SOHAIL[4], J. SALCEDO[4], V. K. GRIBKOFF[1,4], B. MURTISHI[1], H. ROLYAN[1], R. J. LEVY[5], E. A. JONAS[1,4];
[1]Dept. of Intrnl. Medicine, Section of Endocrinol., [2]Dept. of Cell Biol., Yale Univ. Sch. of Med., New Haven, CT; [3]Dept. of Human Nutr. and Hospitality Mgmt., Univ. of Alabama, Tuscaloosa, AL; [4]Marine Biol. Lab., Woods Hole, MA; [5]Columbia Univ. Med. Ctr., New York, NY

Abstract: Loss of function of the gene (Fmr1) encoding Fragile X mental retardation protein (FMRP) results in unregulated, elevated mRNA translation and aberrant synaptic morphology. We find that mitochondria in neurons of the Fmr1-/y mouse have an inner membrane leak that undermines ATP synthesis and contributes to a metabolic phenotype similar to that of certain immature cells. Previous work in cardiomyocytes showed that developmental maturation is dependent on closure of a mitochondrial membrane leak. We now find that mild depletion of ATP synthase c-subunit or inhibition of the c-subunit leak with ATP synthase interacting agents decreases mRNA translation in Fmr1-/y mouse neurons and fibroblasts from Fragile X patients. Leak inhibition during synaptic stimulation alters metabolism in favor of oxidative phosphorylation over glycolysis, in part by encouraging the translation of components of the ATP synthase. In addition, the stimulus-induced, ATP-dependent, phosphorylation of translation elongation factor EF2, a crucial stimulus-response mechanism for synaptic plasticity, is lacking in Fmr1-/y synapses, and is rescued by closing the c-subunit leak. This suggests that synaptic stimulation leads to a switch in mitochondrial metabolism and phenotype that accompanies, and may be required for, the change in subsets of synaptic proteins synthesized. In keeping with this, exaggerated repetitive behaviors in Fmr1-/y mice are ameliorated by mitochondrial membrane

leak closure. We therefore suggest that FMRP regulates a stimulus-dependent change in mitochondrial metabolism required for synaptic development.

Disclosures: P. Licznerski: None. **H. Park:** None. **P. Miranda:** None. **R. Chen:** None. **M. Graham:** None. **N. Mnatsakanyan:** None. **J. Wu:** None. **N. Cruz-Reyes:** None. **N. Mehta:** None. **S. Sohail:** None. **J. Salcedo:** None. **V.K. Gribkoff:** None. **B. Murtishi:** None. **H. Rolyan:** None. **R.J. Levy:** None. **E.A. Jonas:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.13/A70

Topic: A.07. Developmental Disorders

Support: NIH Grant HD084289

Title: Maternal stress and prenatal fluoxetine exposure on offspring of fragile X knockout mice

Authors: *A. L. ARZUAGA[1], M. PATEL[1], J. MROCZEK[2], J. LARSON[1,3,4,5], M. E. RAGOZZINO[2,4];
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Abstract: Fragile X Syndrome (FXS) is a genetic disorder that results in mild to moderate intellectual disability. FXS is caused by transcriptional silencing of Fmr1, a gene encoding an RNA-binding protein that plays a crucial role in typical synapse development. FXS is the most common monogenic syndrome of Autism Spectrum Disorders (ASD), although only about one-third of individuals meet criteria for an ASD diagnosis. The increased prevalence of ASD in recent decades and the heterogeneity of symptom severity may arise from a complex interaction of environmental and genetic risk factors that induce alterations in synaptic function. The present study investigated whether exposure to restraint stress in pregnant female Fmr1 knockout (KO) and wildtype (WT) mice affects repetitive behaviors, anxiety and/or hippocampal synaptic plasticity in male and female offspring. Pregnant female mice were subjected to either a no restraint condition or a restraint condition that consisted of chronic restraint stress from gestational days 4-18 (three daily 30-minute sessions). Offspring were tested as young adults (7 weeks of age) on self-grooming behavior, anxiety behavior in elevated plus maze and for spatial learning and reversal learning with probabilistic reinforcement. Subsequently, hippocampal slices from offspring were tested for alterations in long-term potentiation (LTP) induced by theta burst stimulation (TBS) at Schaffer-commissural synapses in field CA1. Restraint stress, used to model depression, did not affect anxiety in either Fmr1 KO or WT mice. Maternal restraint stress selectively increased grooming behavior in male WT offspring. All groups were comparable on

learning a spatial discrimination task. However, male and female Fmr1 KO mice in both restraint and no - restraint conditions were impaired on spatial reversal learning compared to WT mice. Ongoing experiments are determining whether there is a relationship between impaired spatial reversal learning and hippocampal LTP in Fmr1 KO mice. Initial findings suggest that maternal stress during pregnancy in Fmr1 KO mice does not affect anxiety, self-grooming behavior, or alter the behavioral flexibility deficit in male and female offspring. Additional studies are examining whether maternal stress and antidepressant (SSRI) exposure during pregnancy affect the phenotype in Fmr1 KO offspring that can build a greater understanding of how early life experiences interact with genetics to affect synaptic plasticity and behavior in offspring.

Disclosures: A.L. Arzuaga: None. **M. Patel:** None. **J. Mroczek:** None. **J. Larson:** None. **M.E. Ragozzino:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.14/A71

Topic: A.07. Developmental Disorders

Support: Jim and Betty Ann Rodgers Chair Fund
Avekshan LLC

Title: Norbinaltorphimine, a kappa opioid receptor antagonist influences behavioral phenotypes in a fragile X mouse model

Authors: *D. M. M. MCCARTHY, M. X. TRUPIANO, L. ZHANG, P. G. BHIDE;
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Abstract: Fragile X syndrome (FXS) is a genetic condition characterized by a spectrum of neuro-behavioral symptoms including attention deficit, hyperactivity, anxiety, unstable mood, autistic behavior, acoustic hypersensitivity, delayed speech and seizures. It is caused by inactivation of the X-linked fragile-X mental retardation -1 (*FMR1*) gene and it is the number one inherited cause of intellectual disabilities, and the most common known cause of autism worldwide. Using the *B6.129P2-Fmr1tm1Cgr*/J strain of *Fmr1* KO mouse we confirmed that both male and female *Fmr*1 KO mice display hyperactivity, poor nest construction, motor-impulsivity and impaired social interaction. These phenotypes are consistent with impaired dopamine and/or noradrenaline signaling in the brain. Kappa opioid receptor antagonists, such as norbinaltorphimine (norBNI) increase dopamine and noradrenaline content in the brain by increasing synaptic release of the neurotransmitters. Therefore, norBNI has the potential to influence behavioral phenotypes in the *Fmr1* KO mouse via dopamine and noradrenaline signaling mechanisms. We found that a single administration of nor-BNI (20mg/kg; ip)

significantly reduced hyperactivity in male but not female *Fmr1* KO mice, and reduced motor-impulsivity in female but not male *Fmr*1 KO mice. The effects lasted 24 hours. However, a single oral administration of nor-BNI (20mg/kg; oral gavage) reduced hyperactivity significantly in both male and female *Fmr1* KO mice, and the effects lasted up to 48 hours. Moreover, a lower dose of norBNI (1 mg/kg) administered daily for 5 days via oral gavage improved nest construction in the *Fmr1* KO mouse. Thus, norBNI produced significant effects on multiple behavioral phenotypes in the *Fmr1* KO mouse, and some of the effects were dependent on sex and the route of administration.

Disclosures: D.M.M. McCarthy: None. **M.X. Trupiano:** None. **L. Zhang:** None. **P.G. Bhide:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.15/A72

Topic: A.07. Developmental Disorders

Support: NIH Grant 311179
SIMONS Foundation

Title: Uncovering the role of mTORC2 in fragile X syndrome

Authors: *S. ROUDABUSH[1], J. YAN[1], R. ZUKIN[2];
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Abstract: Fragile X syndrome (FXS) is one of the most common heritable forms of intellectual disability and the leading genetic cause of autism in humans. The neuroanatomical hallmark of Fragile X is an increased density of immature spines, a factor thought to underlie synaptic dysfunction and impaired cognition in Fragile X mice. While a role for mTORC1 in FXS and autism spectrums disorders is well established, little is known about the role of mTORC2, which is thought to mediate actin polymerization. Moreover, mTORC2 is thought to be upstream of Rac/cofilin signaling and actin polymerization. Our laboratory previously identified cofilin, an actin depolymerizing agent that regulates spine structure, and its upstream effector Rac1, a Rho GTPase, to be dysregulated in Fragile X mice (Fmr1 KO) and casually related to dendritic spine abnormalities. Specifically, delivery of constutively active cofilin into juvenile Fmr1 KO mice was sufficient in rescuing the immature dendritic phenotype and aberrant spine density in the somatosensory cortex of Fmr1 KO mice. We hypothesized that genetic reduction of Rictor, a defining component of mTORC2 and binding partner critical to mTOR function and stability, might rescue synaptic defects in Fragile X mice. Since Rictor-null mice are embryonically-lethal,

we generated Fmr1 KO mice in which Rictor could be conditionally knocked out, Fmr1 KO Rictor conditional knockout (cKO), by means of CRISPR/Cas9 and the Lox-Cre systems. In preliminary experiments, we showed that delivery of lentivirus synapsin-Cre into the somatosensory cortex of neonatal Rictor cKO mice successfully knocked out Rictor protein and is sufficient to return phosphorylated (overactive) cofilin and components of the Rac/PAK pathway to wildtype levels. Preliminary findings indicate that cKO of *Rictor* in layer V of the somatosensory cortex of juvenile/neonatal Fmr1 KO mice is sufficient to rescue aberrant dendritic spine morphology. Ongoing studies will determine if cKO of Rictor in layer V of the somatosensory cortex of neonatal Fmr1 KO mice is sufficient to rescue aberrant dendritic spine morphology and density. Future studies will determine whether cKO of Rictor can rescue aberrant synaptic plasticity and somatosensory-mediated behavioral phenotypes in Fmr1 KO mice.

Disclosures: S. Roudabush: None. **J. Yan:** None. **R. Zukin:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.16/A73

Topic: A.07. Developmental Disorders

Support: NIH Grant NS110100
NIH Grant MH078041
NIH U54 HD079125

Title: Cortical morphology in children with the FMR1 premutation

Authors: *J. Y. WANG[1], M. DANIAL[2], C. SOLEYMANZADEH[2], B. KIM[2], Y. XIA[2], K. KIM[2], F. TASSONE[2], T. J. SIMON[3], R. J. HAGERMAN[2], S. M. RIVERA[4];
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Abstract: Premutation alleles (55-200 CGG repeats) of the fragile X mental retardation 1 (*FMR1*) gene are associated with developmental problems, such as cognitive impairment in specific domains and autism-like symptoms. *FMR1* protein is widely expressed in precursors of neurons and oligodendrocytes during brain maturation, stressing the importance of the gene in neurodevelopment. Consistently, defects of neuronal migration and altered expression of neuronal lineage markers have been revealed in knock-in mouse models of the premutation. Such deficits likely affect cortical folding (*i.e.*, gyrification). We performed a retrospective study of cortical morphology in 33 children with the *FMR1* premutation (19 boys, age 8-12 years) and 25 age-matched controls (12 boys). CGG repeat length (N = 55), *FMR1* mRNA level (N = 50), and

IQ scores (N = 47) were also available. Local gyrification indices (LGI) were acquired semi-automatically to quantify folding complexity in 68 cortical regions. Multiple linear regression was used to examine the effect of age, group, and sex on LGI. To identify individuals with hyper- and hypo-gyrification, percentiles were determined based on Z scores of the age- and sex-adjusted LGI relative to controls. Bartlett's test of homogeneity of variances was conducted to compare variances in LGI Z scores between the groups. Pearson's correlation analysis was used to examine the correlations between abnormal LGI and *FMR1* markers and IQ scores. After adjusting for multiple comparisons using the false discovery rate method, we detected significant effects of age and/or sex in 29 regions among which bilateral posterior cingulate and right pars obritalis showed significant sex by group interaction (p = 0.012-0.045) driven by less sex effect in the premutation groups compared with the control groups. High variances of LGI Z scores were also revealed in the right isthmus in the premutation boys, and in the left entorhinal, right rostral anterior cingulate, and right caudal anterior frontal cortices in the premutation girls (p <0.001-0.007). Using Z = ±2.58 (upper and lower 1 percentile) as the cutoff, 17/19 of the premutation boys displayed hyper- and/or hypo-gyrification in 1-10 regions and 11/15 premutation girls showed such scores in 1-14 regions. In contrast, only 1 boy and 1 girl in the control groups showed hypergyrification in two different regions. Importantly, strong associations between the LGI Z scores and molecular and IQ measures were demonstrated in some of the regions showing hyper- or hypo-gyrification. These findings suggest a presence of abnormal cortical folding in children with the *FMR1* premutation, which may underlie cognitive problems.

Disclosures: J.Y. Wang: None. **M. Danial:** None. **C. Soleymanzadeh:** None. **B. Kim:** None. **Y. Xia:** None. **K. Kim:** None. **F. Tassone:** None. **T.J. Simon:** None. **R.J. Hagerman:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Novartis, Roche Parmaceuticals, Alcobra, Neuren. F. Consulting Fees (e.g., advisory boards); Zynerba, Fulcrum. **S.M. Rivera:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.17/A74

Topic: A.07. Developmental Disorders

Support: FRQS
FRAXA Foundation
Association de Neurofibromatose du Québec

Title: Glutamate and GABA in fragile-X syndrome and neurofibromatosis type 1

Authors: M. MABIKA, A. LACROIX, M. PROTEAU-LEMIEUX, P. MAURICE, F. CORBIN, *J.-F. LEPAGE;
Sherbrooke Univ., Sherbrooke, QC, Canada

Abstract: Fragile-X syndrome (FXS) and Neurofibromatosis type 1 (NF1) are two genetic conditions with overlapping symptoms, including seizures and a high risk of neurodevelopmental disorders, namely autism spectrum disorder (ASD). Moreover, previous work suggests that a dysbalance between inhibitory and excitatory mechanisms may be central to the neuropathophysiology of both disorders. Because aberrant excitation/inhibition ratio is a common feature of several animal models of ASD, we sought to assess the level of Glutamate+glutamine (Glx) and GABA in the brain of patients with FXS and NF1 in relationship with ASD symptomatology. So far, magnetic resonance spectroscopy (MRS) was acquired in 35 participants (11 FXS; 14 NF1; 10 Controls; expected N=45) using the MEGA-PRESS sequence (Voxel 3X3X2 cm placed in the occipital lobe) and symptoms severity was assessed using the Aberrant Behavior Checklist (ABC-C) and the Social Communication Questionnaire. Preliminary analyses conducted on MRS data show a marked reduction of GABA concentration in NF1 patients, who differ from controls ($p<0.001$), and also tend to differ from FXS patients ($p=0.055$). GABA level in FXS tend to be lower than controls ($p=0.055$), but show a high inter-individual variability. Reduced level of Glx was noted in FXS compared to NF1 ($p=0.019$), but they did not differed from controls. Correlational analyses with behavioural measures with be conducted once the sample is complete (N=45). This study will be the first to compare genetic conditions at high risk of ASD with regards to the excitation/inhibition hypothesis, and clarify the potential links between an imbalance in neurotransmitter concentration and ASD symptomatology.

Disclosures: M. Mabika: None. **A. Lacroix:** None. **M. Proteau-Lemieux:** None. **P. Maurice:** None. **F. Corbin:** None. **J. Lepage:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.18/A75

Topic: A.07. Developmental Disorders

Support: FRAXA Postdoctoral Fellowship
R01-MH106469-01

Title: Understanding and overcoming pharmacological tolerance in the treatment of fragile-X syndrome

Authors: *P. K. MCCAMPHILL[1], R. K. SENTER[1], H. J. DE JESÚS-CORTÉS[1], M. F. BEAR[2];
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Abstract: Recent clinical trials investigating the efficacy of metabotropic glutamate receptor 5 (mGluR5) inhibitors to treat Fragile-X syndrome (FXS) have had mixed results, with several studies reporting a lack of efficacy in patient populations. Specifically, negative allosteric modulators (NAMs) of mGluR5 were found to work well in treating several disease markers in animal studies, but did not show the same results in human trials. One potential explanation for the failure of these trials is that tolerance rapidly develops to the drugs, and indeed caregiver reports have suggested some early improvement did not continue for the length of treatment. In this study we show that animals also develop tolerance to mGluR5 NAMs, similar to patient populations. Specifically, we demonstrate that a single acute dose of the mGluR5 NAM CTEP reduces 1) the incidence of audiogenic seizures (AGS), 2) the aberrant increase in hippocampal protein synthesis and 3) cortical hyperexcitability of *Fmr1* KO animals; however these effects are lost after repeat administrations. To test whether the development of tolerance is due to a compensatory upregulation of mGluR5 we chronically treated wild type and *Fmr1* KO animals with CTEP for 5 days and then harvested both hippocampal and visual cortical tissue 4 hours after the final dose. There was no significant enhancement of mGluR5 receptor expression in tissue homogenates, however whether membrane or synaptic levels of the receptor are specifically changed following chronic treatment remains to be tested. The viability of pharmacologically targeting intracellular signaling hubs rather than surface receptors to better circumvent tolerance from prolonged dosing was also tested. We have previously used a novel GSK3α specific inhibitor BRD0705 to reverse many core FXS pathophysiologies, however whether chronic administration of BRD0705 could maintain durable efficacy was unknown. We now show that the ameliorative effect of BRD0705 on AGS incidence, seen after a single acute dose, was maintained after chronic administration. This lack of tolerance to BRD0705 in the AGS assay is of considerable interest and suggestive of durable efficacy of chronic GSK3α inhibition for the treatment of other FXS-related pathophysiologies. These findings will be essential to the future development of effective treatments for FXS, therefore it is of critical importance to understand when and how tolerance occurs and explore approaches to circumvent it.

Disclosures: P.K. McCamphill: None. **R.K. Senter:** None. **H.J. De Jesús-Cortés:** None. **M.F. Bear:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.19/A76

Topic: A.07. Developmental Disorders

Support: Brain Canada
Azrieli Neurodevelopmental Research Program
Canadian Institutes of Health Research Doctoral Award (CIHR CGS-D)

Title: Astrocyte purinergic signaling and thrombospondin-1 expression are dysregulated within the Fragile X Syndrome mouse cortex

Authors: *K. E. REYNOLDS[1], C. R. WONG[2], L. C. DOERING[3], A. L. SCOTT[3];
[1]Neurosci. Grad. Program, [3]Dept. of Pathology and Mol. Med., [2]McMaster Univ., Hamilton, ON, Canada

Abstract: The neurological symptoms of Fragile X Syndrome (FXS), the leading genetic cause of Autism Spectrum Disorder (ASD), largely arise due to dysregulations in glial-neuronal communication. While these glial-neuronal interactions may be mediated through various signaling pathways, an integral form of intercellular communication both in the brain and throughout the body is purinergic signaling. Purinergic signaling is broadly defined as the use of ATP, UTP, and their metabolites as excitatory signaling molecules, and is extensively used by astrocytes to facilitate reciprocal glial-neuronal and glial-glial crosstalk. We therefore sought to identify alterations in this signaling pathway within the FXS cortex, using an *Fmr1* knockout (*Fmr1-/-*) mouse model of FXS. In *Fmr1-/-* primary astrocyte cultures, treatment with exogenous UTP evoked a sustained intracellular calcium response compared to wildtype (*Fmr1+/+*) levels. This response was silenced following co-treatment with the general P2 receptor antagonist suramin, suggesting a role for UTP-sensitive P2Y receptors. We then quantified P2Y receptor expression in *Fmr1-/-* vs. wildtype astrocytes, and found elevated expression of P2Y2 and P2Y6 receptors in *Fmr1-/-* primary astrocyte culture, as well as in *Fmr1-/-* magnetically sorted astrocytes obtained from postnatal cortex. Focusing on potential downstream effects, the astrocyte protein thrombospondin-1 (TSP-1) is known to be regulated through UTP-induced activation of P2Y receptors and acts to establish immature excitatory synapses in the cortex. In *Fmr1-/-* early postnatal cortex, we observed transiently elevated TSP-1 expression compared to wildtype levels. *Fmr1-/-* astrocytes also demonstrated proportionally greater TSP-1 production than wildtypes in response to low levels of exogenous UTP, along with dysregulated TSP-1 secretion. Ongoing studies seek to quantify levels of purinergic signaling molecules using liquid chromatography-mass spectrometry, and to identify the effects of heightened astrocyte purinergic signaling on FXS versus wildtype neurons. Together, our results suggest that purinergic signaling is differentially regulated in *Fmr1-/-* astrocytes, and through its regulatory action on TSP-1, may have therapeutic relevance to the excitatory-inhibitory imbalances seen in FXS.

Disclosures: K.E. Reynolds: None. **C.R. Wong:** None. **L.C. Doering:** None. **A.L. Scott:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.20/A77

Topic: A.07. Developmental Disorders

Title: Investigations of oxidative stress in astrocytes of the Fragile X syndrome cortex

Authors: G. VANDENBERG[1], N. DAWSON[2], A. HEAD[1], *A. L. SCOTT[1];
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Abstract: Fragile X Syndrome (FXS) is caused by the instability of a CGG-repeated tract at the 5' end of the Fmr1 transcript. This instability causes silencing of the gene coding for FMRP. Higher levels of reactive oxygen species, lipid peroxidation, and protein oxidation within brain tissue have been found to be associated with the disease. These imbalances, along with altered levels of components of the glutathione system, provide evidence for increased oxidative stress. Astrocytes, glial cells within the brain, have many functions within neurodevelopment. Specifically, they regulate growth and synaptic contacts of neurons, regulate the level of excitability of synapses, and protect neurons at high levels of activity. To protect neurons from oxidative stress, astrocytes maintain oxidative homeostasis through their mitochondrial electron transport and antioxidant systems. This study examines the relationship between oxidative stress and FXS by assessing mitochondrial function and the antioxidant system of astrocytes. Using the Fmr1 knockout (KO) mouse model, astrocytes collected from male and female mice were grown in either normoxic or hypoxic conditions. After collection of the cultured cortical astrocytes, mitochondrial respiration and reactive oxygen species (ROS) production was analyzed. In addition, western blots were conducted on both cortical tissue and cultured cortical astrocytes to determine potential differences in enzyme expression. Lastly, enzyme assays were conducted on cultured cortical astrocytes to determine potential differences in enzyme activity. Preliminary results indicate sex differences in the oxidative capacity of KO cultured cortical astrocytes. Future steps involve assessing mitochondrial function and the antioxidant system of astrocytes while in the presence on neurons, with the intent to provide greater insight into the relationship between FXS and oxidative stress. Characterization of mitochondrial function and the antioxidant system of astrocytes will be highly valuable to the understanding of glial roles during brain development and could provide future insight to direct clinically relevant studies of FXS and other neurodevelopment disorders.

Disclosures: G. Vandenberg: None. **N. Dawson:** None. **A. Head:** None. **A.L. Scott:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.21/A78

Topic: A.07. Developmental Disorders

Support: CNR-Italy
IRCCS Oasi Research Institute
Telethon Italy grant GGP07264

Title: Mitochondrial bioenergetic impairment in the cerebral cortex of the Fmr1 knockout mouse model of Fragile X syndrome

Authors: S. D'ANTONI[1], L. DE BARI[2], D. VALENTI[2], C. M. BONACCORSO[3], R. VACCA[2], ***M. CATANIA**[1,3];
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Abstract: Fragile X syndrome (FXS) is the most common form of inherited intellectual disability and a leading genetic cause of autism. In most cases, FXS is caused by mutations in the FMR1 gene ultimately leading to the absence of the Fragile X mental retardation protein (FMRP), a pleiotropic RNA binding protein involved in multiple aspects of RNA metabolism. A growing body of evidence supports a role for mitochondrial dysfunction and defective oxidative phosphorylation (OXPHOS) in autism spectrum disorders; likewise, the involvement of mitochondrial alterations in the FXS pathogenesis has been suggested. While changes in brain metabolism, such as an increased rate of glucose metabolism, altered levels of energy metabolites and increased oxidative stress markers, have been described in the Fmr1 knock out (KO) mouse model of FXS, the effect of FMRP deficiency on mitochondrial energy metabolism remains to be investigated. To disclose whether and how mitochondrial bioenergetics is affected in *Fmr1* KO mouse brain, we monitored the ATP production via OXPHOS, which represents the major source of ATP in the central nervous system, particularly during development, and investigated some aspects of cell bioenergetics, namely the activity of key glycolytic enzymes, glycerol-3-phosphate shuttle and mitochondrial respiratory chain (MRC) complexes, in the cerebral cortex of the *Fmr1* KO mice. We found that, despite a hyper-activation of MRC complexes, ATP production OXPHOS is compromised, resulting in brain energy impairment in juvenile and late-adult Fmr1 knockout mice. Furthermore, we detected an increased activity of mitochondrial glycerol-3-phosphate dehydrogenase (mG3P-DH) in the cortex of young and late adult Fmr1 KO mice compared to age-matched wild type mice, whereas no change occurred in the activity of cytoplasmic glycerol-3-phosphate dehydrogenase and key glycolytic enzymes.

Our findings provide the first evidence of a compromised and inefficient mitochondrial bioenergetics in brain cortex of Fmr1 KO mice, which is present in young animals and persists into adulthood, and further support the idea that mitochondrial dysfunctions may contribute to neurological impairment in FXS.

Disclosures: S. D'Antoni: None. **L. de Bari:** None. **D. Valenti:** None. **C.M. Bonaccorso:** None. **R. Vacca:** None. **M. Catania:** None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.22/A79

Topic: A.07. Developmental Disorders

Support: NICHD, 1U54HD082008-01

Title: Audiogenic seizures in the Fmr1 knockout mouse are induced by Fmr1 deletion in subcortical, vGlut2-expressing excitatory neurons and require deletion in the inferior colliculus

Authors: D. GONZALEZ[1], M. TOMASEK[1], S. A. HAYS[3], V. SRIDHAR[1], S. AMMANUEL[1], C.-W. CHANG[1], K. PAWLOWSKI[2], ***J. R. GIBSON**[1], K. HUBER[1];
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[3]Bioengineering, Univ. of Texas At Dallas, Richardson, TX

Abstract: Fragile X syndrome (FXS) is the most common form of inherited intellectual disability and the leading monogenetic cause of autism. One symptom of FXS and autism is sensory hypersensitivity (also called sensory over-responsivity). Perhaps related to this, the audiogenic seizure (AGS) is arguably the most robust behavioral phenotype in the FXS mouse model - the *Fmr1* knockout (KO) mouse. Therefore, the AGS may be considered a mouse model of sensory hypersensitivity. Hyperactive circuits are hypothesized to underlie dysfunction in a number of brain regions in FXS patients and *Fmr1* KO mice, and the AGS may be a result of this. But the specific cell-types and brain regions underlying AGSs in the *Fmr1* KO are unknown. We used conditional deletion or expression of *Fmr1* in different cell populations to determine whether *Fmr1* deletion in those cells was sufficient or necessary, respectively, for the AGS phenotype. Our data indicate that *Fmr1* deletion in glutamatergic neurons that express vesicular glutamate transporter 2 (vGlut2) and located in subcortical brain regions is sufficient and necessary to cause AGSs in the *Fmr1* KO. Furthermore, deletion of *Fmr1* in glutamatergic neurons of the inferior colliculus is necessary for AGSs. When we demonstrate necessity, we show that *Fmr1* expression in either the larger population of vGlut2-expressing glutamatergic neurons or the smaller population of inferior collicular glutamatergic neurons - in an otherwise

Fmr1 KO mouse - eliminates AGSs. Therefore, targeting these neuronal populations in FXS and autism may be part of a therapeutic strategy to alleviate sensory hypersensitivity.

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Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.23/A80

Topic: A.07. Developmental Disorders

Title: Fear and fragile x syndrome: "Alternative facts" from the amygdala

Authors: *S. CHATTARJI[1,2,3];
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Abstract: .

Disclosures: S. Chattarji: None.

Poster

366. Genetic and Neural Mechanisms for Development Disorders

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 366.24/A81

Topic: A.07. Developmental Disorders

Support: Michael Smith Foundation for Health Research
Canadian Institutes for Health Research (CIHR)
Fragile X Research Foundation of Canada
Fragile X Syndrome Research & Treatment (FRAXA)

Title: Adiponectin can rescue hippocampal synaptic plasticity in a mouse model of fragile X syndrome

Authors: *L. BETTIO[1], J. THACKER[1], E. BROCKMAN[2], S. YAU[3], B. R. CHRISTIE[1]; [1]Div. of Med. Sci., [2]Univ. of Victoria, Victoria, BC, Canada; [3]Rehabil. Sci., Hong Kong Polytechnic Univ., Hong Kong, Hong Kong

Abstract: Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability and a leading cause of autism. This neurological condition is caused by the transcriptional silencing of the *Fmr1* gene, which codes for fragile X mental retardation protein (FMRP, a negative regulator of protein translation). The lack of FMRP is associated with an overactivation of mTOR signaling in the brain, which in turn leads to the excessive translation of several proteins that regulate spine structure and function. Treatments that increase AMPK activity hold promise for rescuing some of the deficits in synaptic plasticity seen in FXS. AMPK is a highly conserved protein that acts as an energy sensor and regulates several processes impaired in FXS such as mTOR activity, autophagy, insulin signaling and mitochondrial function. In the present study, we investigated the influence of adiponectin (APN, an adipocyte-derived hormone that stimulates AMPK activity) on deficits in synaptic plasticity induced by the lack of FMRP in mice. Short-term incubation with APN (10 min, 50 nM) was able to reverse deficits in both long-term potentiation (LTP) and long-term depression (LTD) induced by the lack of FMRP. Conversely, we found that prolonged incubation with APN (1.5-3 h, 50 nM) exacerbated deficits in LTP. Our findings indicate that APN may be a promising treatment for the management of FXS, but further studies are necessary to elucidate the optimal mode for delivery and timing of treatment.

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Poster

367. Animal Models I

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R21EY026702
R21 NS105119
R21NS097664

Title: Prefrontal parvalbumin interneurons require juvenile social experience to establish adult social behavior

Authors: *L. BICKS[1], K. YAMAMURO[2], M. FLANIGAN[5], J. M. KIM[3], D. KATO[2], E. K. LUCAS[6], H. KOIKE[2], M. S. PENG[6], D. BRADY[2], S. CHANDRASEKARAN[7], M. R. SMITH[5], K. J. NORMAN[7], R. L. CLEM[8], S. J. RUSSO[2], S. AKBARIAN[7], H. MORISHITA[4];
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Abstract: Social isolation during developmental critical windows could be highly detrimental to proper functioning of mature prefrontal cortex (PFC) and establishment of appropriate adult behaviors. However, the specific circuits that undergo social experience-dependent maturation to regulate social behavior development are poorly understood. *In vivo* imaging of mPFC-PVI activity by fiber photometry demonstrated that mPFC-PVI activity immediately preceded active social exploration in adult male mice, and short activation of mPFC-PVIs in a 3-chamber test led to increased social, but not object, approach. Selective chemogenetic suppression of mPFC-PVI activity during the 3-chamber test showed mPFC-PVI activity is required for normal levels of social interaction. Disrupted social experience during a juvenile window resulted in reduced intrinsic excitability and input drives of mPFC-PVIs, with absent mPFC-PVI activity prior to a social encounter. Chemogenetic restoration of mPFC-PVI activity in the adult animal selectively rescued juvenile isolation-induced social deficits. Therefore, PVI development in the juvenile mPFC is critically linked to long-term impacts on social behavior.

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Poster

367. Animal Models I

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Topic: A.09. Adolescent Development

Support: NIH R21 NS105119

Title: Rhythmic recruitment of prefrontal projection to visual cortex is essential for attentionally demanding behavior

Authors: *K. J. NORMAN[1], H. KOIKE[1], S. LOPEZ[1], E. NABEL[1], K. CARO[1], J. BATEH[1], M. FLANIGAN[1], D. KATO[1], Y. GARKUN[1], K. YAMAMURO[1], Z. DONG[1], M. DEMARS[1], D. BRADY[1], M. G. BAXTER[1], S. J. RUSSO[1], H. MORISHITA[2];
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Abstract: Attention is a goal-directed cognitive process that facilitates the detection of task-relevant sensory stimuli from our dynamic environment. Attention deficits are frequently observed in several psychiatric disorders; including autism spectrum disorders, schizophrenia, and depression, yet the underlying neural circuits that regulate attentional behavior are not well understood. Across species, previous studies have demonstrated that the frontal cortex—particularly the anterior cingulate cortex (ACC) — contributes to "top-down" control of sensory processing in the visual cortex (VIS). Here, we aim to investigate the contribution of these evolutionarily conserved, long-range ACC to VIS projecting neurons in top-down control of visual attention behavior. We achieve this by integrating circuit-based techniques to monitor and manipulate selective top-down neural activity in mice performing freely moving attention behavior with a translational automated touchscreen system. Our study has shown that selective chemogenetic suppression of ACC to VIS projections impairs attentional performance in the 5-choice serial reaction time task without disrupting additional detectable readouts of decision-making capacity, motivational state, motor activation, impulsivity, and compulsivity. *In vivo* calcium imaging of projection-specific top-down neurons using fiber photometry imaging in behaving mice revealed their preferential activation during correct trials of attentionally demanding task conditions. Optogenetic modulation of top-down projection neuron during a period of sustained attention, but not during visual cue presentation, improved attentional performance selectively at low gamma frequency (30Hz) stimulation. Conversely, direct optogenetic inactivation of top-down projection terminals at visual cortex during the later period of sustained attention disrupts attentional behavior selectively in high attentionally demanding conditions. Collectively, our data demonstrate that rhythmic recruitment of long-range prefrontal-sensory projections right before the appearance of task relevant stimuli is essential for successful task performance in attentionally demanding conditions. Our findings may provide circuit-based insight into the pathophysiology and neuromodulation intervention strategies for impaired visual attention in neuropsychiatric disorders.

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Poster

367. Animal Models I

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Support: The Naito Foundation
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Title: Prefrontal circuitry in control of the limbic thalamus requires juvenile social experience to establish adult sociability

Authors: K. YAMAMURO[1,2,3,4,5], ***M. B. LEVENTHAL**[1,2,3,4,5], L. BICKS[1,2,3,4,5], S. IM[1,2,3,4,5], D. KATO[1,2,3,4,5], Y. GARKUN[1,2,3,4,5], M. FLANIGAN[2,1,5], K. J. NORMAN[1,2,3,4,5], J. KIM[1,2,3,4,5], M. SADAHIRO[1,2,3,4,5], K. CARO[1,2,3,4,5], S. J. RUSSO[2,1,5], H. MORISHITA[1,2,3,4,5];
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Abstract: Juvenile social isolation causes long-lasting dysfunction in the medial prefrontal cortex (mPFC) and disruption of adult sociability. However, the neural circuit mechanisms underlying these phenomena are poorly understood. Among various subcortical targets, we identified the limbic thalamus, which relays signals to various components of the classical reward circuitry, as the most prominent projection target from the mPFC that is preferentially recruited by social interaction. Chemogenetic or optogenetic suppression of this projection was sufficient to induce sociability deficits without affecting motor activity or anxiety-related behaviors, showing that this circuit is necessary for normal social preference. Importantly, transient juvenile social isolation (p21-35) leads to a failure to activate adult mPFC->limbic thalamus projection neurons in response to a social encounter, due to their reduced intrinsic excitability and aberrantly increased inhibitory drive from low-threshold spiking inhibitory neurons in deep layers of the mPFC. Sociability deficits caused by juvenile social isolation are rescued by an acute chemogenetic or optogenetic activation of mPFC->limbic thalamus projection neurons. Our study identifies a novel pair of mPFC excitatory and inhibitory circuits whose maturation is profoundly affected by social experience during the juvenile period and points toward potential targets for the amelioration of social processing deficits shared across a range of disorders.

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Poster

367. Animal Models I

Location: Hall A

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Program #/Poster #: 367.04/A85

Topic: A.09. Adolescent Development

Title: Social isolation during development reduces excitability of a subtype of pyramidal cell in mouse prefrontal cortex which projects to subcortical areas

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Abstract: Social Isolation during development is crucial for the functional development of forebrain regions, especially the prefrontal cortex (PFC). Social isolation during development causes severe PFC dysfunction in humans, but their neural bases remain poorly understood. Our previous studies of 'social isolation' animal model showed that isolate-housing mouse (for 2 weeks after weaning) had autistic-like behavior, PFC dysfunction and hypomyelination in medial PFC (mPFC). Especially because hypomyelination due to social isolation is prominent in deep-layer of mPFC, we focused on layer-5 pyramidal cells in the mPFC and classified them into 2 types depending on the magnitude of h-current: prominent h-current (PH) cells and nonprominent h-current (non-PH) cells. We found that 2-week social isolation after weaning leads a reduction in excitatory synaptic inputs in PH cells. According to other previous studies, this subtype of pyramidal cells could have axonal projection to subcortical areas as mediodorsal thalamus (MD), striatum or pontine nuclei. However, it is unknown which subcortical area the pyramidal cells affected by social isolation project axons to. To identify which the pyramidal cells of mPFC project axons to, we performed intracranial stereotaxic surgery for mouse and injected the retrograde neural tracer into three subcortical areas (MD, Striatum and Pontine Nuclei) by coordinate and performed whole-cell patch-clamp recordings on the labeled neurons to find the change of electrical activity of pyramidal neuron. We analyzed the electrophysiological data and found that social isolation during development affects excitability of a subtype of pyramidal cell in mouse prefrontal cortex which projects to subcortical areas.

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Poster

367. Animal Models I

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Topic: A.09. Adolescent Development

Support: NIH P60 AA011605

NIH U24AA020024 UNC-CH NADIA Admin Core

Title: Adolescent binge ethanol effects on cholinergic and gabaergic interneurons in the prefrontal cortex, striatum and amygdala

Authors: *A. GOMEZ-A[1], R. P. VETRENO[1], C. DANNENHOFFER[1], S. OUCHOU[1], M. DOUGLAS[1], L. SHAPPELL[1], R. JAWAD[1], C. A. BOETTIGER[2], F. T. CREWS[1], D. L. ROBINSON[1];
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Abstract: Underage alcohol binge drinking in humans is associated with altered behavior and cognition in adulthood. Rodent studies have linked binge-like adolescent intermittent ethanol (AIE) to adult deficits in inhibitory control, behavioral flexibility, and memory encoding, along with changes in neuroimmune signaling, synaptic plasticity and neurogenesis. AIE exposure reduces choline acetyltransferase (ChAT)-expressing neurons in the basal forebrain, and the loss of ascending cholinergic projections may lead to behavioral and cognitive deficits. However, it is unknown if AIE basal forebrain reductions in ChAT+ neurons are exclusive or extend to cholinergic interneurons (ChI) or parvalbumin-expressing interneurons (PVI). These interneurons are key regulators of network function by modulation of local circuits and are important in motor and cognitive function. We hypothesized that AIE exposure decreases ChI and PVI in the PFC, striatum and amygdala relative to controls. We exposed rats to ethanol or water during adolescence (5g/kg, 2-days-on/2-days-off, P25-55). When rats reached adulthood, brains were removed and processed for immunohistochemical visualization of ChAT+ or PV+ cells. ChAT+ interneurons were counted in PFC (orbitofrontal and medial) and striatum (dorsomedial, dorsolateral and ventral). However, we found no difference in cell counts from AIE-exposed and control rats in any of these regions (unpaired t-tests, all p's>0.15). PV+ cell counts are ongoing. Thus, forebrain ChAT+ cholinergic projection neurons are sensitive to AIE, however frontal cortical and striatal ChI are not reduced by AIE. These results highlight the potential relevance of projection neurons in the cognitive and behavioral deficits observed after AIE. Additional ongoing studies on mechanisms underlying AIE behavioral deficits will be presented.
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Poster

367. Animal Models I

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Topic: A.09. Adolescent Development

Support: P01 ES002848 Project 3
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NIMH ESD26896

Title: The effect of adolescent phthalate exposure on cognitive behavior in rats

Authors: *E. P. SELLINGER[1], J. WILLING[2], J. STANLAND[3], L. MAHLOCH[3], J. M. JURASKA[4];
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Abstract: Adolescence is a unique period of development associated with the onset of puberty and rising gonadal hormone levels that restructure neural areas, especially the prefrontal cortex, leading to changes in associated cognitive behaviors. Critically, this period of neurodevelopment may allow for greater susceptibility to environmental factors. Phthalates are a class of endocrine-disrupting chemicals widely used as plasticizers, solvents, fixatives, and emulsifiers in plastics, food processing, and a variety of personal care products. Phthalates are primarily antiandrogenic but have also been shown to influence estrogen signaling (Takeuchi et al., 2005). Our lab has shown that exposure to an environmentally relevant phthalate mixture during perinatal development, a time when gonadal hormones are also playing a role in neurodevelopment, leads to a lasting reduction in synapse and neuron number in the mPFC as well as impaired cognitive flexibility in adulthood (Kougias et al., 2018). The current study aims to understand if exposure to phthalates during adolescence impacts cognitive behaviors.
Male and female Long Evans rats were fed the same environmentally relevant mixture of phthalates mentioned above from postnatal day (P) 27 through P50 at a dose of 0, 0.20, or 1 mg/kg bodyweight. A battery of behavioral tests were run beginning on P85 with an N= 10-11 of each sex per group. The elevated plus maze measures anxiety-like behavior where a higher number of open arm entries indicates lower levels of anxiety. In females, we observed a significant effect of dose in number of trips to the end of the open arm ($p = 0.02$) where the 1mg/kg exposure group had significantly more trips to the end than controls ($p = 0.05$). In males, there was a significant effect of dose in number of closed arm entries during the first five minutes ($p = 0.02$), providing more of an index of locomotor activity than anxiety-like behavior, where the 1mg/kg group had significantly fewer closed arm entries than controls ($p = 0.02$). We

observed no effects of phthalates on performance in the attentional set shift task, which measures cognitive flexibility. The final behavioral paradigm, pre-pulse inhibition, measures sensorimotor gating, and there was no effect of phthalate exposure. However, in analyzing habituation of startle reactivity, we saw a trend of dose ($p = 0.052$) in females only, but post hoc comparisons revealed no significant differences between phthalate and control groups. These results indicate adolescent phthalate exposure does not greatly impact cognitive behaviors including those associated with the mPFC.

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Poster

367. Animal Models I

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Topic: A.09. Adolescent Development

Support: NIH grant R01-MH086507
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Title: Age-dependent α7nAChR modulation of prefrontal inputs and associated fear memory behavior

Authors: *A. M. M. MIGUELEZ FERNÁNDEZ, H. M. MOLLA, D. R. THOMASES, K. Y. TSENG;
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Abstract: Increased brain concentrations of the tryptophan metabolite kynurenic acid (as found in schizophrenia) disrupt the balance of excitatory and inhibitory transmission in cortical circuits through an α7nAChR-dependent mechanism. In the present study, we conducted local field potential recordings along with local administration of the α7nAChR antagonist MLA to determine to what extent α7nAChR signaling regulates prefrontal processing of afferent drive *in vivo*. We found blocking α7nAChR signaling in the prefrontal cortex markedly attenuated the potentiation of local field potential responses elicited from the basolateral amygdala both in adult and adolescent rats. However, prefrontal infusion of MLA failed to disrupt the potentiation of local field potential responses elicited from the ventral hippocampus. Instead, blocking α7nAChR signaling diminished the inhibitory component of the hippocampal drive such that a shift from suppression of local field potential responses to facilitation emerges. Interestingly, this effect of MLA on ventral hippocampal transmission does not arise in the prefrontal cortex after P45, indicating an age-dependent recruitment of α7nAChR signaling by the ventral hippocampus that is input-specific. To examine the behavioral impact of prefrontal α7nAChR signaling, MLA

was locally infused and changes in fear conditioning behavior were assessed. A trace fear conditioning task was selected because it contains a delay period between the conditioned tone and the delivery of a foot shock, which requires normal prefrontal integration of inputs from both the amygdala and the ventral hippocampus. We found that blocking α7nAChR signaling in the prefrontal cortex diminished the acquisition of fear induced freezing behavior and disrupted the normal extinction pattern in adult rats. Such behavioral modulation by α7nAChR was absent in adolescent rats, indicating that prefrontal α7nAChR signaling emerges to control trace fear behavior only in adulthood. Collectively, these results point towards an age-dependent α7nAChR modulation of fear memory likely through a developmental mechanism in which the emergence of an α7nAChR component shapes the appropriate balance between amygdalar and hippocampal inputs to the prefrontal cortex. Supported by NIH grants R01-MH086507 and R01-MH105488 to KYT.

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Poster

367. Animal Models I

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Topic: A.09. Adolescent Development

Support: P01 ES002848 Project 3
USEPA 83543401 Project 3
NIMH ESD26896

Title: Sex differences and influences of puberty on the trajectory of perineuronal net development in the rat medial prefrontal cortex

Authors: *C. DRZEWIECKI[1], J. WILLING[3], E. P. SELLINGER[4], J. M. JURASKA[2]; [2]Psychology, [1]Univ. of Illinois, Champaign, IL; [3]Psychology, Bowling Green State Univ., Bowling Green, OH; [4]Neurosci. Program, Univ. of Illinois Urbana-Champaign, Champaign, IL

Abstract: Perineuronal nets (PNNs) are specialized components of the extracellular matrix that surround the soma, dendrites, and axon segments of neurons. Within the prefrontal cortex (PFC), they play a critical role in maintaining proper inhibitory/excitatory balance and have been implicated in learning and cognitive flexibility. The development of PNNs is thought to coincide with brain maturation, and several studies have found that male rodents reach adult levels of PNN density in the PFC in mid to late adolescence. Consequently, aberrant PNN development may be associated with psychological disorders that commonly emerge during adolescence. However, previous work from our laboratory has shown that the medial prefrontal cortex

(mPFC) decreases in volume across adolescence, and because density is confounded by changes in volume, a more thorough approach for PNN quantification is necessary. The goal of this study is to stereologically quantify the total number of PNNs by collecting the mPFC from male and female hooded rats at various ages between P30 and P60. Additionally, because puberty coincides with broad behavioral and neuroanatomical changes, we included pubertal status as a factor by collecting tissue from age-matched pre- and post- pubertal siblings within a litter. This design allows us to track longitudinal changes in the number of PNNs as well as study the influence of pubertal onset on the overall number of PNNs. Preliminary data analysis (N = 52) replicates previous findings that PNN density increases between P30 and P60 in both males and females ($p < 0.001$). However, when comparing post-pubertal females to their age-matched, pre-pubertal sisters, there is a significant decrease in PNNs at puberty in the infralimbic cortex (IL) ($p = 0.02$, N = 5/group). A similar decrease is observed in the prelimbic cortex (PL), though not significant ($p = 0.4$, N = 5/group). In males, this effect is reversed, such that pubertal onset seems to increase in PNN density in the PL and IL, though a larger sample is needed before making broad conclusions (N = 3/group). Whether these findings are influenced or amplified by changes in cortical volume has yet to be determined. Nonetheless, alterations to PNNs could provide a mechanism by which the mPFC matures during pubertal onset.

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Poster

367. Animal Models I

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Program #/Poster #: 367.09/B4

Topic: A.09. Adolescent Development

Title: Postnatal prefrontal myelination and associated maturation of social behaviors are regulated by Npas4 in mice

Authors: E. LEMANSKI[1], **C. PAGE**[2], *L. COUTELLIER[3];
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Abstract: The juvenile prefrontal cortex (PFC) undergoes several maturational processes including myelination. Studies indicate that experience and neuronal activity drive myelin formation in the brain, which contributes to the acquisition of adult social functions. The molecular mechanisms underlying experience-dependent prefrontal myelination remain to be fully elucidated. We previously showed that the transcription factor Npas4 is highly expressed in the juvenile PFC of mice and regulates aspects of prefrontal adolescent maturation. Furthermore, the activity-dependent expression of Npas4 is highly sensitive to experience. These previous findings led us to hypothesize that Npas4 could contribute to juvenile prefrontal myelination. We

first exposed Npas4 conventional wild-type (WT) and heterozygote (HET) mice to juvenile chronic stress (previously shown to decrease Npas4 expression in the PFC) to determine whether Npas4 deficiency modulates the effects of developmental stress on markers of myelination (MBP and PLP/DM20). In WT mice, juvenile stress did not impact MBP mRNA levels and reduced PLP/DM20 by 25% ($p=0.051$ vs. WT non-stressed mice). The effects of juvenile stress were more pronounced in HET mice: MBP and PLP/DM20 were both decreased vs. HET mice not exposed to stress (MBP: 40% reduction $p=0.003$; PLP/DM20: 57% reduction $p=0.007$). We then tested juvenile and adult Npas4 WT and knock-out (KO) mice for their social performances, as well as related prefrontal mRNA levels of MBP and PLP/DM20. Our preliminary data indicate that while sociability tends to mature in WT mice from the juvenile period to adulthood, the same maturation of social behaviors is not observed in KO mice. Interestingly, we also observed that reducing Npas4 expression in the adult brain decreases sociability to juvenile levels ($p=0.03$). RT-PCR analysis of mRNA expression of myelination markers indicate that juvenile, but not adult, Npas4 KO mice have lower MBP mRNA expression. Altogether, this first set of data is suggestive of a potential role of Npas4 in prefrontal myelination during postnatal development. Our on-going experiments assessing markers of myelination and social behaviors throughout the postnatal development of Npas4 deficient mice will further confirm these results.

Disclosures: E. Lemanski: None. **C. Page:** None. **L. Coutellier:** None.

Poster

367. Animal Models I

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Topic: A.09. Adolescent Development

Support: UBACyT Grant 20020160100005BA

Title: Early chronic noise exposure can induce aminoacidergic neurotransmission changes and reactive gliosis in the hippocampus of adolescent rats. Influence of enriched environment housing

Authors: S. J. MOLINA[1], L. D. UDOVIN[2], C. F. KUSNIER[2], G. E. BUJÁN[3], F. CAPANI[2], *L. R. GUELMAN[3,1];
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Abstract: We have previously shown that exposure of immature rats to moderate noise can induce hippocampus (HC)-related behavioral, molecular and histological alterations, including oxidative imbalance and neural damage, during the peri-adolescence period. In addition, housing

animals in an enriched environment (EE) has shown to be effective in the reversal of most of these alterations. As the involvement of excitotoxicity has been proposed in different brain injuries, the aim of the present work was to test the effects of early noise exposure on aminoacidergic neurotransmission. In addition, considering that glial cells might influence neuronal environment and integrity, hippocampal histology was also evaluated. Finally, the possibility that EE could prevent these changes was further considered. Male Wistar rats of 7 and 15 postnatal days (PND) were exposed to noise (95-97 dB, 2h) for one (N1) or five (N5) consecutive days. After weaning, rats were transferred to an EE, consisting of toys, a wheel, plastic tunnels and ramps, whereas others were placed in standard cages. One week later, western blot experiments were performed to evaluate the levels of GAD 65/67 (a marker of GABAergic neurotransmission) and EAAT-1 (glutamate transporter, a marker of glutamatergic neurotransmission). GFAP reactive area was studied through immunohistochemical procedures to evaluate the presence of reactive gliosis. Results showed that although an increase in EAAT-1 levels and GFAP reactive area was induced only in rats exposed to N5 at PND 7 when compared with non-exposed rats, housing these animals in an EE was effective in restoring these differences. In contrast, no significant changes were observed in GAD 65/67 levels in neither group. These findings suggest that early noise exposure might differentially affect rats by inducing excitotoxicity, demonstrating a high vulnerability of repeated exposures (N5) at earlier ages (PND7). Furthermore, the associated increase of the GFAP reactive area might suggest reactive gliosis as a mechanism aimed to protect HC against excitotoxicity. In consequence, it could be proposed that this defensive response could have been produced in reaction to a previous excitotoxic extra-cellular challenge in order to prevent neuronal damage. Finally, EE has shown to be an effective strategy to reverse all the alterations found, suggesting that visual, social and physical stimulation during the peri-adolescence period could be an effective strategy to prevent HC-related molecular and histological changes.

Disclosures: **S.J. Molina:** None. **L.D. Udovin:** None. **C.F. Kusnier:** None. **G.E. Buján:** None. **F. Capani:** None. **L.R. Guelman:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.11/B6

Topic: A.09. Adolescent Development

Title: The differential impact of periodic and acute enrichment on evoked activity in the adolescent rat amygdala

Authors: C. A. PEGGS, C. E. GAILLARD, C. T. FENNELL, M. P. CARD, A. W. CARPENTER, ***M. C. ZRULL**;
Appalachian State Univ., Boone, NC

Abstract: Adolescence is a stressful time often marked by emotion-driven decision making that can lead to increased risk-taking behavior during both novel and familiar experiences. Environmental enrichment (EE) can provide sensory and motor stimulation, opportunity to interact with same-sex conspecifics, and can evoke behavioral responses to an emotionally arousing situation. Given the relative importance of the amygdala in learned and unlearned emotional response as well as the evaluation of social cues, we examined how periodic and/or acute EE might evoke neural activity across lateral (LA), basolateral (BLA), and central amygdala (CeA). Eleven Long-Evans rats were enriched between postnatal days (pnd) 25 and 48, and 10 controls were not. The daily, 90 min EE sessions allowed same-sex enriched rats to interact in enclosures with ramps, platforms, and objects, which were changed each day. Control rats were handled each day, and all rats lived in same-sized, same-sex groups. Prior to sacrifice on pnd 49, 6 enriched (EE+EE) and 5 control (No+EE) rats experienced a final, acute EE session and other rats did not (EE+No, No+No, n=5 each). Floating section immunohistochemistry was used to process tissue and visualize the neural activity marker c-FOS, and alternate sections were processed for cell body staining using thionin. Neuron densities were quantified in LA, BLA, and CeA using digital microscopy and stereological technique. We observed 25% more neurons in LA of rats with periodic EE than in controls ($p<.019$, $eta^2=0.30$) but no difference in other amygdala subdivisions. However, only the No+EE group exhibited enhanced evoked activity in LA (+95%, $p<.015$, $eta^2=0.14$) relative to controls not experiencing a final EE session. In contrast, both EE+EE and No+EE rats exhibited a greater proportion of c-FOS+ neurons than EE+No and No+No rats in BLA (+64%, $p<.001$, $eta^2=0.43$) and CeA (+71%, $p<.001$, $eta^2=0.41$). Our data suggest a history of enrichment may increase capacity for rapid emotional responses and memory by sparing LA neurons, but that only a first EE experience seems to boost activation of those neurons, which may indicate greater emotional response is elicited only by the novelty of a situation. The enhancement of neural activity in both BLA and CeA of all groups experiencing an acute EE session suggests current information, whether extremely novel or not, is integrated with contextual information to appropriately influence behavior and inform other neural circuits. This interpretation suggests the possibility of emotion-driven assessment prior to response in these adolescent rats.

Disclosures: M.C. Zrull: None. **C.A. Peggs:** None. **C.E. Gaillard:** None. **C.T. Fennell:** None. **M.P. Card:** None. **A.W. Carpenter:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.12/B7

Topic: A.09. Adolescent Development

Support: Hungarian Scientific Research Fund Grant K125390
Hungarian Brain Research Program Grant 2017-1.2.1.-NKP-2017-00002

Title: Post-weaning social isolation-induced social deficits and disrupted network activity across prefrontal subregions

Authors: *L. BIRO[1], C. MISKOLCZI[1], B. BRUZSIK[1], H. SZEBIK[1], Z. K. VARGA[1], L. SZENTE[1], O. HORVATH[1], J. HALASZ[1,2], M. TOTH[1], E. MIKICS[1];
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Abstract: Early childhood and the periadolescent period represent time windows during which brain regions modulating social behavior undergo major network reorganization. Aversive social experience during these sensitive periods can lead to disrupted development. Here we aimed to investigate social behavioral changes induced by post-weaning social isolation (a model of childhood neglect) in mice and characterize underlying network disturbances in distinct subregions of the prefrontal cortex (PFC), a crucial regulator of social behavior. After weaning at P21, mice were housed either socially or isolated (alone) until reaching adulthood, and social interaction and resident-intruder (RI) tests were used to investigate social and aggressive behaviors. We demonstrate that mice subjected to post-weaning social isolation display social disturbances, shown by increased defensive and abnormal aggressive behavior that disregards species-specific rules. We characterize how these behavioral changes are reflected at the neuronal activity level within infralimbic (IL), prelimbic (PrL), anterior cingulate (Cg1) cortices in socially-reared and isolated animals under resting conditions and following RI test. To study RI test induced co-activation patterns across prefrontal subregions, we generated matrices from correlation coefficients of c-Fos activation. Quadratic assignment procedure correlations revealed that social experience exerts differential c-Fos co-activation patterns in isolated animals. Parvalbumin-positive interneurons enwrapped by perineuronal nets (PV+PNN+) are implicated in network organization and closure of critical periods of plasticity but little is known about their activity during social encounters. We found that aggressive social encounter increased the activity of PV+PNN+ neurons within Cg1 and PrL cortices of socially-reared and isolated animals. In contrast, in the IL social encounter decreased the activity of PV+PNN+ neurons in socially-reared mice, an effect absent in isolated animals. Social isolation leads to social behavioral abnormalities and impaired PV-PNN activity following a social encounter. Our results contribute to understanding how disruption of neuronal network organization during development translates into social abnormalities in adulthood.

Disclosures: L. Biro: None. **C. Miskolczi:** None. **B. Bruzsik:** None. **H. Szebik:** None. **Z.K. Varga:** None. **L. Szente:** None. **O. Horvath:** None. **J. Halasz:** None. **M. Toth:** None. **E. Mikics:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.13/B8

Topic: A.09. Adolescent Development

Support: MIH Grant R01MH106460

Title: Clock∆19 mutation leads to increased oxidative damage and altered adolescent development of parvalbumin interneurons in mouse frontal cortex

Authors: *J. BURNS[1], Z. KARANIKOS[2], K. N. FISH[3], J. F. ENWRIGHT, III[3], C. A. MCCLUNG[3];
[1]Psychiatry, [2]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [3]Psychiatry, Univ. of Pittsburgh Med. Sch., Pittsburgh, PA

Abstract: Adolescence represents a critical period of heightened plasticity in the prefrontal cortex (PFC) and is a time in which individuals are at an increased risk for mental illness. The development of parvalbumin (PV) cells is thought play an important role in critical period timing, as their maturation is thought to underlie the initiation of the critical period, and perineuronal nets (PNNs), which form around PV cells at the end of the critical period, act to restrict plasticity. Given that alterations in PV cells and PNNs have been observed in multiple psychiatric diseases, this suggests that a developmental deficit in PV cells may play a role in psychiatric disease pathophysiology. Additionally, PV cells are particularly vulnerable to oxidative stress and studies have shown increased levels of oxidative stress in the brains of patients with psychiatric diseases. As genes in the endogenous antioxidant system (EAS) are thought to be under the control of the molecular clock, we used *Clock*∆19 mice to investigate the relationship between the molecular clock, oxidative stress, and PV cell maturation in frontal cortical regions across adolescence in order to gain insight into how this process may be perturbed in disease. Quantitative fluorescence microscopy was used to measure oxidative stress, PV expression, and PNN formation in the anterior cingulate cortex (ACC) and prelimbic region (PL) of wildtype and *Clock*∆19 mice at postnatal days 20, 40, and 90 (n=4-5/group). qRT-PCR was performed on micropunches from the ACC and PL of wildtype and *Clock*∆19 mice (n=4-5/group) to investigate changes in the expression of genes in the EAS, as well as genes related to PV cell development and PNN formation. We find that adult *Clock*∆19 mice display a cell-type specific increase in oxidative stress in PV cells in adulthood. Furthermore, *Clock*∆19 mice show decreased PV expression at postnatal day 40 and into adulthood and decreased staining for PNNs in adulthood. *Clock*∆19 mice also show reduced expression of genes in the EAS and altered expression of genes involved in PV cell maturation. Given that *Clock*∆19 mice display increased oxidative stress in PV cells only in adulthood, we hypothesize that the increase in oxidative

stress, due to an impairment in the EAS, may negatively affect PV cell development. Furthermore, the decreased expression of PV and decreased PNN formation in adult *Clock*Δ19 mice suggests impaired critical period timing in these mice. Given that PV cells are crucial for the proper adolescent development of the PFC, by understanding the effect of oxidative stress on PV cell maturation we may gain insight into the development of PFC dysfunction in psychiatric disease.

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Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.14/B9

Topic: A.09. Adolescent Development

Support: R01MH086507 to KY Tseng
R01MH105488 to KY Tseng

Title: Endocannabinoid modulation of prefrontal afferent transmission and associated extinction of fear memory

Authors: *H. M. MOLLA[1], A. M. M. MIGUELEZ FERNANDEZ[2], K. Y. TSENG[2];
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Abstract: Prefrontal cortex (PFC) maturation during adolescence involves remodeling of glutamatergic and GABAergic transmission in the PFC, and is partly dependent upon proper modulation of afferent drive. Previous studies have also implicated changes in signaling components of the endocannabinoid system (CB1 receptor and endocannabinoids such as 2-AG and anandamide) in the PFC during adolescence. However, the manner by which endocannabinoid signaling impacts PFC synaptic transmission during the transition to adulthood remains unknown. Here we conducted in vivo local field potential recordings in rats and examined how pharmacological manipulations of prefrontal endocannabinoid signaling affects transmission originating from the basolateral amygdala (BLA) and ventral hippocampus (vHIP). We found the recruitment of endocannabinoid-CB1R signaling in the PFC is developmentally regulated, which emerges to exert a powerful inhibitory control of BLA and vHIP inputs after P45. Our data indicate both 2-AG and anandamide contribute to limit vHIP-to-PFC transmission, yet only 2-AG was recruited to modulate BLA inputs. At the behavioral level, PFC elevation of endocannabinoids delays the extinction of fear memory, an effect that cannot be mimicked by prefrontal activation of CB1Rs. Together, these results show that endocannabinoid-CB1R

signaling in the PFC emerges to control the gain of afferent transmission in an age-dependent and input-specific manner.

Disclosures: H.M. Molla: None. **A.M.M. Miguelez Fernandez:** None. **K.Y. Tseng:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.15/B10

Topic: A.09. Adolescent Development

Support: NIH Grant R01-MH086507
NIH Grant R01-MH105488

Title: Systemic administration of the cannabinoid CB1 receptor agonist WIN during adolescence disrupts the maturation of trace fear extinction behavior

Authors: *A. Y. OROZCO[1], H. M. MOLLA[2], A. M. MIGUELEZ FERNÁNDEZ[1], A. CABALLERO[1], K.-Y. TSENG[1];
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Abstract: During the transition from adolescence to adulthood, the prefrontal cortex (PFC) undergoes marked development accompanied by increased risk for developing psychiatric disorders which display prefrontal dysfunction. In preclinical studies, administration of synthetic cannabinoids during adolescence has been shown to disrupt the functional connectivity between the ventral hippocampus and PFC when tested in adulthood (Cass et al., *Molecular Psychiatry* 2014). Accordingly, a disruption of PFC-mediated extinction of trace fear memory emerged. What remains unknown is whether the effect of adolescent cannabinoid exposure on the PFC can be observed immediately or it is manifested only when reaching adulthood. To fill this gap in knowledge, i.p. injection of the synthetic CB1 receptor agonist WIN was delivered once daily for 5 days to adolescent (postnatal days P35-40) rats at 2 mg/kg. Behavioral assessment of ventral hippocampal-PFC function was determined using the trace fear conditioning and extinction paradigm at 24 hours or 10 days post-last injection of WIN or vehicle. Results show that the rate of extinction was significantly decreased in WIN-treated rats when compared to vehicle controls. Moreover, rats exposed to WIN had extinction rates equivalent to those observed in P30-35 juveniles. Collectively, the results show that exposure to CB1 receptor cannabinoids during adolescence is sufficient to elicit behavioral deficits which can be detected shortly after administration. However, the disruption endures and becomes more apparent in adulthood as ventral hippocampal-PFC functional connectivity is recruited to regulate behavior.
This study is supported by NIH grants R01-MH086507 and R01-MH105488 to KYT.

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Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.16/B11

Topic: A.09. Adolescent Development

Support: MITACs
CIHR

Title: Effects of hippocampal dysregulation on the hyper-dopaminergic state induced by chronic adolescent THC exposure

Authors: *M. DE FELICE[1], T. VINE[1], R. M. HUDSON[1], M. RODRÍGUEZ-RUIZ[1], C. CHEN[2], M. LAM[2], S. N. WHITEHEAD[3], K. K. C. YEUNG[2], W. J. RUSHLOW[4], S. R. LAVIOLETTE[1];
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Abstract: Clinical and preclinical studies have associated adolescent cannabis exposure with a higher risk to develop neuropsychiatric disorders later in life. It is widely accepted that glutamate and dopamine (DA) dysfunctions are critical to the pathophysiology of schizophrenia. Moreover, aberrant mesolimbic DAergic signaling is associated with dysregulation of hippocampal activity, as shown in both human and rodent studies.
We have demonstrated in a neurodevelopmental rodent model that adolescent administration of delta-9-tetrahydrocanabinol (THC), the primary psychoactive component of marijuana, induces a schizophrenia-like phenotype, characterized by a hyper-DAergic state in the VTA (Renard J. et al, 2016; 2017), and linked to a loss of inhibitory control in the prefrontal cortex. However, given the established role of hippocampal dysregulation in schizophrenia-related DA dysfunction, we hypothesized that adolescent THC exposure may similarly induce DAergic dysregulation through functional, polysynaptic connections between the hippocampus and VTA. In this study we examined whether neuronal and molecular dysregulation of the hippocampal dorsal subiculum (DS) might disinhibit dopamine neural activity, through direct modulation of the nucleus accumbens (NAc), following adolescent THC exposure.
Adolescent rats were treated from postnatal day (PND) 35 to 45 with increasing doses of THC (days 1-3 2.5mg/kg, days 4-7, 5mg/kg, days 8-11, 10mg/kg i.p., twice a day) or vehicle. At adulthood (PND 75), we used Matrix-assisted laser desorption ionization Imaging Mass Spectrometry (MALDI-MS) to investigate neuroanatomical distributions of GABA and

glutamate levels in both, DS and NAc. Moreover, we quantified receptor levels using Western Blot technique.
We report that chronic adolescent THC exposure enhances NMDA2B and NMDA1 glutamate receptor expression in the nucleus accumbens (NAc) as well as altering DA receptor expression levels, inducing an increase in D1R expression and a reduction in D2R. Moreover, MALDI-MS neurotransmitter quantification revealed a significant reduction of GABA and dopamine in DS as well as a decrease in GABA and glutamate in both, NAc shell and core.
Overall, our findings provide evidence that adolescent THC exposure induces a persistent glutamatergic/DAergic imbalance in DS-NAc-VTA circuitry. We are currently performing *in vivo* electrophysiology experiments to investigate the neural activity in DS and NAc.
Results from these studies will improve our knowledge of the neurobiological mechanisms underlying the pathophysiology of THC-induced neuropsychiatric disorders.

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Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.17/B12

Topic: A.09. Adolescent Development

Support: NIH grant P50AA017823
NIH training grant T32AA025606

Title: Acute ethanol challenge differentially regulates the expression of growth factors in the dorsal hippocampus: A comparison of adolescent and adult rats of both sexes

Authors: *T. M. BARNEY[1], A. S. VORE[2], T. DEAK[3];
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Abstract: Acute ethanol exposure produces rapid alterations in neuroimmune gene expression that are both time- and cytokine-dependent. For instance, IL-6 increased rapidly in the CNS after ethanol challenge, peaking at about 3 hr post-injection, whereas IL-1β and TNFα increased during acute ethanol withdrawal (Doremus-Fitzwater et al., 2014; Gano et al., 2017). Interestingly, adolescent rats, who often consume binge-like quantities of alcohol, displayed reduced neuroimmune responses to acute ethanol challenge (Doremus-Fitzwater et al., 2015). The mechanisms underlying reduced neuroimmune reactivity in adolescents remains unclear. Since growth factors and their signaling pathways are intimately intertwined with

cytokine/inflammatory signaling, we hypothesized that age-related differences in growth factor expression and their response to acute ethanol exposure may shed important light on these mechanisms. To investigate this, adolescent (P29-P34) and adult (P70-P80) Sprague Dawley rats of both sexes were injected with either ethanol (3.5 g/kg) or saline, and brains were harvested at 3 hr post-injection for assessment of growth factor expression using RT-PCR. Initial analyses examined the dorsal hippocampus with a panel of growth factors. As expected, acute ethanol challenge significantly increased IL-6 expression, replicating our prior findings. When growth factors were examined, acute ethanol significantly decreased BDNF and increased FGF2. In contrast, GDNF and PDGF were unresponsive to ethanol, but displayed tendencies toward heightened expression among adolescents. NGF and VEGF did not vary as a function of ethanol, age or sex. Overall, these findings indicate selective regulation of growth factors by acute ethanol challenge, yet little evidence emerged to support the hypothesis that high expression of growth factors during adolescence might contribute to reduced neuroimmune reactivity among adolescents, though future follow-up studies are necessary to test this causally. Furthermore, no significant sex differences were observed in any of the factors examined. These findings may have implications for understanding unique features of the adolescent brain that confer unique sensitivity to, and proclivity for, binge-like ethanol exposure.

Disclosures: **T.M. Barney:** None. **A.S. Vore:** None. **T. Deak:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.18/B13

Topic: A.09. Adolescent Development

Support: NIH grant P60AA011605
NIH grant U24AA020024
CAPES/CNPQ

Title: Adolescent ethanol exposure alters frontolimbic neuronal firing patterns to a reward predictive cue in adult female rats

Authors: *J. A. POCHAPSKI[1], S. J. STRINGFIELD[2], A. GOMEZ-A[3], C. A. DANNENHOFFER[3], H. D. JAGGERS[3], C. DA CUNHA[1], C. A. BOETTIGER[3], D. L. ROBINSON[3];
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Abstract: Adolescent ethanol exposure has been associated with long-term adverse outcomes, including cognitive deficits and neurochemical changes, due to effects of ethanol on adolescent brain development. Many consequences of adolescent ethanol consumption and exposure in male rodents are described in the literature, but the behavioral and neuronal changes promoted by adolescent intermittent ethanol (AIE) exposure in females are less well described. As we previously found that AIE-exposed rats showed reduced conditioned approach to a reward receptacle, we hypothesized that AIE would alter neuronal firing patterns in brain regions involved in reward conditioning. Thus, the present study examined the effect of AIE on reward learning and underlying neurocircuits by measuring activity of neurons in orbitofrontal cortex (OFC) and nucleus accumbens (NAc) during Pavlovian conditioned approach (PCA). Sprague-Dawley female rats received AIE (5g/Kg intragastric ethanol, 2-day-on/2-days-off) or water through adolescence (P25-54). At ~P70 rats began PCA training, where a 30-second conditioned stimulus (CS), presented as a cue light and lever extension, predicted delivery of a 20% sucrose reward. After training, electrode arrays were implanted in the OFC and NAc and electrophysiological recordings were performed during PCA sessions. Neuronal firing in both the OFC and NAc exhibited phasic excitation at CS onset. Control rats had larger population-level excitation in the NAc (~85% increase in firing within 500 ms after CS onset) than the OFC (~20%). In contrast, AIE-exposed rats presented a more similar magnitude of CS-induced population-level excitation in both regions (~60% in NAc and ~45% in OFC). Moreover, at the first receptacle entry after the CS onset, 35% of NAc neurons exhibited phasic excitations and 21% exhibited inhibitions in control rats. In contrast, only 17% of NAc neurons exhibited phasic excitations and 10% exhibited inhibitions in AIE-exposed rats. Additional analyses on the neuronal firing patterns during specific conditioned responses are in progress. Taken together, these preliminary data suggest altered fronto-limbic participation in neuronal processing of reward-motivated behavior.

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Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.19/B14

Topic: A.09. Adolescent Development

Title: Anxiety and sociability in adult rats after adolescentcannabinoid exposure

Authors: *R. D. LUNDY, C. E. YULE, H. M. PARRISH, L. M. PACK, L. M. BUYNACK, G. D. MEDLEY, D. M. HAYES, P. A. JACKSON;
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Abstract: During adolescence, the brain undergoes developmental stages that are necessary for a healthy cognitive state. Studies have found that rats exposed to a cannabinoid during these key developmental stages exhibit learning deficits as well as potentially negative effects on levels of anxiety and sociability (Biscaia et al., 2003; O'Shea & McGregor, 2006; Schneider, 2009). This may be attributable to the cannabinoid, however, another possible explanation for these deficits is a lack of nutrients and healthy weight gain during the injection period (Biscaia et al., 2003; Schneider, 2009). For this study, Long-Evans male rats were semi-randomly assigned to one of ten conditions on post-natal day (PND) 34. These conditions determined the amount of nutritional supplement and food that the rats received throughout the injection period (PND 35-48), in addition to whether they received a synthetic cannabinoid (CP55, 940) injection, or the vehicle, at a dose of 0.35 mg/kg. The animals were subjected to an extensive series of behavioral measures including the Elevated Plus Maze (EPM), Open Field (OF), and Social Interaction (SI) engagement with a conspecific rat. On PND 78, the animals performed their first task on the EPM, and were observed for anxiety levels via time spent in open or enclosed arms and locomotor activity. Results suggested that drug animals not receiving nutritional supplement exhibited greater anxiety. In addition, the groups that received drug but no supplement were significantly less active than supplemented animals or vehicle controls. Following this, on PND 83 the animals performed an OF task where they were placed on the apparatus for ten minutes to explore and habituate while researchers observed thigmotaxis and rear count in relation to anxiety, as well as locomotor activity. Results seem to indicate that the groups which received higher amounts of additional nutrition were less active than the untreated control group while groups that received no additional supplement during their injection period showed lower levels of anxiety. On PND 84 the rats performed a social interaction task on the open field along with an unfamiliar conspecific rat located inside a circular wire cage. The results suggest that the vehicle groups which received lower supplementation were significantly more likely to seek out social interaction. In conclusion, nutritional supplementation during adolescent cannabinoid exposure alters behavior and could explain some of the long-term changes observed.

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Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.20/B15

Topic: A.09. Adolescent Development

Title: Perineuronal net development during adolescence is critical for optimal decision-making in adulthood

Authors: *E. JACOBS-BRICHFORD[1], J. D. ROITMAN[2];
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Abstract: A hallmark of adolescent brain development is the establishment of a finely-tuned excitatory-inhibitory (E-I) balance in the prefrontal cortex (PFC). This balance, which comes online as inhibitory signaling is refined, helps regulate neuron activity and is essential for the acquisition of higher cognitive function, such as decision-making, in adulthood. It is widely accepted that the maturation of parvalbumin-containing (PV^+) interneurons is critical for developing E-I balance, as PV^+ interneurons are known to regulate excitability of other cells. Approximately 70% of PV^+ interneurons in PFC are surrounded by perineuronal nets (PNNs), a lattice-like structure that wraps around them and regulates their plasticity. Extensive research has established that the emergence of PNNs around PV+ cells is associated with the closing of critical periods of development and a reduction in the neural placticity typical of these periods, but the functional relevance of PNN development remains unclear.
To address this gap in our knowledge, we assessed whether PNN development in prelimbic cortex (PrL) and orbitofrontal cortex (OFC) is essential for optimal decision-making in adulthood in rats. We used chondroitinaseABC (chABC) to digest PNNs in PrL or OFC during adolescence (P35) or adulthood (P75). In adulthood, animals were trained on a probabilistic choice task, where one lever pays a small, certain reward (1 sucrose pellet at 100%) and the other pays a larger, risky reward (3 sucrose pellets at 16, 33 or 67%). After animals completed the task, they were sacrificed, and we use immunohistochemistry to assess any long-lasting changes in parvalbumin-PNN colocalization or in the number or fluorescence intensity of PNNs using *Wisteria floribunda* agglutinin (WFA), a plant lectin that often colocalizes with PNNs.
All groups (P35-control, P35-chABC, P75-control, P75-chABC) showed increasing preference for the larger, risky reward with increasing probability of reward delivery. Degradation of PrL PNNs during adolescence, but not adulthood, led to increased preference for risky rewards in adulthood, even when selecting the risky option was suboptimal. Findings in OFC are forthcoming, but the results from PrL indicate that higher cortical functions are disrupted when PNNs are not present during PV cell maturation, suggesting a mechanism of adolescent vulnerability.

Disclosures: E. Jacobs-Brichford: None. **J.D. Roitman:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.21/B16

Topic: A.09. Adolescent Development

Support: NIH Grant R01AA025035
VA Merit Grant BX004091

Title: Effects of THC on schizophrenia-related gene DNA methylation and expression in mouse primary neuron culture and adolescent mice

Authors: G. P. VALLERINI[1,2], M. KAZMI[1], S. KAZMI[1], *D. P. GAVIN[1,2];
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Abstract: Tetrahydrocannabinol (THC) is the main psychoactive component of marijuana and exerts its pharmacological actions through activation of specific receptors highly expressed in the brain, namely cannabinoid receptors 1 (CB1) and 2 (CB2). Increasing evidence suggests a link between cannabis use during adolescence and a higher risk of developing psychosis later in life. Increased expression of DNA methylating enzymes, such as DNMT1 and DNMT3A, and increased BDNF promoter methylation associated with reduced BDNF expression have been reported in postmortem brain samples in schizophrenia. A decrease in *Bdnf* levels is reported in rodent studies as a result of THC treatment. To date, there have been few studies that have examined whether enduring gene expression changes observed in schizophrenia may be the result of THC-induced changes in DNA methylation. In the current study, we examined whether THC affects *Dnmt* expression and genes associated with schizophrenia using mouse primary neuron culture and *in vivo.* We tested the effects of cannabinoid 1 and 2 receptor agonists, and THC with and without cannabinoid 1 and 2 receptor antagonists on mouse E18 primary cortical neuron cultures. Using mice, we also examined the effects of a single dose of THC 10 mg/kg and adolescent chronic THC exposure on gene expression changes in the prefrontal cortex. In mouse primary cortical neuron cultures we found concentration-dependent increased levels of the DNA-methylating enzymes *Dnmt1* and *Dnmt3a* following THC or cannabinoid agonists application. Interestingly, the observed increase in *Dnmts* mRNA expression was abolished or reversed by co-treatment with either a CB1 antagonist or a CB2 inverse agonist, indicating a causal role for THC through interaction with both its main brain pharmacological targets. Further, schizophrenia-related genes *Gad1*, *Reln*, and *Bdnf* were all down-regulated in the presence of THC, and the effect was reversed by cannabinoid antagonists and the DNMT inhibitor RG-108. In adult male mice treated with THC 10 mg/kg and in adolescent mice treated with IP injections of THC twice a day for 10 days *Dnmt3a* mRNA expression was significantly increased. In the adolescent mice there was also a significant reduction in *Gad1*, *Reln*, and *Bdnf* mRNA expression. These early studies suggest that THC produces robust changes in expression of epigenetic pathways that may contribute to gene expression changes observed in schizophrenia.

Disclosures: G.P. Vallerini: None. **M. Kazmi:** None. **S. Kazmi:** None. **D.P. Gavin:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.22/B17

Topic: A.09. Adolescent Development

Support: R21 DA040228;

Title: Developmental nicotine exposure elicits multigenerational neurotrophic, neuroendocrine, and neuroepigenetic alterations in the frontal cortices, striata, and hippocampi of adolescent mice

Authors: *J. M. BUCK[1], H. C. O'NEILL[3], J. A. STITZEL[2];
[2]Inst. Behav Genet., [1]Univ. of Colorado Boulder, Boulder, CO; [3]Univ. of Colorado Boulder Inst. for Behavioral Genet., Boulder, CO

Abstract: Maternal smoking during pregnancy, a form of developmental nicotine (NIC) exposure (DNE), is associated with neurodevelopmental disorders such as ADHD in children. Modeling the transmissible impacts of smoking during pregnancy in the first- (F1) and second- (F2) generation adolescent offspring of NIC-exposed female C57BL/6J mice, we have previously reported that DNE elicits multigenerational nicotine preference, hyperactivity and risk-taking behaviors, aberrant rhythmicity of activity, altered nicotinic acetylcholine receptor expression and function, impaired dopamine transporter function, and global DNA hypomethylation in the frontal cortices and striata. This ensemble of multigenerational behavioral, neuropharmacological, and DNA methylomic anomalies recapitulates multiple domains of ADHD pathosymptomatology. Further characterizing this mouse model via an array of immunoblot experiments, the present study reveals multigenerational DNE-induced disruptions of both BDNF processing and glucocorticoid receptor (GR) activation in the frontal cortices (n = 8-12), striata (n = 9-11), and hippocampi (n = 9-12) of male and female adolescent mice compared to saccharin (vehicle)-exposed controls. These findings are consistent with the BDNF deficits and HPA axis hypoactivity reported in both ADHD patients and the children of maternal smokers. Moreover, as BDNF is a quintessential mediator of neurodevelopment, our findings of multigenerational DNE-induced ADHD-like behavioral and neuropharmacological anomalies may stem from neurodevelopmental insults conferred by the aberrant BDNF and pro-BDNF signaling observed in DNE mice. In addition, our findings of multigenerational GR hypoactivity may contribute to the increased risk-taking behaviors displayed by F1 and F2 DNE mice. Given that BDNF and GR signaling are epigenetically regulated, and in light of our prior findings of corticostriatal global DNA hypomethylation in F1 and F2 DNE mice, the current study next probed for alterations in the expression and phosphorylation of key epigenetic factors via immunoblotting. The resulting data indicate multigenerational downregulation and aberrant phosphorylation of methyl-CpG-binding protein 2 (MeCP2), DNA methyltransferase 3A

(DNMT3A), and histone deacetylase 2 (HDAC2) in the frontal cortices (n = 8-12), striata (n = 9-11), and hippocampi (n = 9-12) of male and female adolescent DNE mice. Given the extensive regulatory roles of MeCP2, DNMT3A, and HDAC2, these findings suggest that neuroepigenetic abnormalities may constitute a mechanistic hub for the multigenerational transmission of DNE-induced ADHD-like phenotypes.

Disclosures: J.M. Buck: None. **H.C. O'Neill:** None. **J.A. Stitzel:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.23/B18

Topic: A.09. Adolescent Development

Support: NIDA

Title: Phytocannabinoids strongly modulate BDNF/TrkB expression in the dorsal hippocampus of adolescent female mice

Authors: J. WINSTONE, H. S. SHAFIQUE, K. MACKIE, ***J. WAGER-MILLER**;
Indiana Univ., Bloomington, IN

Abstract: The increasing acceptance and use of marijuana in the US and abroad require a better understanding of the effects its constituents may have on the developing brain. In this exploratory study, adolescent female *CD1* mice (n=7, each group) were exposed to delta9-tetrahydrocannabinol (THC) and/or cannabidiol (CBD) (each at 3mg/kg, IP), two major phytocannabinoids in cannabis, from P28 to P49. Mice were sacrificed at P50 and mRNA prepared from the dorsal hippocampi. Levels of four brain derived neurotrophic factor (BDNF) variants and the TrkB receptor were analyzed. While treatment with THC or CBD alone did not significantly affect BDNF transcript levels, there was a significant increase in BDNF4 mRNA when both THC and CBD were present. Furthermore, combining THC and CBD led to a large decrease in BDNF6 mRNA. Treatment with either THC or CBD, but not the combination, significantly decreased TrkB transcript levels. These results suggest that different combinations of phytocannabinoids have distinct effects on the BDNF/TrkB system, a finding that could be related to some of the transient and enduring consequences of adolescent cannabis use. [KM1]CD1, C57 etc. [KM2]Say how many (or which ones) were analyzed.

Disclosures: J. Winstone: None. **H.S. Shafique:** None. **K. Mackie:** None. **J. Wager-Miller:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.24/B19

Topic: A.09. Adolescent Development

Title: Growing up hamster: Analysis of social behavior across adolescence in the Syrian hamster

Authors: K. C. DE LORME[1], **A. M. BAUMANN**[2], I. R. GORE[2], *B. M. ARSZNOV[2]; [1]Psychological Sci., Gustavus Adolphus Col., Saint Peter, MN; [2]Minnesota State Univ. Mankato, Mankato, MN

Abstract: Syrian hamsters must appropriately display complex agonistic behaviors used to communicate social status, ensure successful reproduction, and defend territory and resources. These behaviors include aggression, submission, and flank marking, which are all essential to survival and develop across adolescence. Most research investigating agonistic behavior has focused on male hamsters; however, female hamsters display aggression earlier than male hamsters do and are as aggressive as males. Additionally, agonistic behavior has been studied extensively using the resident/intruder paradigm, but few studies have investigated how hamsters interact in a neutral territory. Thus, we are interested in exploring how prepubescent, adolescent, and adult female hamsters differ in their display of agonistic behavior using the neutral arena paradigm.Twenty-four female Syrian hamsters were divided into three groups of eight: prepubertal, adolescent, and adult. All females were age and weight-matched with another experimental female for behavior testing. During their dark cycle, all pairs were placed in a neutral arena, that neither female had occupied prior to testing, for 10 minutes in dim red light. Behavior was recorded using a digital camera and later quantified using Behavioral Observation Research Interactive Software (BORIS). Preliminary analyses revealed significant main effects of age for both attacks ($p = 0.023$) and flank marking ($p < 0.000$). Post-hoc analysis revealed prepubertal hamsters engaged in significantly greater number of attacks compared to both adolescent ($p= 0.001$) and adult hamsters ($p = 0.002$) with adolescents and adult not differing from each other. Furthermore, adults displayed a significantly greater amount of flank marking compared to prepubertal hamsters ($p= 0.027$), but not compared to adolescent hamsters. These data are in line with previous studies on the adolescent development of agonistic behavior in Syrian hamsters using the resident/intruder paradigm. However, the mean number of attacks is lower and mean number of flank marks is higher when using the neutral arena paradigm compared to previous studies using the resident/intruder paradigm. Taken together, these data suggest that the patterns of agonistic behavior are similar across adolescent development when establishing (neutral arena) and maintaining (resident/intruder) territory, but the behavioral strategies differ between the establishment and maintenance of territory.

Disclosures: **K.C. De Lorme:** None. **A.M. Baumann:** None. **I.R. Gore:** None. **B.M. Arsznov:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.25/B20

Topic: A.09. Adolescent Development

Support: NIH Grant MH117459-01

Title: Development of memory for conspecifics and caregivers

Authors: *B. J. LAHAM, E. C. COPE, E. DIETHORN, E. GOULD;
Princeton Univ., Princeton, NJ

Abstract: Social recognition, or memories of familiar individuals, is imperative for the development and stability of large social groups consisting of unrelated individuals. Recent investigation has pinpointed the CA2 region of the hippocampus as necessary for the memory of adult conspecifics in mice, with silencing of CA2 pyramidal neurons resulting in a temporary loss of memory for familiar conspecifics (Hitti and Siegelbaum, 2014; Dudek et al., 2016; Smith et al., 2016). While previous research has elucidated the role of the CA2 in social memories formed by adult mice, little is known about the development of social memory. Using a direct social investigation test, we found evidence of social recognition memory as early as P14 in mouse pups. This behavior is qualitatively similar to that observed in adulthood, with increased investigation times associated with novel, compared to familiar, conspecifics. Shortly after weaning, mice display social recognition memory that is quantitatively similar to that observed in adulthood in terms of investigation times. We also explored the earliest form of social memory, the memory a mouse pup has for its caregiver. Using a modified social test, we observed that genetically related, as well as cross-fostered, mouse pups exhibit a preference for their caregiver over a novel lactating female as early as P3. After separation from the caregiver at the time of weaning, mice retain the memory of the caregiver but exhibit a transition in preference for a novel female over the caregiver. The memory of the caregiver persists long into adulthood, for up to at least 150 days. Ongoing studies will explore the role of the CA2 and other hippocampal subregions in the development and maintenance of social memory, for age-matched conspecifics as well as for the caregiver.

Disclosures: **B.J. Laham:** None. **E.C. Cope:** None. **E. Diethorn:** None. **E. Gould:** None.

Poster

367. Animal Models I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 367.26/B21

Topic: A.09. Adolescent Development

Support: CAPES/PROSUP 001

Title: Reflexological and physical developmental impairment of male rats offspring induced by maternal stress through the nesting material reduction

Authors: *P. S. RODRIGUES, T. MENDES-LIMA, A. C. S. SAMPAIO, L. S. MEDEIROS, M. C. GALVÃO, K. E. KIATAQUI, T. M. REIS-SILVA, N. MOREIRA, T. B. KIRSTEN, M. M. BERNARDI;
Envrn. and Exptl. Pathology, Paulista Univ., São Paulo, Brazil

Abstract: Reductions in maternal care during the development of young rats may contribute to different impairments such as predisposition to depressive and anxiety-like behaviors, cognition deficits such as learning and memory deficits, and changes in the immune system. Considering that these behavioral changes may lead to a different behavioral pattern in the adulthood, the objective of this study was to analyze the effects of maternal neglect induced by the reduction of the nesting material during lactation in the physical and reflexological development of male offspring. For this, lactating rats were divided into two groups: control group (CG), in which the amount of nesting material was preserved and an experimental group (EG) where the amount of nesting material was reduced in 50% from post natal day 2 to 21 (PND). General activity and maternal behavior were analyzed in the mothers on PND 5-7 while the puppies were evaluated for their physical and reflexological development. The parameters with two groups were determinate with a student t test. Two-way ANOVA was used considering weight and time period as variables when pertinent, and finally, a Fisher exact test was performed to analyze the survival percentage. Regarding the results in the mother, despite no differences in general activity or maternal behavior being observed, the EG displayed increased frequency ($p = 0.02$), time ($p = 0.002$) and maximal duration ($p = 0.04$) of self-grooming compared to CG. In the pups, the maternal neglect decreased the survival percentage ($p = 0.01$), the body weight at 31 days of age [$F(1,84) = 3.37$, $p = 0.07$] as well as the delay in the average day of hair appearance (t test, $p = 0.04$), descent of the testicles ($p = 0.0007$) and reflex of palmar grasp ($p = 0.03$). Thus, it was possible to conclude that the maternal neglect direct affect the physical and reflexological development of the male offspring despite maternal behavior has been preserved in the mothers. These results may be attributed to maternal stress expressed by increased self-grooming of the mother at this first moment. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES/PROSUP) - Finance Code 001.

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Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.01/B22

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: JSPS KAKENHI Grant Number JP17H02117

Title: Combination of exercise and pharmacological neuromodulation targeting $GABA_A$ receptors promotes motor recovery and BDNF protein expression in the M1 after focal brain ischemia in rats

Authors: *T. INOUE[1,3], M. KITAHARA[1], M. OKAMURA[1], Y. TAKAMATSU[2], H. MAEJIMA[2];
[1]Grad. Sch. of Hlth. Sci., [2]Dept. of Rehabil. Science, Fac. of Hlth. Sci., Hokkaido Univ., Sapporo, Japan; [3]Res. Fellow of Japan Society for the Promotion of Sci., Tokyo, Japan

Abstract: It has been well recognized that aerobic exercise increases the expression of brain-derived neurotrophic factor (BDNF), a member of neurotrophins, which has a crucial role for neuroplasticity in the central nervous system. Recently, neuromodulation has attracted attention as a tool for promoting the recovery in stroke rehabilitation. Specifically, studies showed that pharmacological neuromodulation reducing GABAergic inhibition enhanced motor functional recovery after stroke. Thus, it was expected that both exercise and pharmacological neuromodulation beneficially contribute to the recovery after stroke. However, there is a dearth of research on the interactive effect of exercise and pharmacological neuromodulation targeting GABA in stroke rehabilitation. The objective of this study was to examine the interactive effects of exercise and low-level inhibition of $GABA_A$ receptors on the recovery of motor function and BDNF expression in the primary motor cortex (M1) using stroke model rats. Thirty-seven male Sprague-Dawley rats were divided into 5 groups: a sham group (SHAM, n=8), a control group (CON, n=7), an exercise group (EX, n=7), a bicuculline group (BIC, n=7), and a bicuculline and exercise group (BICEX, n=8). Animals except the SHAM group received the middle cerebral artery occlusion (MCAO) surgery to induce an ischemic stroke. We administered the $GABA_A$ receptor antagonist bicuculline intraperitoneally to the BIC and BICEX groups at a non-epileptic dose of 0.25 mg/kg. The EX and BICEX groups received treadmill exercise for 30 min a day. Following 2-week intervention after MCAO surgery, mRNA expression and protein level of BDNF in the ipsilateral and contralateral M1 were assayed using RT-PCR and ELISA. All study procedures were approved by the ethics committee for animal research of Hokkaido University

in Japan. Slight recovery was found in the EX and BIC groups, whereas exercise in the presence of bicuculline administration significantly enhanced the recovery of motor function in the BICEX group. Furthermore, BDNF protein level in the ipsilateral M1 was significantly greater in the BICEX group compared with those of other groups, whereas no significant difference among the groups was found in BDNF protein level in the contralateral M1. These findings suggested that the enhancement of BDNF protein level in the ipsilateral M1 could contribute to the specific recovery in the BICEX group more than that in the contralateral. Altogether, it was expected that exercise combined with low-level inhibition of $GABA_A$ receptors would promote the recovery of motor function after stroke accompanying the upregulation of BDNF expression in the ipsilateral M1.

Disclosures: T. Inoue: None. **M. Kitahara:** None. **M. Okamura:** None. **Y. Takamatsu:** None. **H. Maejima:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.02/B23

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NHMRC
iRTP
PGRF

Title: BDNF V66M variant exhibits reduced functional compliance in mediating neuronal survival

Authors: *C. JOSEPH, A. MANGANI, V. GUPTA, S. GRAHAM;
Macquarie Univ., Sydney, Australia

Abstract: Brain Derived Neurotrophic factor (BDNF) dysregulation is attributed to progressive cell loss leading to pre-disposition to neurodegenerative disorders. A common single nucleotide polymorphism associated with BDNF, BDNF V66M has been reported in conjunction with pathological features found in several neuronal diseases, however the precise mechanism underlying the regulation of BDNFV66M on downstream survival kinases is not well known. Here we compared the effects of BDNF and BDNF V66M molecules on different neuronal types to understand BDNF V66M specific effects on downstream receptors. Differentiated SHSY5Y cells (sensory neurons) and NSC34 cells (motor neurons) were treated with 1uM Cyclotraxin-B (TrkB inhibitor) and 1uM TAT-Pep5 (p75NTR inhibitor) overnight. (n=3) Cells were then subjected to either 50ng/ml BDNF or BDNF V66M treatment for 10 minutes. Cells were then immediately washed with ice-cold PBS, lysed and quantified using BCA assay. Western blotting

was performed with respective antibodies and analyzed using ImageJ software. BDNF treatment induced phosphorylation of survival kinases ERK and AKT in both cell types. TrkB inhibition rendered a loss in protein phosphorylation and BDNF treatment could restore the effects only partially. However, BDNF V66M induced effects were less significant compared to BDNF (pTrkB 3.6±0.16, pAkt 6.2±0.2, pERK 1.8±0.12) and, TrkB inhibition significantly ablated the BDNF V66M biochemical effects (pTrkB 1.6±0.21, pAkt 2.5±0.2, pERK 1.5±0.26). p75NTR inhibition compromised BDNF induced effects much more than TrkB inhibition and it was irrecoverable with BDNF V66M treatment (pTrkB 0.6±0.02, pAkt 1.6±0.13, pERK 0.4±0.08). Also, a differential activation pattern of kinases was observed in different neuronal cells. Together these results demonstrate that BDNF V66M lacks the usual neuroprotective effect of BDNF in inducing survival pathways and has a cell type-specific phosphorylation pattern in distinct neuronal cells. Survival pathways are more severely compromised with Cyclotraxin-B treatment than TAT-Pep5 treatment reiterating the role of TrkB in BDNF signaling essential for neuronal development and survival.

Disclosures: **C. Joseph:** None. **A. Mangani:** None. **V. Gupta:** None. **S. Graham:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.03/B24

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: JSPS KAKENHI Grant 15K12570
JSPS KAKENHI Grant 17H02117

Title: Voluntary exercise combined with repetitive inhibition of GABAergic synapses modulates the expression of BDNF in the motor cortex

Authors: *H. MAEJIMA[1], M. HAYASHI[2], T. INOUE[2], M. KITAHARA[2];
[1]Dept. of Rehabil. Science, Fac. of Hlth. Sci., [2]Grad. Sch. of Hlth. Sci., Hokkaido Univ., Sapporo, Japan

Abstract: Recent researches focus on pharmacological treatments using $GABA_A$ receptor antagonists or inverse-agonist to inhibit GABAergic synapses and enhance excitability of cortical neurons in patients with CNS disorders, like the treatment using transcranial direct current stimulation (tDCS). Neurotrophins play crucial roles in neuroplasticity, neurogenesis, and neuroprotection in the central nervous system. Specifically, aerobic exercise increases the expression of brain-derived neurotrophic factor (BDNF) in the brain. In our previous studies, exercise combined with low-level $GABA_A$ receptor inhibition increased the expression of BDNF in the brain, i.e., low-level $GABA_A$ receptor inhibition with intraperitoneal administration of 0.25

mg/kg bicuculline, an antagonist for $GABA_A$ receptor, potentiated exercise-induced expression of BDNF in the motor cortex and the cerebellar cortex. Meanwhile, it was reported that bicuculline administration improved the scores of behavioal tests in a dose-dependent manner. Therefore, we assessed the interactive effects of exercise and higher inhibition of $GABA_A$ receptors with the intraperitoneal administration of 1.00mg/kg bicuculline for two weeks on the expression of BDNF in the motor cortex. The objective of this study was to consider the clinical treatment of GABAergic inhibition for neurorehabilitation in the patients with CNS disorders such as cerebrovascular accident (CVA). ICR mice were randomly divided into 4 groups based on the factors of exercise and $GABA_A$ receptor inhibition. We administered bicuculline intraperitoneally (1.00 mg/kg) every day and mice exercised on a running wheel for 2 weeks. Protein expression and mRNA expression of BDNF and its receptors (TrkB and p75) in the motor cortex were assayed using ELISA and real time PCR. Bicuculline administration enhanced daily running distance. However, repetitive administration of 1.00 mg/kg bicuculline reduced protein BDNF and increased transcriptional expression of p75, a receptor for BDNF related to neuronal death, whereas exercise tended to attenuate the exacerbation caused by bicuculline administration. Therefore, the present study suggested that exercise combined with low-level $GABA_A$ receptor inhibition could beneficially adjust the excitability of cortical neurons and modulate the expression of BDNF in the motor cortex as demonstrated in our previous studies using 0.25 mg/kg bicuculline in neurorehabilitation for the patients with CNS disorders.

Disclosures: H. Maejima: None. **M. Hayashi:** None. **T. Inoue:** None. **M. Kitahara:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.04/B25

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Title: Functional expression of TrkB receptors in hippocampal area CA3 interneurons

Authors: *E. J. GALVAN[1], G. HERRERA-LOPEZ[2], R. GUTIERREZ[3], G. BARRIONUEVO[4]; [1]Pharmacobiology, Cinvestav Sur, Mexico, Mexico; [2]Pharmacobiology, Cinvestav Sur, Mexico City, Mexico; [3]Ctr. for Res. and Advanced Studies, Mexico DF, Mexico; [4]Dept Neurosci, Univ. Pittsburgh, Pittsburgh, PA

Abstract: The present study examines the expression of the Tropomyosin-related kinase B (TrkB) receptor and the electrophysiological consequences of its activation on GABAergic interneurons of hippocampal area CA3. Triple immunolabeling for glutamate decarboxylase 67, the calcium-binding proteins parvalbumin, calbindin or calretenin, and the TrkB, shows that TrkB is widely expressed in horizontally-oriented bipolar and rounded-multipolar Stratum Oriens interneurons, bipolar and multipolar Stratum Lucidum interneurons and rounded-

multipolar GABAergic cells somatically located in the Stratum Radiatum/Stratum Lacunosum-Moleculare of area CA3. Electrophysiological characterization showed that Stratum Oriens interneurons exhibited a fast-spiking pattern of firing output, whereas Stratum Lucidum cells exhibited either fast-spiking or regular spiking output. In the Stratum Radiatum/Lacunosum-Moleculare, interneurons fired slow regular spiking trains of action potentials. Activation of the TrkB receptor with the perfusion of the specific agonist 7-8, dihydroxyflavone, modulated the amplitude and frequency of spontaneous excitatory events recorded in acute hippocampal slices. On Stratum Oriens interneurons, both amplitude and frequency of events increased significantly. On Stratum Lucidum, fast-spiking interneurons also exhibited increased amplitude and frequency of the spontaneous events. In contrast, regular spiking interneurons exhibited a significant reduction in the amplitude of the spontaneous events accompanied by increased spontaneous events frequency. The rounded-multipolar interneurons of the Stratum radiatum/Lacunosum-Moleculare exhibited increased amplitude and frequency of spontaneous excitatory events. The increased excitability of the inhibitory transmission was mirrored in the GABAergic inputs impinging on CA3 pyramidal cells, as the spontaneous inhibitory activity was dramatically increased on CA3 pyramidal cells during the perfusion of 7,8-DHF. Our data show that activation of TrkB receptors via Brain-Derived Neurotrophic Factor modulates the strength of synaptic transmission of area CA3 and restrain the excitability of CA3 pyramidal neurons.

Disclosures: E.J. Galvan: None. **G. Herrera-Lopez:** None. **R. Gutierrez:** None. **G. Barrionuevo:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

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Program #/Poster #: 368.05/B26

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: CONACYT Grant 456272

Title: Functional expression of TrkB receptor in hippocampal area CA3 interneurons

Authors: *E. GRIEGO-MELO[1], G. HERRERA-LOPEZ[1], G. GOMEZ LIRA[1], R. GUTIERREZ[1], G. BARRIONUEVO[2], E. J. GALVAN[1];
[1]Cinvestav Sur, Mexico City, Mexico; [2]Dept Neurosci, Univ. Pittsburgh, Pittsburgh, PA

Abstract: Brain-Derived Neurotrophic Factor (BDNF), an endogenous ligand for the Tropomyosin-related kinase B (TrkB) receptor, mediates multiple neural functions including synaptic transmission and plasticity. In this work, we are exploring the expression of TrkB receptors on anatomically identified CA3 interneurons and the physiological consequences of TrkB activation on the synaptic transmission of CA3 interneurons. Confocal analysis revealed

TrkB immunoreactivity in GAD-67 positive neurons that are also immunopositive to the calcium-binding proteins parvalbumin, calbindin or calretinin. TrkB is abundantly expressed in interneurons somatically located in the strata Oriens, Lucidum, Radiatum, and Lacunosum-Moleculare. Whole cell recordings in acute hippocampal slices showed that stimulation of TrkB receptor with the specific agonist 7, 8-dihydroxyflavone, differentially modulates amplitude and frequency of spontaneous excitatory postsynaptic currents (sEPSCs) of CA3 interneurons. In S. Oriens interneurons, both amplitude and frequency of sEPSCs increased significantly. In S. Lucidum, depending on its firing pattern, stimulation of TrkB increased amplitude and frequency of sEPSCs (fast-spiking interneurons) or decreased the sEPSCs amplitude without changes in the frequency (regular spiking interneurons). For interneurons located at the S. Radiatum and S. Lacunosum-Moleculare stimulation of TrkB consistently increased amplitude and frequency of sEPSCs. As expected, the increase in the glutamatergic transmission on CA3 interneurons was accompanied by an increase in the spontaneous inhibitory postsynaptic currents (sIPSCs) recorded on CA3 pyramidal neurons. Our results provide experimental evidence that activation of the TrkB receptor modulates the synaptic transmission of GABAergic interneuron and restrain the excitability of CA3 pyramidal cells.

Disclosures: **E. Griego-Melo:** None. **G. Herrera-Lopez:** None. **G. Gomez Lira:** None. **R. Gutierrez:** None. **G. Barrionuevo:** None. **E.J. Galvan:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

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Takeda Science Foundation
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Research grant from Toyama First Bank Foundation

Title: Visualizing activity-dependent BDNF expression in living mouse brain

Authors: *M. FUKUCHI[1], R. SAITO[2], S. MAKI[2], N. HAGIWARA[1], S. MITAZAKI[1], H. MORI[3];
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Abstract: BDNF (Brain-derived neurotrophic factor), a member of neurotrophin family, plays a crucial role in expressing a variety of neural functions such as memory consolidation. In addition, alterations in BDNF level has been reported in psychiatric and neurodegenerative diseases including depression and Alzheimer's disease, suggesting that BDNF could be a biomarker and drug target for these diseases. We previously generated a novel transgenic mouse strain, termed *Bdnf-Luciferase* transgenic (*BDNF-Luc*) mouse, to visualize changes in *Bdnf* expression in living mice, using a luciferase as an imaging probe. We successfully detected bioluminescence signals from *BDNF-Luc* mice after the administration of D-luciferin, a substrate for luciferase. However, it was quite difficult to visualize changes in *Bdnf* expression in the brain region of the Tg mice, because the bioluminescence light producing D-luciferin and luciferase poorly penetrates biological tissues. We here used a novel substrate for luciferase, TokeOni, water-soluble substrate producing highly penetrable near-infrared bioluminescence light. Comparing with D-luciferin, we clearly detected the bioluminescence signal from the brain region of *BDNF-Luc* mice after the administration of TokeOni. Using TokeOni, we could also visualize kainic acid- or light stimulation-induced *Bdnf* expression in the brain region of the Tg mice. Taken together, TokeOni would be a beneficial substrate for luciferase to visualize changes in BDNF expression in the brain region, and this could be a powerful tool for clarification of the role of BDNF expression in pathophysiological and physiological conditions.

Disclosures: M. Fukuchi: None. **R. Saito:** None. **S. Maki:** None. **N. Hagiwara:** None. **S. Mitazaki:** None. **H. Mori:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.07/B28

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: DFG, SFB 665
Thyssen foundation

Title: Neuregulin-3 promotes excitatory synapse formation on hippocampal interneurons in a Bace1 dependent manner

Authors: *T. MULLER[1], R. JÜTTNER[1], B. C. VOIGT[1], S. BRAUD[2], F. RATHJEN[1], J. GEIGER[2], J. F. A. POULET[1], C. BIRCHMEIER[1];
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Abstract: NRG3 and ERBB4 were implicated by human genetics in schizophrenia and other neuropsychiatric diseases. Nrg3 is a ligand of the Neuregulin gene family. Fast spiking

GABAergic interneurons are crucial for cortical network function and have been implicated in psychiatric disorders. They express high levels of the molecular interaction partner of Nrg3, the ErbB4 tyrosine kinase receptor. We found that Nrg3 is a functionally important and even the dominant interaction partner of ErbB4 in parvalbumin-positive (PV) GABAergic interneurons. Nrg3 and ErbB4 are located pre- and postsynaptically, respectively, in excitatory synapses on PV interneurons *in-vivo* and in-*vitro.* Ablation of Nrg3 results in a similar phenotype as the one described for ErbB4 ablation, i.e. reduced numbers of excitatory synapses on PV interneurons, altered short-term plasticity, and a disinhibition of the hippocampal network. In culture, presynaptic Nrg3 increases excitatory synapse numbers on ErbB4+ interneurons. Conversely, Nrg3 mutant neurons were poor donors of presynaptic terminals, which was particularly evident in the presence of competing neurons that produce recombinant Nrg3. Furthermore, we found that when presented by non-neuronal cells, Nrg3 induced postsynaptic membrane specialization. ErbB4, the tyrosine kinase receptor that binds Nrg3, is mainly known for its signaling function. However, we show that Nrg3 is a poor signaling molecule and instead provides adhesive cues in neuronal culture. These cues facilitate excitatory neurons to synapse onto ErbB4+ interneurons. Furthermore, we show that the synaptogenic activity of Nrg3 requires processing by the protease Bace1. Our work revealed the molecular mechanisms underlying the synaptic function of Nrg3, and provides insight into the role of Nrg3/ErbB4 functions in psychiatric disease.

Disclosures: T. Muller: None. **R. Jüttner:** None. **B.C. Voigt:** None. **S. Braud:** None. **F. Rathjen:** None. **J. Geiger:** None. **J.F.A. Poulet:** None. **C. Birchmeier:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.08/B29

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: IN216817

Title: PI3K and AKT signaling pathway activated by prolactin in the rat hippocampus

Authors: *G. MOLINA SALINAS[1], V. RODRIGUEZ CHAVEZ[2], M. CERBON CERVANTES[2];
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Abstract: Prolactin (PRL) is a peptide hormone, produced primarily by lactotrophs of the adeno-hypophysis and belongs to group I of cytokines family, together with growth hormone (GH) and placental lactogen (LP). PRL has been reported that it is synthesized in extra pituitary tissues, such as mammary gland, ovaries, decidua, also in cells of the immune system as well as

endothelial cells and more recently it has been suggested its synthesis in the brain. PRL It is associated with functions such as: neuronal plasticity, stimulation of neurogenesis in the olfactory bulb, induction of maternal behavior, reduction of anxiety, activation of glia and remyelination of oligodendrocytes and in recent reports as a neuroprotective against excitotoxicity produced by glutamate (Glu) or kainic acid (KA). The signaling pathways associated with its effects are JAK2/SATA5, ERK1/2 and PI3K/AKT. However, the signaling pathway by which PRL exerts its effects in the hippocampus, a region of the brain related to memory and learning, is not known in detail. The aim of this work is to determine, whether the PI3K/AKT pathway is responsible in part for the PRL-activated molecular mechanisms in the rat hippocampus. The animal model to answer this issue was ovariectomized wistar rats which were administered PRL via intraperitoneal (500µg/kg). The time course assay was done at 15, 30 and 60 minutes. mRNA and proteins were obtained from dissection of hippocampus. Analysis of the expression of AKT, pAKT CREB, pCREB, BDNF and NFkB proteins which are located downstream of the PI3K/AKT pathway and RT qPCR to evaluate some genes such as Bcl2 and PRLR that are activated by PRL were performed. Results show differential expression in the phosphorylated proteins from 30 min of PRL administration as well as increased expression of both genes. This suggests that PI3K/AKT pathway is early activated by PRL treatment and it would be responsible in part for the molecular actions induced by prolactin in the hippocampus of rats.

Disclosures: G. Molina Salinas: None. **V. Rodriguez Chavez:** None. **M. Cerbon Cervantes:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.09/B30

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Australian Government; Innovation Connect Grant

Title: Quantification of urinary neurotrophins by ELISA assay in prostate cancer patients

Authors: B. MARCH[1], ***L. M. FOSTER**[2], S. FAULKNER[3], M. SMOLNY[2], R. A. RUSH[2], H. HONDERMARCK[3];
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Abstract: Background: The autonomic nervous system regulates the development and progression of prostate cancer. Prostate cancer-derived neurotrophins promote nerve infiltration

into the tumour microenvironment, whereby the local release of neurotransmitters stimulates cancer cell progression, growth, and invasion. Immunohistochemical studies have shown an association between prostatic neurotrophins (in particular BDNF, proNGF, NGF, GDNF)[1-3] and tumour aggressiveness, therefore we hypothesised that urinary neurotrophins may be candidate biomarkers for prostate cancer detection and prognosis.
Method: We used and validated the Biosensis *Rapid*™ ELISA kits for the quantification of neurotrophins in human urine (ELISA kits for NGF, BDNF, NT3, NT4/5, proNGF, proBDNF and GDNF). Reproducibility, spike recovery, parallelism and selectivity tests were performed for each kit. Thereafter, urine specimens were tested from a prospectively collected cohort of men with suspected or established prostate cancer (n=60). Control urine specimens were collected in healthy volunteers of similar age (n=15). All samples were undiluted and tested in duplicate. Total protein and creatinine were measured in each sample to normalise urinary neurotrophin concentration. Assay reliability was analysed by several criteria including potential interference by sample matrices and heterophilic antibodies.
Results: Neurotrophins were detected in both cancer and healthy urine, but there was no significant difference in the mean concentration between groups in any of the tested neurotrophins. Normalising the neurotrophin concentration to urinary creatinine or total protein concentration did not affect the outcome.
Conclusion: Neurotrophins are detectable in human urine samples and at this stage we have not evidenced any significant difference between prostate cancer patients and healthy controls.
Keywords: neurotrophins, prostate cancer, biomarker, therapeutic target
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2.Pundavela J, et al, Am J Pathol.2014 Dec;184(12):3156-62 PMID:25285721
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Disclosures: B. March: None. **L.M. Foster:** None. **S. Faulkner:** None. **M. Smolny:** None. **R.A. Rush:** None. **H. Hondermarck:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.10/B31

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NIH Grant AR047410

Title: Increased accumulation of Rab7 and nerve growth factor receptor TrkA and $p75^{NTR}$ in rat sciatic nerve during peripheral inflammation

Authors: *V. GUJAR, K. E. MILLER;
Anat. and Cell Biol., Oklahoma State University, CHS, Tulsa, OK

Abstract: In addition to modulating the synthesis of neuropeptides like substance P (SP), calcitonin gene-related peptide (CGRP) and neurotransmitter glutamate (GLU), nerve growth factor (NGF) signaling is crucial for the regulation of growth and survival of sympathetic and sensory neurons. A small GTPase Rab7 has been shown to mediate the trafficking of NGF/TrkA signaling endosomes in sympathetic neurons. To determine whether Rab7 performs the similar function in primary afferents during the inflammatory state, we examined the immunoreactivity of Rab7 along with NGF and its receptors TrkA and p75NTR in rat sciatic nerve during adjuvant-induced arthritis (AIA). AIA was induced by a single unilateral subcutaneous injection of 150 µl of 1:1 of saline/CFA emulsion (complete Freund's adjuvant, 150 µg/150 µl) in the hind paw plantar surface. AIA was allowed to develop for 6hr prior to sciatic nerve ligation surgery and then after 24hr the distal end to the ligature is collected and processed for determining immunoreactivity. We found that the immunoreactivity of Rab7 was elevated in the distal side of a ligated peripheral nerve. NGF, pTrkA, and p75NTR also showed elevated immunoreactivity in sciatic nerve during peripheral inflammation. These results indicate the enhanced axoplasmic trafficking of Rab7 and NGF signaling molecules in the sciatic nerve during the process of peripheral inflammation. Further, these results suggest a possible association between NGF signaling and Rab7 GTPase in primary afferents during AIA.

Disclosures: V. Gujar: None. **K.E. Miller:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.11/B32

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: NICHD ZIA-HD000711

Title: Neurodevelopmental, behavioral and transcriptomic analyses of isoform-specific ErbB4 Cyt-1 mutant mice

Authors: *L. M. ERBEN[1,2], M. CRONIN[1], R. MURPHY[1], M. SKIRZEWSKI[1], I. KARAVANOVA[1], D. VULLHORST[1], S. L. CARROLL[3], A. BUONANNO[1];
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Abstract: Genetic variants of Neuregulins (NRGs), and their cognate neuronal receptor ErbB4 have been associated with a risk for schizophrenia. Null ErbB4 knock-out (KO) mice exhibit neurodevelopmental impairments and behavioral deficits relevant to psychiatric disorders. ErbB4 transcripts are alternatively spliced at two locations: alternative splicing of JMa or JMb exons

encoding the extracellular juxtamembrane region renders the receptor susceptible or resistant to metalloprotease-mediated cleavage, respectively. In the cytoplasmic domain, inclusion of the Cyt-1 exon 26 confers upon the receptor the ability to signal via the PI3K pathway, in contrast to Cyt-2 variants lacking this exon. The ErbB4 Cyt-1 isoform comprises approximately 40% of ErbB4 in most brain areas, and independent studies have reported that ErbB4 transcripts including the Cyt-1 exon are increased in postmortem brains of schizophrenia patients. To investigate the functional role of ErbB4 Cyt-1, we targeted exon 26 by site-directed recombination. Ablation of the Cyt-1 exon was confirmed by the absence of Cyt-1 transcripts in brain sections using a novel *in situ*hybridization approach that detects single-exon boundaries (BaseScope), and by quantitative real-time PCR using TaqMan probes. Importantly, absence of the Cyt-1 exon did not alter overall expression levels of ErbB4, the JMa/JMb isoform ratios or the related NRG receptor ErbB3 in mutant mice. We then addressed phenotypes reported in ErbB4 KO mice. While GABAergic interneurons are reduced in the adult cortex and hippocampus of ErbB4 KO mice, we found no changes in density or distribution of interneurons in Cyt-1 KO mice. Unexpectedly, Cyt-1 KO mice performed normally in a series of behavioral assays: open field, elevated plus maze, sensorimotor gating and cognitive behaviors; all affected in ErbB4 KO mice. Lastly, our expression analyses in the hippocampus and the mesencephalon revealed only few changes in the transcriptome of Cyt-1 mutant mice. Taken together, we conclude that ErbB4 Cyt-1 receptors are largely dispensable for CNS development and function *in vivo*. *This work was kindly supported by the Eunice Kennedy Shriver NICHD Intramural Research Program, NIH.*

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Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.12/B33

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: Intramural Research Program of the Eunice Kennedy Shriver Institute of Child Health and Human Development (ZIA- HD000711)

Title: NMDA receptors regulate both neuregulin 2 accumulation at ER-PM junctions and ectodomain shedding by ADAM10

Authors: *D. VULLHORST[1], A. L. BUONANNO[2];
[1]NICHD, NIH, Bethesda, MD; [2]Head of Cell Biol. Affinity Group, NICHD, Chief of Sect Mol Neurobiol (SMN), NICHD, NIH, Bethesda, MD

Abstract: Unprocessed pro-Neuregulin 2 (pro-NRG2) and the voltage-gated potassium channel Kv2.1 co-localize on neuronal cell bodies at junctions between the endoplasmic reticulum and plasma membrane (ER-PM junctions). NMDA receptors (NMDARs) trigger NRG2 ectodomain shedding from these sites followed by activation of ErbB4 receptor tyrosine kinases, and ErbB4 signaling cell-autonomously downregulates intrinsic excitability of GABAergic interneurons by reducing voltage-gated sodium channel currents. NMDARs also promote dispersal of Kv2.1 clusters from ER-PM junctions and cause a hyperpolarizing shift in its voltage-dependent channel activation, suggesting that NRG2/ErbB4 and Kv2.1 act in concert to regulate intrinsic interneuron excitability in an activity-dependent manner. Here we explored the cellular processes underlying NMDAR-dependent NRG2 shedding in cultured rat hippocampal neurons. We report that NMDARs control shedding by two separate but converging mechanisms. First, NMDA treatment disrupts pro-NRG2 clusters at ER-PM junctions by post-translationally modifying conserved Ser/Thr residues in its intracellular domain, remarkably similar to the molecular mechanisms underlying NMDAR-dependent downregulation of Kv2.1 clusters. Second, using a mutant NRG2 that cannot be modified at these residues and that fails to accumulate at ER-PM junctions, we demonstrate that NMDARs directly promote NRG2 shedding by ADAM-type matrix metalloproteinases. Using pharmacological and shRNA-mediated knock-down, and metalloproteinase overexpression, we unexpectedly find that ADAM10, but not ADAM17/TACE, is the major NRG2 sheddase acting downstream of NMDAR activation. Together, these findings reveal how NMDARs exert tight control over the NRG2/ErbB4 signaling pathway in GABAergic interneurons, and strengthen the notion that NRG2 and Kv2.1 are co-regulated components of a pathway that homeostatically regulates intrinsic excitability.

Disclosures: D. Vullhorst: None. **A.L. Buonanno:** None.

Poster

368. Neurotransmitters: Transporters and Signaling Molecules

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 368.13/B34

Topic: B.01. Neurotransmitters/ Transporters/ and Signaling Molecules

Support: National Research Foundation of Korea NRF-2017R1A2B4002922

Title: Ultrastructural and molecular characterization of PDGFR-β-positive leptomeningeal cells in adult rat brain

Authors: *T.-R. RIEW[1,3], X. JIN[1,3], H. KIM[4], S. KIM[1,3], Y. PARK[1,3], K.-W. SUNG[2,3], M.-Y. LEE[1,3];
[1]Dept. of Anat., [2]Pharmacol., Col. of Medicine, The Catholic Univ. of Korea, Seoul, Korea, Republic of; [3]Catholic Neurosci. Institute, The Catholic Univ. of Korea, Seoul, Korea, Republic of; [4]Integrative Res. Support Center, The Catholic Univ. of Korea, Seoul, Korea, Republic of

Abstract: The leptomeninges referring to arachnoid and pia mater and their projections into the perivascular compartments in the central nervous system actively participate in diverse biological processes including fluid homeostasis, immune cell infiltrations, and neurogenesis, yet their detailed cellular and molecular identities remain elusive. The present study provides the first comprehensive characterization of platelet-derived growth factor beta (PDGFR-β)-expressing cells in the leptomeninges in the adult rat brain using light and electron microscopy. PDGFR-β^+ cells comprised the inner arachnoid, arachnoid trabeculae, and pia mater as well as the leptomeningeal sheath of the subarachnoid vessels, thereby forming a cellular network throughout the leptomeninges. Leptomeningeal PDGFR-β^+ cells were commonly characterized by large euchromatic nuclei, thin branching processes forming web-like networks, and expression of intermediate filaments nestin and vimentin. They were typical of active fibroblasts showing well-developed rough endoplasmic reticulum and close spatial correlation with collagen fibrils. Leptomeningeal PDGFR-β^+ cells ensheathing the vasculature in the subarachnoid space joined with pial PDGFR-β^+ cells upon entering the cortical parenchyma, yet perivascular PDGFR-β^+ cells in these penetrating vessels showed an abrupt changes in their morphological and molecular characteristics: they became more flattened with loss of immunoreactivity for nestin and vimentin and deficient collagen deposition, indicative of inactive fibroblasts termed fibrocytes. In cortical parenchyma, PDGFR-β immunoreactivity was almost exclusively localized to larger caliber vessels, and significantly decreased in capillary-like microvessels. Collectively, our data identify PDGFR-β as a novel cellular marker for leptomeningeal fibroblasts comprising the leptomeninges and perivascular adventitial cells of the subarachnoid and penetrating large-sized cortical vasculatures. Funding: This research was supported by the grants from the National Research Foundation of Korea (NRF) [grant number NRF-2017R1A2B4002922].

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Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.01/B35

Topic: B.06. Synaptic Transmission

Support: Wellcome Trust (Technology Development Grant 202932)
EU FP7 HEALTH-F2-2009-241498
EU FP7 720270
EU SYNNOVATE grant agreement 695568

Title: Synapse population distributions reveal synaptome architecture including regional differentiation

Authors: *E. A. FRANSÉN[1], M. REHN[1], Z. QIU[2], M. J. CIZERON[2,3], B. KONIARIS[2], S. G. N. GRANT[2];
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Abstract: Synaptic properties have been studied extensively, temporally over a range of time scales, and spatially either at high resolution in small volumes, or at low resolution across the brain, however systematic characterization of types and subtypes of individual synapses within and across brain regions remains poorly understood.
We have produced the first synaptome atlas of the vertebrate brain by examining the expression of fluorescently labelled MAGUK proteins in individual excitatory synapses in mice (Zhu et al., 2018). Results show large diversity of synaptic types as well as regional specific composition and perturbations of these maps in disease-relevant mutations. Previous studies on the distribution of excitatory postsynaptic potential (EPSP) amplitudes or spine sizes show skewed distributions with heavy tails, but interpretations are limited by the small number of synapses and regions studied. As these distributions constitute fingerprints of learning as well as development, theoretical work on learning rules has nevertheless been informed by these findings.
To further analyze the synaptic organization from single synapses to brain-wide scale we have developed and used statistical methods. Using very large datasets obtained from the mouse synaptome atlas (+100 million synapses), we have evaluated the population (frequency) distributions of PSD95 positive excitatory synapses as described by protein numbers, morphology of postsynaptic density protein expression and associated subtype features. We observed skewed heavy-tailed distributions, consistent with previous electrophysiological and morphological findings. We further find differences between brain regions with regard to both the locations of the distribution peaks (constituting the most common synapses) and the width of the distributions (constituting the diversity) and support for spatial patterning and gradients. Previous studies have shown correlations between PSD95 expression, spine morphology and EPSP amplitude, allowing our results to be interpreted also in functional terms and to address hypothesis based on theoretical work. These analyses and other approaches are being developed to understand the remarkably complex architecture of the mouse synaptome.

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Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.02/B36

Topic: B.06. Synaptic Transmission

Support: Wellcome Trust (Technology Development Grant 202932)
European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (SYNNOVATE grant agreement 695568)

Title: The dynamic synaptome: A single-synapse resolution atlas of synaptic protein turnover across the mouse brain

Authors: *E. BULOVAITE[1], Z. QIU[1], M. HORROCKS[2,3], B. KONIARIS[1], N. H. KOMIYAMA[1], E. FRANSEN[4], S. G. N. GRANT[1,2,5];
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Abstract: We previously generated a mouse synaptome atlas that reveals high diversity of synapse types distributed into an architecture across the brain (Zhu, Cizeron & Qui, *et al*, Neuron 2018). This atlas is based on the static levels of expression of synaptic proteins. Here we introduce a novel method to visualize and systematically analyse endogenous synaptic protein turnover at single synapse resolution across the living brain. We created a line of genetically modified mice in which the endogenous PSD95 was fused to the HaloTag domain. These mice were injected intravenously with silicone-rhodamine (SiR) HaloTag ligand, which covalently binds the HaloTag domain, enabling the visualization of excitatory synapses with confocal and super-resolution microscopes. The turnover of PSD95 can be measured by the loss of fluorescence observed in brain tissue sections. Furthermore, the newly synthesised PSD95 can be detected by labelling with a second ligand (TMR-Halo) applied post-fixation. Combining this labelling method with synaptome mapping technology enabled us to quantify the changes in synapses in many brain regions. Strikingly, the vast majority (>90%) of excitatory synapses in the brain had replaced all detectable PSD95 within two weeks. However, there were residual populations of “long-lived” synapses in the cortex, hippocampus and amygdala showing discrete anatomical localisations. In addition to creating a dynamic synaptome atlas, this versatile technology enables us to study the mechanisms governing synapse diversity and its relevance to behaviour and disease.

Disclosures: **E. Bulovaite:** A. Employment/Salary (full or part-time):; Genes to Cognition Program, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh EH16 4SB,

UK. **Z. Qiu:** A. Employment/Salary (full or part-time):; Genes to Cognition Program, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, EH16 4SB, UK. **M. Horrocks:** A. Employment/Salary (full or part-time):; UK Dementia Research Institute, University of Edinburgh, Chancellor's Building, Edinburgh Medical School, E. **B. Koniaris:** A. Employment/Salary (full or part-time):; Genes to Cognition Program, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh EH16 4SB, UK. **N.H. Komiyama:** A. Employment/Salary (full or part-time):; Genes to Cognition Program, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh EH16 4SB, UK. **E. Fransen:** A. Employment/Salary (full or part-time):; Department of Computational Science and Technology, School of Electrical Engineering and Computer Science, KTH Royal Institute of Technology, 10044 Stockholm, Sweden. **S.G.N. Grant:** A. Employment/Salary (full or part-time):; Genes to Cognition Program, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh EH16 4SB, UK. 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.; UK Dementia Research Institute, University of Edinburgh, Chancellor's Building, Edinburgh Medical School, Edinburgh. Other; Simons Initiative for the Developing Brain.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.03/B37

Topic: B.06. Synaptic Transmission

Support: NIH Grant NS065920 (JIW)

Title: Climbing fiber-mediated spillover transmission to interneurons is regulated by EAAT4

Authors: *S. MALHOTRA[1], G. BANUMURTHY[2], J. H. VADEN[2], L. S. OVERSTREET-WADICHE[2], J. I. WADICHE[2];
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Abstract: A single cerebellar climbing fiber (CF) makes hundreds of individual contacts with one Purkinje cell (PC) and releases multiple vesicles at each synapse, a process termed multivesicular release (MVR)[1]. The high synaptic glutamate concentration resulting from MVR is mostly limited to the synaptic cleft by Excitatory Amino Acid Transporters (EAATs) on Bergmann glia that express EAAT1/2 and on PCs that express EAAT4. Nevertheless, sufficient glutamate spills over to activate glutamate receptors on nearby molecular layer interneurons (MLIs) despite the absence of anatomically-defined synaptic specializations. The expression of EAAT4 follows a parasagittal banding pattern and may limit CF-MLI glutamate spillover,

similar to its regulation of extrasynaptic neuroglial signaling[2,3]. Here, we use mice expressing Venus under the Aldolase C promoter to visualize EAAT4 expression[4] to test the idea that cerebellar regions are endowed with distinct spillover properties. We made patch-clamp recordings from MLIs and electrically isolated CFs in microzones with both high and low EAAT4 expression. Consistent with our hypothesis, spillover responses are smaller in areas where EAAT4 is expression is high, and larger where EAAT4 expression is low. Because many proteins have patterned expression that matches that of EAAT4, we used the pan-EAAT inhibitor TBOA to block glutamate transporters. The amplitudes of CF-mediated spillover EPSCs onto MLIs were similar between microzones in the presence of TBOA, confirming that these differences resulted from glutamate transport. This suggests that a lower concentration of glutamate escapes from CF-PC synapses when EAAT4 is prevalent. Since spillover signaling to MLIs triggers feedforward inhibition and disinhibition of PCs[5], these results suggest that non-synaptic circuity generates distinct patterns of inhibition in EAAT4 microzones.

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4 Fujita, H. *et al. PloS one* **9**, e86679 (2014).
5 Coddington, L. T. *et al. Neuron* **78**, 1050-1062 (2013).

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Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.04/B38

Topic: B.06. Synaptic Transmission

Support: NIGMS P20GM109089-01
NSF NSF1632881
NIMH R21NS093442

Title: NSG2 confers unique postsynaptic properties via AMPAR surface expression in a discrete subset of hippocampal synapses

Authors: *P. CHANDER, G. M. ELIASON, J. P. WEICK;
Neurosciences, Univ. of New Mexico, Albuquerque, NM

Abstract: Previous studies demonstrate that members of the Neuron-Specific Gene family (NSG1 and NSG2) are actively transported in multiple endo-lysosomal vesicles throughout dendritic arbors, and regulate secretory trafficking of postsynaptic AMPARs. Interestingly, we

recently demonstrated that NSG2 resides in a subset (~30%) of excitatory postsynaptic densities (PSDs) in hippocampal cultures, yet robustly affects postsynaptic efficacy. Thus, we hypothesized that either: 1) NSG2 is targeted to a unique subset of hippocampal synapses, or 2) NSG2 transiently visits all or most excitatory synapses over time, but occupies an average of 30% at any given timepoint. Using timelapse imaging we now demonstrate that postsynaptic NSG2 is stable during prolonged imaging periods (>3hr), supporting its preferential targeting to a subset of excitatory PSDs. Further, a proportion of synaptic NSG2 co-localizes with PSDs that contain large, multi-headed spines that resemble dentate gyrus-CA3 Mossy fiber synapses. To determine the function of NSG2 within PSDs we analyzed surface GluA1 and GluA2, and found that NSG2-containing PSDs displayed increased surface expression of AMPARs compared to PSDs that lacked NSG2. Furthermore, evoked excitatory postsynaptic currents (EPSCs) were significantly greater at NSG2-containing synapses, consistent with a role for NSG2 in promoting surface AMPAR expression. In order to determine the molecular determinants of NSG2 localization we deleted the entire amino (ΔN) or carboxy (ΔC) termini of NSG2. We found that the ΔN-NSG2 localized to significantly fewer synapses, while ΔC-NSG2 was trafficked to PSDs to a similar extent as wild-type NSG2. However, we found that compared to wild-type NSG2, neither ΔN-NSG2 nor ΔC-NSG2 mutants were able to cause increases in the amplitude of miniature EPSCs. This suggests that the N-terminus regulates PSD localization while the C-terminus is important for promoting AMPAR surface expression. Together, these and other data suggest that NSG2 promotes AMPAR surface expression at a subset of excitatory synapses and utilizes unique targeting and functional domains within the N- and C-termini, respectively.

Disclosures: P. Chander: None. **G.M. Eliason:** None. **J.P. Weick:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.05/B39

Topic: B.06. Synaptic Transmission

Support: NIH Grant 5R01MH107182

Title: Usp9X controls ankyrin repeat domain protein homeostasis during dendritic spine development

Authors: *S. YOON[1], E. PARNELL[1], M. KASHERMAN[2], M. FORREST[1], K. MYCZEK[1], S. PREMARATHNE[3], M. C. SANCHEZ VEGA[4], M. PIPER[2], T. BURNE[4], L. JOLLY[5], S. WOOD[3], P. PENZES[1];
[1]Northwestern Univ., Chicago, IL; [2]The Univ. of Queensland, Brisbane, Australia; [3]Griffith Univ., Brisbane, Australia; [4]The Queensland Brain Inst., Brisbane, Australia; [5]Robinson Institute, The Univ. of Adelaide, Adelaide, Australia

Abstract: Variants in the *ANK3* gene encoding ankyrin-G are associated with neurodevelopmental disorders, including intellectual disability, autism, schizophrenia, and bipolar disease. However, no upstream regulators of ankyrin-G at synapses are known. Through an unbiased screen, we show that ankyrin-G interacts with Usp9x, a neurodevelopmental disorder-associated deubiquitinase (DUB). Usp9x phosphorylation enhances their interaction, decreases ankyrin-G polyubiquitination, and stabilizes ankyrin-G to maintain dendritic spine development. In forebrain-specific Usp9X knockout mice ($Usp9X^{-/Y}$), ankyrin-G as well as multiple ankyrin-repeat domain (ANKRD)-containing proteins are transiently reduced at two, but recovered at 12-weeks, postnatally. However, reduced cortical spine density in knockouts persists into adulthood. $Usp9X^{-/Y}$ mice display ankyrin-G aggregates, increased anxiety, and novelty-induced hyperactivity. *USP9X* mutations in patients with intellectual disability and autism ablate its catalytic activity or ankyrin-G interaction. Our data reveal a DUB-dependent mechanism of ANKRD protein homeostasis, impairment of which only transiently affects ANKRD protein levels, but leads to persistent neuronal, behavioral, and clinical abnormalities.

Disclosures: S. Yoon: None. **E. Parnell:** None. **M. Kasherman:** None. **M. Forrest:** None. **K. Myczek:** None. **S. Premarathne:** None. **M.C. Sanchez Vega:** None. **M. Piper:** None. **T. Burne:** None. **L. Jolly:** None. **S. Wood:** None. **P. Penzes:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.06/B40

Topic: B.06. Synaptic Transmission

Support: NIH Grant DA041876

Title: The spinophilin, SAPAP3, and mGlu5 complex as a potential regulatory node of obsessive-compulsive disorder-like behavior

Authors: *C. W. MORRIS[1], D. S. WATKINS[1], A. J. BAUCUM II[2];
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Abstract: Scaffolding proteins enriched in dendritic spines are poised to organize protein complexes and facilitate downstream signaling in response to neuronal activation. Genetic loss of scaffolding proteins that are highly expressed in the striatum lead to altered behavioral outputs. For example, loss of spinophilin, the major dendritic spine protein phosphatase 1-targetting protein, induces striatal motor deficits. Specifically, these mice have deficits in rotarod learning and do not sensitize to the hyperlocomotive effects of amphetamine. Biochemically, spinophilin has been implicated in regulating the surface expression of the metabotropic

glutamate receptor 5 (mGlu5). Interestingly, we have reported that spinophilin interacts with SAPAP3 in the striatum. SAPAP3 is also a scaffolding protein enriched in dendritic spines, where loss of this protein yields an excessive grooming phenotype, making it a validated mouse model of obsessive-compulsive disorder (OCD). Work investigating SAPAP3 KO mice has revealed this excessive grooming phenotype is largely due to exacerbated mGlu5 signaling. However, the role spinophilin plays in regulating this mGlu5-driven excessive grooming phenotype is largely unknown. The present study explores this knowledge gap by utilizing biochemical and behavior approaches to address the physiological importance of the spinophilin/SAPAP3/mGlu5 complex. Elucidating the mechanisms and consequences of this interaction in the striatum can lead to novel druggable targets capable of rescuing OCD-like phenotypes.

Disclosures: C.W. Morris: None. **D.S. Watkins:** None. **A.J. Baucum II:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.07/B41

Topic: B.06. Synaptic Transmission

Support: National Key R&D Program of China (2017YFA0505300)
National Natural Science Foundation of China (31630030, 31621002 and 31761163006)
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the Anhui Provincial Natural Science Foundation (1908085QC95)
U.S. NIH (GM071940)

Title: Mesophasic organization of $GABA_A$ receptors in hippocampal inhibitory synapse

Authors: Y.-T. LIU[1], ***C.-L. TAO**[1], X. ZHANG[2], L. QI[1], R. SUN[1], P.-M. LAU[1], Z. H. ZHOU[3], G.-Q. BI[1];
[1]Univ. of Sci. and Technol. of China, Hefei, China; [2]Zhejiang Univ., Hangzhou, China; [3]Univ. of California, Log Angles, Los Angeles, CA

Abstract: Neurotransmitter receptors play pivotal roles in synaptic functions. However, the structure and organization of individual receptor molecules has not been observed *in situ*. Here, we identified and determined the structure of $GABA_A$ receptor ($GABA_AR$) in the inhibitory synapses of cultured hippocampal neurons by cryo electron tomography. The $GABA_ARs$ form super-complexes with a fixed 11nm inter-receptor distance but variable relative angles. These

super-complexes then form receptor networks with reduced Voronoi entropy than randomly distributed receptors. The receptor networks further organize into a mesophasic assembly with a ~18nm phase boundary. The assembly correlates with condensates of postsynaptic scaffolding proteins, as well as presynaptic neurotransmitter release sites. This hierarchical self-organization maintains both regularity and flexibility, thus could serve for balanced reliability and plasticity in synaptic information processing.

Disclosures: **Y. Liu:** None. **C. Tao:** None. **X. Zhang:** None. **L. Qi:** None. **R. Sun:** None. **P. Lau:** None. **Z.H. Zhou:** None. **G. Bi:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.08/B42

Topic: B.06. Synaptic Transmission

Support: Swiss National Science Foundation

Title: Gephyrin phosphorylation regulates sex-dependent inhibitory connectivity in the mouse brain

Authors: *B. F. N. CAMPBELL[1], M. HLEIHIL[1], S. K. TYAGARAJAN[2];
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Abstract: Recent characterisation of sex differences in inhibitory neurotransmission has revealed both developmental and regulatory dimorphisms, although underlying mechanisms are poorly understood. Fast GABAergic synaptic transmission is enacted via the pre-synaptic output of inhibitory interneurons and the post-synaptic activation of $GABA_A$ receptors ($GABA_ARs$) which in turn are organised by the scaffolding protein gephyrin. Gephyrin is the substrate for a plethora of post-translational modifications which affect the clustering and interactions of gephyrin molecules. Our group has reported that phosphorylation of gephyrin at serines 268 (by ERK1/2) and 270 (by GSK3β) negatively controls gephyrin cluster size and inhibitory synaptic transmission. Consequently, blocking the phosphorylation of these residues leads to increased density and size of gephyrin clusters, enhancing the amplitude and frequency of GABAergic input. We have recently found evidence that gephyrin is differentially phosphorylated between males and females in the brain. To understand how gephyrin phosphorylation may be a substrate for control of GABAergic transmission between the sexes, we developed a mutant mouse model in which serines 268 and 270 are mutated to alanines to constitutively prevent phosphorylation at these residues. Characterisation of behavioural phenotypes in these mutant mice has revealed deficits in learning, with altered inhibitory connectivity in the hippocampal CA1 formation

leading to functional changes in inhibitory synaptic input. This phenotype presents as sexually dimorphic, with opposing changes between male and female mice due to regulation of interneuron number. Taken together, this research reveals the unexpected involvement of a synaptic scaffold protein controlling inhibitory connectivity in a sexual dimorphic manner.

Disclosures: B.F.N. Campbell: None. **M. Hleihil:** None. **S.K. Tyagarajan:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.09/B43

Topic: B.06. Synaptic Transmission

Support: NIH Grant K99MH117235
NIH Grant R37-MH052804

Title: Latrophilin adhesion GPCRs direct synapse specificity by coincident binding of FLRTs and Teneurins

Authors: *R. SANDO, X. JIANG, T. C. SUDHOF;
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Abstract: Emerging evidence supports that bidirectional signaling by cell-adhesion molecules mediates synapse formation, but the mechanisms involved remain elusive. The adhesion-G-protein coupled receptor (GPCR) Latrophilins are large cell-adhesion molecules with extensive extracellular sequences, as well as putative intracellular signal transduction capabilities. We found that the adhesion-GPCRs latrophilin-2 and latrophilin-3 are postsynaptic molecules that selectively direct formation of perforant path and Schaffer-collateral synapses, respectively, in hippocampal CA1-region neurons. Latrophilin-3 binds to at least two trans-cellular ligands, fibronectin leucine-rich-repeat transmembrane proteins (FLRTs) and teneurins. Binding to both ligands *in vivo* was required for input-specific synapse formation, suggesting that coincident binding of both ligands is necessary for synapse specificity. *In vitro*, co-expression of FLRT and teneurin was required to induce excitatory synapse formation. Thus, postsynaptic latrophilins promote excitatory synapse formation by simultaneous binding of two unrelated presynaptic ligands, which is required for the formation of distinct synaptic inputs at specific dendritic localizations. These results suggest a coincident binding and signal integration mechanism for input-specific excitatory synapse formation.

Disclosures: R. Sando: None. **X. Jiang:** None. **T.C. Sudhof:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.10/B44

Topic: B.06. Synaptic Transmission

Title: The nanoscale organization of glutamate receptor subtypes is optimized for synaptic function and plasticity

Authors: *M. HRUSKA[1], R. CAIN[1], M. B. DALVA[2];
[2]Neurosci., [1]Thomas Jefferson Univ., Philadelphia, PA

Abstract: At many synapses in the brain, action potential (AP) evoked synaptic transmission consists of a synchronous and asynchronous phase. The synchronous and asynchronous release at individual synapses underlie diverse computational outcomes that modulate synaptic transmission and plasticity. The synchronous release is dependent on the presence of fast calcium sensor Synaptotagmin-1 (SYT1), whereas Synaptotagmin-7 (SYT7) functions as a sensor for asynchronous release. Despite their importance for regulating the kinetics of release, how the nanosynaptic architecture of SYT1 and SYT7 is related to the functional organization of the synapse is unknown. Using multi-color Stimulated Emission Depletion (STED) nanoscopy, we find a precise relationship between the organization of glutamate receptors and sites of synchronous and asynchronous release at spine synapses. Using cultured cortical neurons, we find that 20% of spines are "silent" consisting of only NMDAR containing nanomodules. As spines increase in size the number, but not the size, of NMDAR nanomodules increases. In AMPAR containing spines, the number of GluA2 nanomodules scales with spine size, but the number of GluA1 nanomodules does not. At most synapses, GluA1 clusters co-localize only with one PSD-95 nanomodule regardless of PSD-95 nanomodule numbers. We find SYT1 and SYT7 localize to most spines containing NMDARs and AMPARs. Similar to GluN1 and GluA2 clusters, the number of both SYT1 and SYT7 nanomodules scales with the number of PSD-95 nanomodules and the spine size. To begin to understand how synaptic release sites are organized with relation to glutamate receptors, we analyzed the distances between pre- and postsynaptic modules. NMDAR modules localize closer to the center of PSD-95 and SYT7 nanomodules. In contrast, GluA1 and GluA2 clusters are found at the edges of PSD-95 nanomodules and are located closer to SYT1 than to SYT7. These findings indicate a remarkably precise organization of the nanostructure of the synapse and suggest that distinct sub-synaptic domains may define regions responsible for synaptic function and plasticity.

Disclosures: **M. Hruska:** None. **R. Cain:** None. **M.B. Dalva:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.11/B45

Topic: B.06. Synaptic Transmission

Support: R01NS100785
R01MH107182
R01MH097216

Title: Proteomics and functions of the synaptic proteins circulating in the CSF in schizophrenia

Authors: *M. DOS SANTOS[1,2], E. K. BOMBA-WARCZAK[3], E. L. PARNELL[1,2], D. D. LOIZZO[2], R. GAO[1], M. D. MARTIN-DE-SAAVEDRA[1,2], J. N. SAVAS[3], P. PENZES[1,2]; [1]Physiol., [2]Ctr. for Autism and Neurodevelopment, [3]Neurol., Northwestern Univ., Chicago, IL

Abstract: Schizophrenia (SZ) is a chronic debilitating psychiatric disorder involving complex interactions between genes and environment. At the neuronal level, this disorder is characterized by a decrease in synapses number in postmortem brain tissues. Due to this large heterogeneity of factors coupled with the lack of specific animal models, the molecular and neuronal bases of the synaptopathies in SZ are still poorly known. In order to develop better therapeutic strategies, it becomes crucial to find new biomarkers of the disease. Our study aims to identify novel synaptic markers of schizophrenia circulating in the cerebrospinal fluid (CSF) and study their physiological functions. The CSF is one of the bodily fluid most in contact with neurons and contains many synaptic proteins due to ectodomain shedding and extracellular vesicles release. Therefore, the CSF can mirror the synaptic proteome environment and requires only a mildly invasive extraction procedure. CSF proteins levels were measured in 5 controls and 5 individuals with schizophrenia using an unbiased tandem mass tag labeling followed by liquid chromatography/Mass spectrometry. We detected 140 proteins with dysregulated levels in the CSF in SZ individuals when compared to controls. Among these proteins, we focused our attention on proteins localized in the postsynaptic density for their fundamental roles in neuronal network development. Interestingly, we found a high proportion of cell adhesion molecules as well as ion channels regulators in this fraction, some of them are already known to be implicated in psychiatric disorders. The second part of the project consists in studying the neuronal functions of these circulating dysregulated proteins by using single cell 2photon calcium imaging on mouse brain slices exposed to recombinant forms of the candidates. This approach shows us the impact of these proteins at the neuronal network level such as the synchrony of the calcium events, their frequency and amplitudes. Ultimately, these results can pave the way to new therapeutic strategies using CSF peptides infusion based on observed dysregulated proteins in SZ.

Disclosures: **M. Dos Santos:** None. **E.K. Bomba-Warczak:** None. **E.L. Parnell:** None. **M.D. Martin-de-Saavedra:** None. **R. Gao:** None. **J.N. Savas:** None. **P. Penzes:** None. **D.D. Loizzo:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.12/B46

Topic: B.06. Synaptic Transmission

Support: NIH 5R01NS102871
DOD SCI-30225

Title: Patch clamp recordings in adult mouse thoracic paravertebral ganglia show changes in spontaneous event frequency and amplitude after spinal cord injury

Authors: D. B. STOCKTON[1], ***M. L. MCKINNON**[2], Y. LI[2], S. HOCHMAN[2], A. A. PRINZ[1]; [1]Dept. of Biol., Emory Univ., Atlanta, GA; [2]Dept Physiol, Emory Univ. Sch. Med., Atlanta, GA

Abstract: Thoracic intraspinal preganglionic neurons project to sympathetic postganglionic neurons (SPNs) within paravertebral ganglia. Major functions of the signals processed or relayed by these neurons include control of vasomotor tone, brown adipose tissue, sweat glands, and piloerector muscles. Spinal cord injury (SCI) can disrupt proper signal transmission and have profound effects, including the onset of autonomic dysreflexia, episodes of extreme hypertension that can lead to seizures, strokes, or death.

In a recently published paper, we developed an *in vitro* approach for whole-cell recordings in intact murine thoracic ganglia (T3-T12) to characterize cellular and synaptic properties. In the present abstract, we describe exploratory efforts to characterize the effects on synaptic properties due to SCI.

Our control group consisted of 3 male and 2 female C57Bl/6J mice and the treatment group of 1 male and four female C57Bl/6J mice. In the treatment group, we transected the spinal cord above the T2 vertebra three weeks or six weeks before sacrificial surgery. In both groups, we dissected the spinal column to perform *ex vivo* whole cell patch clamp electrophysiological recordings. We analyzed the recordings to characterize differences between groups. We found that spontaneous excitatory post-synaptic current (sEPSC) events approximately doubled in frequency post-SCI, while amplitude showed a trend to increase. In addition, amplitude statistical characteristics showed greater variability after SCI.

In summary, thoracic SPNs show marked changes in sEPSC event characteristics after spinal cord injury. This may suggest a compensatory reorganization of synaptic input to regain loss of function after SCI. These data highlight the potential role of spontaneous event mechanisms in understanding and treating spinal cord injuries.

Disclosures: **D.B. Stockton:** None. **M.L. McKinnon:** None. **Y. Li:** None. **S. Hochman:** None. **A.A. Prinz:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.13/B47

Topic: B.06. Synaptic Transmission

Title: Neuroligin4 regulates excitatory synaptic transmission in human neurons

Authors: *S. G. MARRO[1], S. CHANDA[2], T. C. SUDHOF[1], M. WERNIG[3];
[2]MCP, ISCBRM, [3]Werning Lab, Stem Cell Inst., [1]Stanford Univ., Stanford, CA

Abstract: The autism-associated synaptic-adhesion gene Neuroligin-4 (NLGN4) is poorly conserved evolutionarily, limiting conclusions from Nlgn4 mouse models for human cells. Here, we show that the cellular and subcellular expression of human and murine Neuroligin-4 differ, with human Neuroligin-4 primarily expressed in cerebral cortex and localized to excitatory synapses. Overexpression of NLGN4 in human neurons resulted in an increase in excitatory synapse numbers but a remarkable decrease in synaptic strength. Human neurons carrying the syndromic autism mutation NLGN4-R704C also formed more excitatory synapses but with increased functional synaptic transmission due to a postsynaptic mechanism, while genetic loss of NLGN4 did not significantly affect synapses in the human neurons analyzed. Thus, the NLGN4-R704C mutation represents a change of function mutation. Our work reveals contrasting roles of NLGN4 in human and mouse neurons, suggesting human evolution has impacted even fundamental cell biological processes generally assumed to be highly conserved.

Disclosures: **S.G. Marro:** None. **S. Chanda:** None. **T.C. Sudhof:** None. **M. Wernig:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.14/B48

Topic: B.06. Synaptic Transmission

Title: DKK2 regulates neuronal development and synaptic transmission in hippocampal CA1 neurons

Authors: *W. SONG, S. H. YOON, M.-H. KIM;
Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: DKK2 (Dickkopf-related protein 2) is a member of the DKK family (DKK1-4) proteins which modulate the Wnt signaling pathway through the interaction with Wnt co-receptors LRP5/6. Previous studies have suggested that DKK2 can modulate the Wnt signaling pathway either positively or negatively, depending on the cellular or tissue context. However, the role of DKK2 in the nervous system, especially in brain neurons, has not been investigated. Here we show that the deficiency of DKK2 in mice promotes neuronal development of hippocampal CA1 pyramidal neurons. DKK2 knockout (KO) mice displayed increased dendritic arborization and spine density in CA1 neurons. These morphological changes were accompanied by an increased frequency of miniature excitatory postsynaptic currents (mEPSCs). Furthermore, DKK2 KO mice showed enhanced long-term potentiation (LTP) at the Schaffer collateral-CA1 synapse, while basal synaptic transmission and the paired-pulse ratios were comparable to wild-type littermates. These results suggest that DKK2-mediated signaling is critical for normal neuronal development and synaptic function.

Disclosures: W. Song: None. **S.H. Yoon:** None. **M. Kim:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.15/B49

Topic: B.06. Synaptic Transmission

Support: SFB2016 ProjectA06

Title: Lack of interaction between gephyrin and tubulin at the inhibitory post-synapse

Authors: *I. ARIOZ[1], A. BECKER[1], D. DIETRICH[2], S. SCHOCH[1];
[1]Inst. of Neuropathology, Bonn, Germany; [2]Univ. Clin. Bonn, Bonn, Germany

Abstract: Disruption of excitation/inhibition balance of neuronal circuits contributes to various brain pathologies. Despite the importance of inhibitory synaptic transmission, our understanding of the structural organization of the inhibitory postsynaptic scaffold and how it is maintained, stabilized and dynamically modulated lacks far behind our knowledge of excitatory synapses. It is well established that Gephyrin a multifunctional protein that plays role in clustering of glycine and GABAA receptors is a major scaffold specific to inhibitory post-synapses. Interestingly, in contrast to excitatory postsynapses a direct link of the postsynaptic scaffold to microtubules has been reported. This was based on biochemical studies, which showed a direct interaction of polymerized tubulin with gephyrin. However, other studies suggest that postsynaptic and

extracted gephyrin was not affected by the depolymerization of microtubules or actin in cultured mature hippocampal neurons. To date, the nanoscale architecture of cytoskeletal elements has not been resolved in inhibitory post-synapses. To visualize the underlying structure, we have used super resolution microscopy (dSTORM) of microtubules and the inhibitory post-synapse scaffold protein gephyrin in cultured neurons. We found that the frequency of clustered gephyrin significantly increased with the number of dendritic microtubule filaments and that single filament-containing dendrites were devoid of gephyrin punctae. However, we did not collect evidence for a direct contact of tubulin with postsynaptically clustered dendritic gephyrin. These results may suggest that tubulin-gephyrin interaction is important for transport and/or initiation of an inhibitory synapse but not for the maintenance of the gephyrin postsynaptic scaffold.

Disclosures: I. Arioz: None. **A. Becker:** None. **D. Dietrich:** None. **S. Schoch:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.16/DP04/B50

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: B.06. Synaptic Transmission

Support: NIH Grant R01MH107182-02

Title: Insights into spinule dynamics, regulation, and function uncovered by 3D enhanced resolution microscopy

Authors: *C. R. ZACCARD[1], L. P. SHAPIRO[1], C. P. PRATT[2], K. MYCZEK[1], M. D. MARTIN-DE-SAAVEDRA[2], P. PENZES[3];
[2]Physiol., [1]Northwestern Univ., Chicago, IL; [3]Physiol., Nothwestern Univ. Feinberg Sch. Med., Chicago, IL

Abstract: Dendritic spinules are thin, membranous protrusions formed by neuronal dendritic spines that are not adequately resolved by diffraction-limited light microscopy. Our understanding of their structure and function is largely inferred from fixed-tissue electron microscopy, but recently developed enhanced-resolution modalities have enabled live-cell nanoscopic imaging of transient, sub-spine structures. Herein, we utilized rapid structured illumination microscopy (SIM) and enhanced resolution confocal microscopy to study spatiotemporal spinule dynamics in live cultured cortical pyramidal neurons. Spinules typically formed on large mushroom spines at the same topographical locations. Most were short-lived, originating near simple post-synaptic densities (PSDs), while a subset was long-lived and elongated, emerging from complex PSDs. We observed calcium transients within spinules

synchronized with spine head transients and a drastic decrease in spinule number following calcium depletion, while providing evidence of differential calcium-mediated regulation of spinule classes. We next investigated the role of Kalirin in spinule formation, a guanine nucleotide exchange factor for Rho GTPases that is a major regulator of actin dynamics in spines. Finally, we utilized live FM dye staining to assess short- and long-lived spinule interactions with presynaptic terminals. In summary, we identified unique spinule classes divergent in lifespan, dynamics, morphology, relationship to the PSD, and mode of regulation. These data together suggest distinct synaptic functions of short-lived and long-lived spinules, informing future studies, and demonstrating a new application for enhanced resolution microscopy to study sub-spine membrane projections.

Disclosures: C.R. Zaccard: None. **L.P. Shapiro:** None. **C.P. Pratt:** None. **K. Myczek:** None. **M.D. Martin-de-Saavedra:** None. **P. Penzes:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.17/B51

Topic: B.06. Synaptic Transmission

Support: 2R01MH097216-06

Title: Ion channel dysfunction and seizure susceptibility in the 16p11.2 duplication mouse model is mediated by epilepsy gene PRRT2

Authors: *M. P. FORREST[1], N. H. PIGUEL[1], Y.-Z. WANG[2], D. SIMKIN[3], L. E. DIONISIO[1], N. HAWKINS[3], V. BAGCHI[1], M. DOS SANTOS[1], A. L. GEORGE, Jr.[3], J. KEARNEY[3], J. N. SAVAS[2], P. PENZES[1];
[1]Physiol., [2]Neurol., [3]Pharmacol., Northwestern Univ., Chicago, IL

Abstract: The 16p11.2 microduplication is a rare copy number variant (CNV) that confers risk of multiple neuropsychiatric conditions including, schizophrenia, autism, and epilepsy. The 16p11.2 chromosomal region contains 27 protein-coding genes however, the mechanisms by which altered gene dosage in this region increases disease susceptibility is still unknown. To uncover novel disease-relevant pathways, we undertook a quantitative proteomic profiling in the 16p11.2 microduplication mouse model (dp/+) and discovered a large set of upregulated synaptic and ion channel proteins, which converged on known epilepsy risk factors. Using electrophysiology and calcium imaging we demonstrate that cortical neurons *in vitro* are hyperexcitable and have hypersynchronous calcium oscillations suggesting abnormal cortical activity in dp/+ mice. Moreover, we show that dp/+ mice are more susceptible to kainate-induced seizures. We searched for a potential driver gene of these excitability-related phenotypes within

the CNV region and show that *Prrt2,* a monogenic epilepsy gene, has a leading role. To illuminate the function of PRRT2, we performed immunoaffinity purification combined with mass spectrometry, and identified a PRRT2 protein-interaction network that is highly disrupted in dp/+ mice. Lastly, we show that genetic correction of *Prrt2* gene dosage in dp/+ mice delays the onset of kainate-induced seizures, functionally validating its role in seizure susceptibility. Our study reveals that the 16p11.2 microduplication disrupts an epilepsy risk network causing seizure-related endophenotypes due to increased *Prrt2* gene dosage. This work improves our understanding of CNV pathogenesis, and outlines novel strategies for identifying causal genes in CNVs.

Disclosures: M.P. Forrest: None. **N.H. Piguel:** None. **Y. Wang:** None. **D. Simkin:** None. **L.E. Dionisio:** None. **N. Hawkins:** None. **V. Bagchi:** None. **M. Dos Santos:** None. **A.L. George:** None. **J. Kearney:** None. **J.N. Savas:** None. **P. Penzes:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.18/B52

Topic: B.06. Synaptic Transmission

Support: AFOSR MURI Grant FA9550-18-1-0051

Title: Computational modeling of crosstalk between different calcium stores in dendritic spines

Authors: *D. OHADI, P. RANGAMANI;
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Abstract: Neurons critically rely on spatially and temporally regulated Ca^{2+} dynamics in subcellular compartments. This exquisite level of Ca^{2+} compartmentalization is achieved through storage and release of Ca^{2+} from various intracellular organelles. Mitochondria and endoplasmic reticulum (ER) are well-known as storage organelles in controlling Ca^{2+} dynamics in neurons. IP_3-induced Ca^{2+} release from ER through Ca^{2+}-induced Ca^{2+} release (CICR) controls long-term potentiation (LTP) and long-term depression (LTD). On the other hand, Ca^{2+} influx in dendritic mitochondria can regulate ATP synthesis and Ca^{2+} homeostasis. The ability of ER to store Ca^{2+} and exchange it with mitochondria at an inter-organelle distance of less than 30 nm, can potentially develop complex spatiotemporal interactions between the organelles. This type of crosstalk may result in diverse synaptic functions including presynaptic short-term plasticity, and postsynaptic long-term plasticity.
Ca^{2+} signal specificity is encoded in the temporal, spatial, frequency, and amplitude of Ca^{2+} transients. Globally, the Ca^{2+} signal is regulated by the close interconnection between plasma membrane channels and intracellular organelles. However, little is known about how Ca^{2+}

signals are regulated locally. This can be specifically critical for efficient Ca^{2+} signaling in small subcompartments such as dendritic spines in neurons. In this study, we propose a 3D computational reaction-diffusion model that investigates the role of dendritic mitochondria and ER-dependent Ca^{2+} dynamics in dendritic spines. This spatiotemporal model accounts for Ca^{2+} oscillations initiated by glutamate stimulation of metabotropic glutamate receptors and Ca^{2+} changes in three different compartments: cytosol, ER, and mitochondrion. Simulations predict that the organization of these organelles and differential distribution of key Ca^{2+} channels such as IP_3 receptor and ryanodine receptor can change Ca^{2+} dynamics. Our findings shed light on the involvement of calcium stores in the generation and maintenance of long-term potentiation.

Disclosures: D. Ohadi: None. **P. Rangamani:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.19/B53

Topic: B.06. Synaptic Transmission

Support: NINDS intramural funds

Title: Stimulation induces gradual increases in the thickness and curvature of the postsynaptic density

Authors: *J.-H. TAO-CHENG;
NINDS, NIH, Bethesda, MD

Abstract: Activity can induce structural changes in glutamatergic excitatory synapses, including increase in thickness and curvature of the postsynaptic density (PSD); these structural changes can only be documented by electron microscopy. Here in rat organotypic hippocampal slice cultures where experimental conditions can be easily manipulated, increases in thickness and curvature of PSDs were noticeable within 30 seconds of stimulation (depolarization with high K^+ at 90 mM or NMDA treatment at 50 μM) and progressed with time up to 3 min. These structural changes were reversible upon returning the samples to control medium for 5-10 min. The gradual increase in thickness of PSD could result from a gradual translocation of some PSD proteins to the PSD. The most likely major contributor is CaMKII because of its great abundance and its substantial translocation to the PSD under excitatory conditions. Under basal conditions, label for CaMKII is typically evenly distributed within the spine. Upon stimulation, CaMKII molecules could initially translocate to the cytoplasmic edge of the PSD core (the proximal layer of the PSD, up to 30-40 nm from the postsynaptic membrane) to bind to NR2B. As stimulation progresses, additional CaMKII molecules could continue to pile into thicker layers that extend into the PSD pallium (the deeper layer of the PSD, ~ 40-120 nm from the postsynaptic

membrane). Alternatively, the additional CaMKII at the PSD brought on by stimulation might initially distribute evenly in the PSD pallium and bind to other scaffold proteins like Shank that exist there. Eventually, after 2 min of stimulation, label for CaMKII became concentrated and confined within the border of the PSD pallium, and the laminar distribution of CaMKII at the PSD became very similar to that of Shank. These additional CaMKII and shank molecules in the PSD pallium may account for the appearance of PSD thickening. The change in curvature of PSD (from "concave" to "convex") upon depolarization by high K^+ could be due to an increase of presynaptic membrane caused by massive release of synaptic vesicles, forcing the presynaptic terminal to wrap around the PSD and bend the PSD to arch into the presynaptic terminal. Alternatively, a postsynaptic mechanism might be involved. Upon stimulation, a change in actin configuration or interaction with PSD proteins could exert a pull toward the interior of the spine and result in a curvature change in the PSD. The present data support a postsynaptic mechanism, since NMDA treatment does not induce synaptic vesicle release, and yet it still increases the curvature of the PSD.

Disclosures: J. Tao-Cheng: None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

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Topic: B.06. Synaptic Transmission

Support: SNF Grant 31003A_170082 (to GK)
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NIH Grant 2R56MH095980-06 (to KMH)
NIH Grant 1R01MH104319-01A1,02,03,04,05 (to KMH)

Title: Towards quantification of plasticity-related protein turnover at central synapses

Authors: *M. KUWAJIMA[1], N. T. N. PHAN[2], J. M. MENDENHALL[1], G. KNOTT[3], S. O. RIZZOLI[2], K. M. HARRIS[1];
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Abstract: Long-term potentiation (LTP) has been widely used as a model to elucidate cellular and molecular mechanisms of learning. Using 3D reconstruction from serial section electron microscopy (3DEM) we previously found changes in synapse structure that manifest after the initial saturation of LTP at CA3→CA1 synapses in the hippocampus: Many of these synapses

contained nascent zones, dynamic edge regions that have a postsynaptic density (PSD) but lack the presynaptic vesicles normally found at active zones. By 30 min following LTP induction, the nascent zones were converted to active zones (i.e., presynaptic vesicles were acquired). By 2 hr, both nascent and active zones were enlarged, with the greatest synapse enlargement occurring on dendritic spines that contained polyribosomes or smooth endoplasmic reticulum (Bell et al., 2014. J Comp Neurol 522: 3861-84; Chirillo et al., 2019, Sci Reports 9: 3861). Thus, we hypothesized that nascent zones may exhibit an increased turnover of proteins to support dynamic regulation of PSD structure and molecular composition during synaptic plasticity. To address this hypothesis, we are developing a correlative imaging approach combining 3DEM and secondary-ion beam mass spectrometry (SIMS). We aim to map protein turnover at a subsynaptic resolution from the brain tissue of animals that underwent metabolic labeling with stable isotopes. As a proof of concept, low-resolution EM imaging followed by SIMS was performed on sections (~70 nm) of resin-embedded neocortical tissue from a perfusion-fixed Wistar rat (6 wk old male) fed with ^{13}C-labeled glucose (10% solution for 24 hr). SIMS analysis showed overall ^{13}C abundance ratio of 2.2% in the analyzed sections, which was higher than the ratio of naturally occurring ^{13}C (~1.1%). When correlated with EM images, areas of ^{13}C enrichment were non-uniformly distributed across the tissue sections, demonstrating the feasibility of our approach. Thus, further analysis at a higher spatial resolution will be done with animals fed with isotope-labeled amino acids to examine which subsynaptic compartments show enrichment of newly synthesized proteins *in vivo*.

Disclosures: M. Kuwajima: None. **N.T.N. Phan:** None. **J.M. Mendenhall:** None. **G. Knott:** None. **S.O. Rizzoli:** None. **K.M. Harris:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.21/B55

Topic: B.06. Synaptic Transmission

Support: 5T32GM008203-28

Title: Partial segregation of spontaneous and evoked neurotransmission at inhibitory synapses

Authors: *P. M. HORVATH[1], L. M. MONTEGGIA[3], E. T. KAVALALI[2];
[2]Vanderbilt Brain Inst., [1]Vanderbilt Univ., Nashville, TN; [3]Vanderbilt Brain Inst., Nashville, TN

Abstract: Neurotransmission can be classified into two broad types: spontaneous and evoked. Much of the work examining these types of neurotransmission and their properties has been done in excitatory synapses. Previous work has shown a segregation of AMPA receptors which respond to spontaneous and evoked neurotransmission at excitatory synapses in both the central

nervous system as well as the *Drosophila* neuromuscular junction. Additionally, central nervous system NMDA receptors also show a near complete segregation of spontaneous and evoked neurotransmission. Although inhibitory synapses also transmit both spontaneous and evoked neurotransmission, they differ from excitatory synapses in both structure and function. Therefore, it has been unclear if the same principle of segregation holds true at inhibitory synapses. We have addressed this question by utilizing the use-dependent properties of picrotoxin, a $GABA_A$ receptor antagonist. By selectively blocking $GABA_A$ receptors activated by spontaneous neurotransmission we can examine the $GABA_A$ receptors specifically activated by evoked neurotransmission. Through these studies we show that there is partial, but not full, segregation of spontaneous and evoked neurotransmission at inhibitory synapses. These data indicate that while the principle of segregation of spontaneous and evoked neurotransmission still applies at inhibitory synapses, it is not as complete as at excitatory synapses.

Disclosures: P.M. Horvath: None. **L.M. Monteggia:** None. **E.T. Kavalali:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.22/B56

Topic: B.06. Synaptic Transmission

Support: Basal Center of Excellence in Aging and Regeneration AFB-170005
FONDECYT 1160724
FONDECYT Postdoctoral 3170043

Title: Wnt5a modulates dendritic spines morphology through the small Rho GTPases and their downstream target Cofilin

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Abstract: Introduction: Dendritic spines are actin-rich small protrusions that act as receiving sites of most excitatory inputs in the central nervous system. Actin cytoskeleton remodeling is required for the formation and consolidation of functional spines, a process regulated by the small Rho GTPases family. Wnt5a is a synaptogenic factor that promotes the formation of new dendritic spines and induces synaptic transmission in hippocampal and cortical neurons. Our main goal is to elucidate the mechanism by which Wnt5a promotes dendritic spine density and controls their morphology. **Methodology:** We employed primary culture of rat cortical and

hippocampal neurons DIV14 and HT22 cell line to analyze by pull-down activation assays, western blot and immunofluorescence the effects of Wnt5a in actin dynamics. **Results:** Wnt5a induces an increase in the number of dendritic spines and promotes their maturation. Moreover, Wnt5a controls the activation of the small Rho GTPases Rac1, Cdc42, and RhoA. Wnt5a also regulates the phosphorylation of LIMK1 and Cofilin in a time-dependent manner. Specifically, Wnt5a regulates Cofilin phosphorylation through Cdc42 and Rac1 by two different mechanisms. **Discussion:** Wnt5a is a synaptogenic factor whose expression increases during development. Synaptogenic factors have been described as inductors of plastic changes through the activation of different signaling pathways. Wnt5a was shown to activate the non-canonical planar cell polarity pathway, essential for neuronal morphogenesis in which the small Rho GTPases are included. Additionally, Wnt5a has been described as a promoter of dendritic spines formation in hippocampal neurons. In the present study we evaluated the hypothesis that treatment with Wnt5a regulates small Rho GTPases, as controls the dendritic spines morphology through the actin cytoskeleton remodeling. Our results suggest that Wnt5a controls actin cytoskeleton dynamics at synaptic level through the modulation of the small Rho GTPases as a part of the underlying mechanism of the postsynaptic function of Wnt5a.

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Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.23/DP05/B57

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
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Support: JSPS KAKENHI (B) 19H03336
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Title: Thalamo cortical synaptic network in motor cortex analyzed with large volume electron microscopy

Authors: *Y. KUBOTA[1,2], J. SOHN[1,3], E. KURAMOTO[4], Y. KAWAGUCHI[1,2];
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Abstract: It is well known that the cortex receives signals from the basal ganglia and cerebellum via thalamo-cortical afferents. About 4 - 16% of the thalamo-cortical excitatory afferents make synaptic contacts on spines each of which is also postsynaptic to an inhibitory terminal, dually innervated spine (DiS) (J Neurosci, 2007, 27, 1139-1150). Our previous morphological and physiological analyses (eLife, 2015, elife 4) as well as optogenetic methods demonstrated that the inhibitory synapse to the DiS can effectively veto the local excitatory synaptic inputs. Furthermore, the inhibitory synapses on the DiS display more dynamic properties than the inhibitory synapses on the dendritic shaft (Neuron, 2016, 90: 662-664). One of the important functions of the thalamo-cortical system is to provide feedforward inhibition. We showed that FS basket cells receive thalamo-cortical innervation on their soma, and believe that this link is likely involved in feedforward inhibition (Cereb Cor, 2016, 26: 2689-2704).
To understand this intriguing cortical microcircuit architecture further, we investigated how the thalamo-cortical axon terminals participate in the cortical microcircuit in rat frontal cortex. The axon arborization pattern across cortical layers varies significantly among thalamocortical afferents from three motor-related thalamic nuclei: the ventral medial nucleus (VM), the ventral anterior (VA) and the ventral lateral (VL) thalamic complex, which relay motor information from the basal ganglia (VM/VA) and the cerebellum (VL), respectively (2015, *Cereb Cor* 25: 221-235). We hypothesized that the synaptic connections of VM/VA and VL afferents in the cortical microcircuits are different. A viral vector (AAV pal-GFP) was injected into each of three motor-related thalamic nuclei. Their target structures in the motor cortex was investigated using a correlated light and electron microscopy (CLEM) with a laser confocal microscopy and automated tape-collecting ultramicrotomy (ATUM) with scanning electron microscopy (SEM). To identify the GFP labeled axonal fibers subsequently at the electron microscopy, we stained blood vessels with lectin, and cellular nuclei with DAPI, and used them as landmarks in cortical tissue sections. Firstly, images were taken with a laser confocal microscopy. Then the tissue sections were embedded in plastic and sectioned with ATUM for SEM observation. GFP-labeled thalamo-cortical fibers and their target structures were identified in serial electron micrographs, and reconstructed three-dimensionally. Our preliminary results indicated that the VA fibers mainly targeted dendritic spines of the layer 5 pyramidal cell.

Disclosures: **Y. Kubota:** None. **J. Sohn:** None. **E. Kuramoto:** None. **Y. Kawaguchi:** None.

Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 369.24/B58

Topic: B.06. Synaptic Transmission

Support: NIH Grant R01 MH107182

Title: ANK3 190 kDa isoform protein palmitoylation mediates dendrite and spine morphogenesis and is altered in response to lithiumdendrite and spine morphogenesis and is altered in response to lithium

Authors: *N. H. PIGUEL[1,3], S. YOON[1,3], F. I. DE SIMONE[4], S. S. SANDERS[5], G. M. THOMAS[6], P. PENZES[1,3,2];
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Abstract: The *ANK3* gene, encoding the protein ankyrin-G (AnkG), is associated with a variety of neuropsychiatric and cognitive disorders including bipolar disorder, autism spectrum disorder, and schizophrenia. These diseases are characterized by abnormal dendritic and synaptic architecture. AnkG is a multifunctional scaffold protein with several isoforms: The 480 kDa and 270 kDa isoforms have roles at the axon initial segment and node of Ranvier, but the function of the 190 kDa isoform (AnkG-190) is less well understood. Moreover, these isoforms are regulated by palmitoylation, but palmitoylation of AnkG-190 has not been investigated in neurons. Here we show that AnkG is required for normal dendrite and spine architecture in vivo and that AnkG-190 stabilizes pyramidal neuron dendrites. We found that Cys70 palmitoylation stabilizes AnkG-190 in spine heads and at dendritic plasma membrane nanodomains, and is necessary for the maintenance of normal spine density, dendrite arborization, and for correct microtubule dynamics. Lithium, a commonly used mood stabilizer, reverses spine and dendrite deficits induced by AnkG knockdown in a manner that is dependent of palmitoylation. Finally, we found that lithium reduces AnkG-190 palmitoylation and increases its mobility in spines. Taken together, our data reveal a novel mechanism regulating dendritic architecture and mood stabilizer action on palmitoylation of an important psychiatric disorder risk factor.

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Poster

369. Synaptogenesis and Activity-Dependent Development III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: B.06. Synaptic Transmission

Support: National Institute of Mental Health
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Start-up fund from Peking University Shenzhen graduate school and Shenzhen Institute of Health Sciences (to B. Zhang)

Title: Neuroligins maintain clustering of AMPA receptors at a central synapse

Authors: *Y. HAN[1], L. Y. CHEN[2], T. C. SUDHOF[3], B. ZHANG[4];
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Abstract: The efficiency of synaptic transmission is largely determined by the composition of postsynaptic neurotransmitter receptors and its distribution. Accumulating evidence suggests that neuroligins (Nlgns) are critical for alpha-amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid receptors (AMPARs) mediated synaptic transmission *in vivo*. However, how Nlgns regulate postsynaptic AMPA receptors *in vivo* is still not fully understood. Electrophysiological recording from the principal neurons in the medial nucleus of the trapezoid body (MNTB) slice of the parvalbumin-specific conditional knockout Nlgn1 and Nlgn3 (Nlgn1/3) mice showed slower kinetic and smaller amplitude of AMPARs-mediated miniature excitatory postsynaptic current (mEPSC) from postnatal day (P) 12-13 MNTB neurons compared to control while viral-mediated Nlgn1/3 knockout reduced amplitude without altering the kinetics of mEPSC from P8-9 MNTB neurons. Furthermore, Nlgn1/3 knockout did not alter the level of postsynaptic scaffold protein Homer-1. Our computational modeling suggested that increasing the inter-GluAs distance at the postsynaptic site and reducing GluAs content caused the slower kinetics and smaller amplitude of mEPSC. Thus, our results suggest that Nlgn1/3 maintain high synaptic transmission efficiency via controlling the content and clustering of postsynaptic AMPARs at a central synapse.

Disclosures: Y. Han: None. **L.Y. Chen:** None. **T.C. Sudhof:** None. **B. Zhang:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.01/B60

Topic: B.10. Epilepsy

Support: MH020065
GM100768
AG051513
Donald F. Steiner Scholarship Fund
Iowa Neuroscience Institute Fellowship

Title: Using *drosophila* models to study PNPO deficiency and PNPO mutations identified in epilepsy patients

Authors: *W. CHI[1], A. S. R. IYENGAR[2], M. ALBERSEN[3], W. LIU[4], M. BOSMA[3], N. VERHOEVEN-DUIF[3], C.-F. WU[2], X. ZHUANG[1];
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Abstract: Gene *pyridox(am)ine 5'-phosphate oxidase* (*PNPO*) encodes a rate-limiting enzyme in the synthesis of pyridoxal 5'-phosphate (PLP), which is the biologically active form of vitamin B6 and a co-factor required for the synthesis of several neurotransmitters including GABA. Mutations in *PNPO* were initially identified in neonatal epileptic encephalopathy patients and recently also in early-onset epilepsy. *PNPO* is also regarded as one of the sixteen epilepsy genes involved in the common epilepsies. In contrast to the increasingly recognized significance of PNPO deficiency in epilepsy, our understanding of the neurobiological mechanisms of PNPO deficiency is limited, and PNPO mutations have never been studied systematically due to the lack of animal models. Based on a nutritional conditional lethal phenotype, we have previously identified a *Drosophila PNPO* gene (*sugarlethal*, *sgll*) and a hypomorphic *sgll* allele ($sgll^{95}$). Here we report that PNPO deficiency causes seizures in flies as it does in humans, and that seizure patterns in these flies resemble that in wild-type flies caused by GABA blockade. Moreover, seizures are correlated with low internal PLP levels and can be rescued by ubiquitous expression of wild-type human PNPO, demonstrating that human and *Drosophila* PNPOs are functionally conserved. Based on that, we further generated four knock-in (KI) fly strains using CRISPR/Cas9. In each strain, *Drosophila sgll* gene was replaced by human WT *PNPO* cDNA or one of three mutant *PNPO* cDNAs: D33V, R116Q, and R95H. We have found that severe hPNPO deficiency leads to lethality in early development, intermediate hPNPO deficiency results in conditional lethality and seizures, whereas mild hPNPO deficiency shortens lifespan. At the molecular and cellular level, different hPNPO mutations reduce its protein stability, change its protein cellular localization, or affect its transcription. Lastly, we report a dominant-negative effect of R95H mutant hPNPO on WT hPNPO protein. Our fly models can be used to study PNPO biology and treatment options for PNPO deficiency. These studies also indicate that *Drosophila* can be used as a diagnostic tool to examine the functional consequences of newly identified *hPNPO* mutations.

Disclosures: **W. Chi:** None. **A.S.R. Iyengar:** None. **M. Albersen:** None. **W. Liu:** None. **M. Bosma:** None. **N. Verhoeven-Duif:** None. **C. Wu:** None. **X. Zhuang:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.02/B61

Topic: B.10. Epilepsy

Support: NIH F31 NS08002
NIH R01 AG051513
Carver Collaborative Project, Univ. Iowa

Title: Alterations of an evolutionarily conserved electroconvulsive seizure-flight motor sequence by two classes of hyperexcitable *Drosophila* mutations

Authors: *A. IYENGAR, C.-F. WU;
Univ. of Iowa, Iowa City, IA

Abstract: Studies of hyperexcitable behaviors in *Drosophila* mutants have yielded insights into the role of epilepsy genes in nervous system function. In flies, high frequency stimulation across the brain triggers a stereotypic sequence of electroconvulsive seizure (ECS) discharges, consisting of an initial seizure discharge (ID), period of paralysis, a delayed seizure discharge (DD), followed by eventual recovery of the fly. These seizure discharge episodes manifest across the nervous system and can be assessed through spiking activity (peak firing rate > 30 Hz) in the large, indirect flight muscles, the Dorsal Longitudinal Muscles (DLMs), in conjunction with microphone recordings of wing beats. During flight, the DLM motor neuron spikes (~5 Hz) provide Ca^{2+} influx for stretch-activated myogenic contractions which power wing beats (~200 Hz).

Unexpectedly, in tethered wild-type (WT) flies with a strictly isolated stimulation configuration, we found that WT flies would reliably display sustained flight activity following DD, with DLM spiking (~ 5 – 10 Hz) and wing beats (~170 Hz). Indeed, this motor sequence was present across a number of *Drosophila* species including *melanogaster*, *robusta*, and *virilis*, divergent by ~60 million years. Ordinary air-puff triggered flight requires intact antennal and haltere sensory inputs. However, DD-evoked flight was present after surgical removal of these organs, suggesting a mode of flight pattern generator activity independent of sensory feedback.

Using Poincaré plots of adjacent inter-spike intervals and related non-linear dynamical systems analyses to produce a spike pattern 'signature', we examined DLM spiking during ECS discharges in two classes of hyperexcitable mutants: 'shakers' (e.g. *Sh*, *qvr*) whose legs twitch under ether anesthesia, and 'bang-sensitives' (e.g. *eas*, *sda*) which display mechanical shock-induced seizures. In 'shaker' mutants, with disrupted I_A K^+ currents, the DD-evoked flight sequence was largely intact, but with a reduced and dwindling wing beat frequency, presumably reflecting alterations direct flight muscle motor programs. In contrast, bang-sensitive mutants

which display distinctive mutant-specific DLM ECS discharge patterns, with varying duration, firing frequency and regularity. Strikingly, a common feature across the bang-sensitive mutants was the complete absence of DD-evoked flight.
Together, these findings highlight the range of mutations-specific vulnerabilities in motor circuits which could be quantitatively characterized to provide signatures of their alterations of nervous system function.

Disclosures: **A. Iyengar:** None. **C. Wu:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.03/B62

Topic: B.10. Epilepsy

Title: Novel cellular and zebrafish model system of DNM1 epileptic encephalopathy

Authors: *L. LLACI, G. C. MILLS, R. PANDEY, R. GUPTA, E. FRANKEL, C. BILAGODY, R. C4RCD, V. NARAYANAN, S. RANGASAMY;
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Abstract: Next generation sequencing (NGS) has led to the identification of several causal genes in individuals with epileptic encephalopathies (EE), and the list has expanded dramatically. Recently, mutations in the DNM1 gene, encoding Dynamin 1 (OMIM: 602377) have been recognized to cause early infantile epileptic encephalopathy-31 (OMIM: 616346). At the Center for Rare Childhood Disorders (C4RCD), we have assembled a cohort of EE patients with de novo heterozygous mutations in dynamin 1 (DNM1) and established patient-derived (skin biopsy) fibroblast cultures to understand the cell biology of DNM1 EE. Patient-derived DNM1 mutant fibroblast cells demonstrate altered phosphorylation levels of the Dynamin 1 protein, and an impaired clathrin mediated endocytosis (CME), showing that DNM1 mutations cause a functional deficit. Surprisingly, mitochondrial dysfunction (complex I or IV deficiency) was the primary finding in two of the patients in our cohort. Muscle biopsy from these patients confirmed mitochondrial dysfunction, and this remained the neurological diagnosis for over ten years, until the discovery of the primary DNM1 mutation. We observed a significant decrease in the ATP generation and mitochondrial spare capacity in DNM1 mutant patient cells compared to control cells. Collectively, our results demonstrate mitochondrial involvement in the DNM1 EE. Interestingly, the dynamin superfamily members share a conserved GTPase domain, which plays a critical role in mitochondrial fission and fusion. However, mammalian Dynamin 1 has never been linked to mitochondrial function, and our results suggest a mechanistic link between mitochondrial function and DNM1. We also created a zebrafish model of epilepsy by inhibiting DNM1 via a chemical inhibitor. Further, through microinjection of human mutant DNM1

(p.Gly43Asp) mRNA at 72-hr post-fertilized embryos, we also recapitulated the seizure-like activity observed in patients. Screening of FDA approved anti-epileptic drugs (AEDs) led to the identification of a new compound (C4RCD-1) as a potential therapy for DNM1 induced seizures. In summary, we identified a novel association of DNM1 EE with mitochondrial dysfunction using a patient derived cell model. Furthermore, we developed a genetic DNM1 model of zebrafish which will advance epilepsy treatment through development of personalized therapy.

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Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

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Program #/Poster #: 370.04/B63

Topic: B.10. Epilepsy

Support: ISCBIF

Title: Sestrin3 and seizures after traumatic brain injury

Authors: *Q. ZHOU[1], Z. LEI[2], L. ZHOU[4], X. DONG[3], Z. C. XU[5];
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Abstract: Traumatic brain injury (TBI) is one of the major causes of epilepsy. However, no effective treatment of post traumatic epilepsy (PTE) is currently available. The mechanisms underlying PTE are under active investigation. Recent studies indicate that Sestrin 3 (Sesn3), a highly conserved protein encoded by the Sesn3 gene whose expression is upregulated in cells exposed to environmental stresses, Sesn3 has been recently identified as a regulator of a pro-convulsant gene network. It is conceivable that the Sestrin3 might be involved in seizure generation following TBI. The present study attempts to reveal the role of Sestrin 3 in PTE and the potential mechanisms. TBI was produced in WT and Sesn3 KO mice using controlled cortical impact method. Seven days after TBI, seizures were induced with Pentylenetetrazole (PTZ, 40mg/kg. i.p). One day after seizure induction, the animals were sacrificed and brain slices were prepared for electrophysiological studies. Whole cell patch-clamp recording was performed on granule cells in hippocampus. Passive membrane properties and miniature excitatory postsynaptic current (mEPSC) were compared in different experimental groups. The seizure rate increases from 60% in naïve animals to 71% after TBI and further increases to 100% in Sesn3

KO mice after TBI, suggesting that Sesn3 has negative impacts on seizure generation after TBI. The spike threshold, the amplitude of afterhyperpolarization and rheobase in granule cells significantly increases in KO mice after TBI as compared with the naïve ones, suggesting the excitability of granule cells in Sesn3 KO mice is reduced after TBI. The frequency of mEPSCs in granule cells increases after TBI as compared with the naïve animals but return to control levels in Sesn3 KO mice. These results suggest that Sesn3 might reduce seizure generation by increase of granule cell excitability after TBI. However, depletion of Sesn3 reduces the mEPSC frequency after TBI.

Disclosures: Q. Zhou: None. **Z. Lei:** None. **L. Zhou:** None. **X. Dong:** None. **Z.C. Xu:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.05/B64

Topic: B.10. Epilepsy

Support: NIH Grant NS084959

Title: Genetic modifiers influence survival in a mouse model of Dravet syndrome

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Abstract: Epilepsy is a common neurological disorder that affects 1% of the worldwide population. Pathogenic variants in voltage-gated sodium channel genes are the most common cause of monogenic epilepsies. More than 1,600 pathogenic variants in *SCN1A* have been reported in individuals with several epilepsy syndromes. The clinical phenotype most frequently associated with *SCN1A* is Dravet syndrome, which often results from *SCN1A* haploinsufficiency. Mice with heterozygous deletion of *Scn1a* (*Scn1a*$^{+/-}$) recapitulate core features of Dravet syndrome, including spontaneous seizures, seizures provoked by hyperthermia, and sudden unexpected death. These phenotypes have prominent strain-dependence that is reminiscent of variable expressivity observed in patients with monogenic epilepsies. *Scn1a*$^{+/-}$ mice on the [129S6/SvEvTac x C57BL/6J]F1 strain exhibit spontaneous seizures and high mortality, whereas these phenotypes are masked on the 129S6/SvEvTac (129) strain. This suggests that 129 contributes protective alleles, while C57BL/6J (B6) contributes risk alleles. Previously, we identified modifier loci on chromosomes 5, 7, 8 and 11 that influence strain-dependent severity of the *Scn1a*$^{+/-}$ Dravet phenotype. The objective of the current study was to refine the map interval and perform candidate gene analysis for the *Dsm5* (Dravet survival modifier) locus on

mouse chromosome 11.
A series of B6.129-Chr11 interval-specific congenic (ISC) strains were generated and crossed with 129.*Scn1a*$^{+/-}$ mice. Resulting offspring were homozygous 129/129 or heterozygous 129/B6 in the interval of interest. Survival to 8 weeks of age was monitored in male and female *Scn1a*$^{+/-}$ offspring and compared between ISC genotypes. Analysis of six ISC strains with overlapping intervals narrowed the modifier locus to a <14 Mb region ($p<0.03$, LogRank). This interval contains ~600 genes, of which 115 are expressed in brain. RNA-seq analysis identified 7 genes with differential brain expression between the 129 and B6 strains. In addition, the region contains 22 genes with moderate-high impact coding sequence variants. Among the protein coding genes with strain-dependent differences, 13 have a prior association with epilepsy or seizures. Furthermore, this interval contains 5 miRNA genes with seed matches in the *Scn1a* 3'UTR, suggesting potential regulation of *Scn1a* transcript. Strain-dependent expression differences were recently reported for some of these miRNAs, and several have been shown to be upregulated by seizures. Identification of modifier genes that influence severity of Dravet syndrome may advance our understanding of disease pathogenesis and suggest novel therapeutic approaches.

Disclosures: N.A. Hawkins: None. **L.D. Copeland-Hardin:** None. **S.E. Duarte:** None. **J.A. Kearney:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

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Program #/Poster #: 370.06/B65

Topic: B.10. Epilepsy

Support: Citizen United for Research in Epilepsy (CURE) Innovator Award

Title: A CRISPRi screen in human iPSC-derived iNeurons to discover novel focal cortical dysplasia genes

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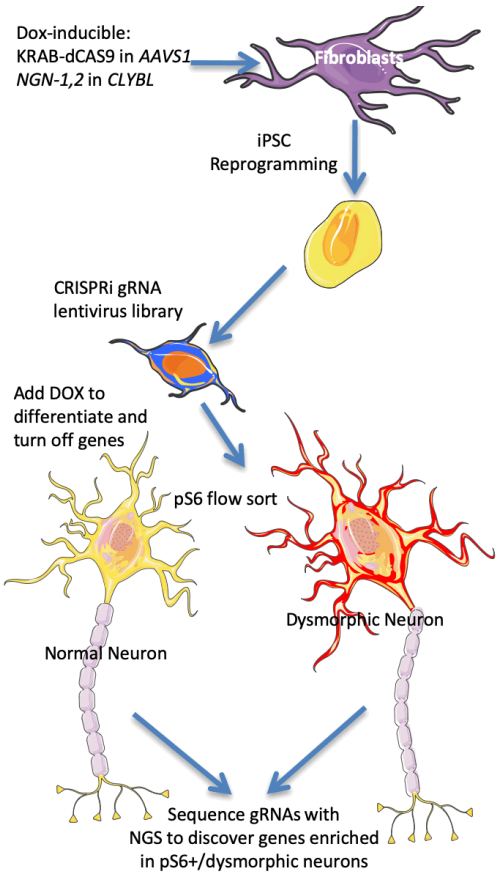
Abstract: Focal cortical dysplasia (FCD) is a common cause of focal epilepsy and often results from somatic, mosaic mutations. The sparse nature of mutated cells (1-6% of cells in surgically resected tissue) presents many experimental barriers to FCD gene discovery, and only a small number of FCD genes have been identified based upon hypothesis-driven targeted sequencing. Therefore, we have developed an unbiased screening platform for identifying novel FCD genes ***in vitro*** using a genome-wide CRISPR interference library. We first reprogrammed human iPSC

lines stably expressing both KRAB-dCAS9 and Neurogenin (*NGN*) 1,2 genes under control of a doxycycline (DOX)-inducible promoter. The inducible expression of NGN1,2 allows for the efficient, uniform differentiation of human excitatory cortical neurons. The KRAB-dCAS9 inhibits the expression of genes targeted by a guide RNA (gRNA) sequence introduced via lentivirus, with 80-95% knockdown of mRNA and protein after DOX treatment in prior studies. We are using phosphorylated S6 ribosomal protein (pS6), a biomarker for FCD type II, as the selection assay in our screen. Using a pS6 antibody and FACS sorting, we were able to obtain genomic DNA from pS6-high and -low cell populations. To validate our screen, we constructed a test library of 12 genes that were expected to be positive, negative, or neutral regulators of pS6. The pS6-sorted neuronal genomic DNA samples were amplified using primers flanking the gRNA portion of the lentiviral insertion. The abundance of each gRNA sequence was then assessed using NGS. As expected, the gRNAs for FCD genes were significantly enriched in the pS6-high sample while gRNAs for genes necessary for S6 phosphorylation were significantly enriched in the pS6-low sample. A genome-wide gRNA library containing 5 unique sequences for each human gene (>100,000 total gRNAs) is now being applied to identify novel genes. Validated FCD gene candidates will offer additional targets for sequencing from patient tissues and the potential for new therapeutic strategies for FCD-associated epilepsies.



Disclosures: **A.M. Tidball:** None. **J.L. Margolis:** None. **T.W. Glenn:** None. **G.L. Carvill:** None. **J.M. Parent:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.07/B66

Topic: B.10. Epilepsy

Title: Genomic dissection of the 5p15-q12 locus implicates the CDC20B gene in a juvenile myoclonic epilepsy family

Authors: *P. BARAK[1], A. ANAND[1], M. KAUR[1], S. SINHA[2], P. SATISHCHANDRA[2], G. KURUTTUKULAM[3];
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Abstract: Juvenile Myoclonic Epilepsy (JME) is a common genetic generalized epilepsy characterized by frequent myoclonic jerks which are accompanied by generalized tonic-clonic seizures and absence seizures. Linkage studies have identified about 30 sub-genomic locations which may harbor genes underlying JME. Here, we present, whole genome-wide linkage analysis of a multi-affected JME family which led to the identification of a novel locus that maps to chromosome 5p15-q12. Whole exome-based sequencing study for affected members of this family helped identify a potentially causative gene, *CDC20B* (Cell Division Cycle 20B). In addition, four rare (MAF<0.005), missense *CDC20B* variants present almost exclusively among a cohort of over 500 JME patients were found. *CDC20B* encompasses 60 kilobase of the genome and encodes a 591 amino acid protein. It has 7 WD repeat domains at its C-terminus. *CDC20B* is paralogous to the *CDC20* gene, which is known to interact with anaphase promoting complex and has a major role in cell cycle progression. Cellular and molecular roles of CDC20B are beginning to be examined. Cellular expression and localization of CDC20B protein has been studied in mammalian cultured cells. While during late telophase and cytokinesis, the protein localizes to the midbody, during other cell cycle stages, it is present in the cytoplasm. CDC20B co-immunoprecipitates with gamma tubulin, a protein present abundantly at midbody during cytokinesis. The missense mutations identified in the JME patients do not seem to alter expression and localization of the CDC20B proteins in mammalian cells. Over-expression of the mutants in HeLa cells led to the accumulation of cells at the cytokinesis stage, suggesting cell cycle associated roles for the protein.

Disclosures: P. Barak: None. **A. Anand:** None. **M. Kaur:** None. **S. Sinha:** None. **P. Satishchandra:** None. **G. Kuruttukulam:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.08/B67

Topic: B.10. Epilepsy

Title: Precision medicine in FGF12 related developmental and epileptic encephalopathy

Authors: *S. SEIFFERT[1], N. SCHWARZ[1], K. HELBIG[2,3], I. HELBIG[2,3,4,5], M. PENDZIWIAT[4], Y. WEBER[1,6];
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Abstract: Voltage-gated sodium channels (Navs) are crucial players of neuronal function, and mutations in genes encoding CNS Navs (Nav1.1 [*SCN1A*], Nav1.2 [*SCN2A*], Nav1.3 [*SCN3A*], and Nav1.6 [*SCN8A*]) are a cause of some of the most common and severe genetic epilepsies and developmental and epileptic encephalopathies (DEE). Fibroblast-growth-factor homologous factors (FHFs) compose a family of 4 proteins that interact with the C-terminal tails of Navs and enhances the kinetics of channel inactivation. Even though mutations have been identified in FGF13, to date only one recurrent mutation in FGF12 p.R52H was reported as a cause for early-onset DEE inducing a strong gain-of-function due to an interaction with the cytoplasmic tail of Nav1.6 (*SCN8A*). We performed an exome sequencing study in 395 patient-parent trios with DEE and could detect a novel *de novo* variant in FGF12 (p.G50S). This variant is located two amino acids upstream of the already known variant. We actually characterize the effect of variant by co-transfecting ND7/23 cells together with Nav1.6. Another aim of this study is to identify potential precision medicine e.g. sodium channel blockers to compensate the effect of the mutation. Additionally, there is a *FGF12* duplication case reported with early-onset DEE. If it is possible, we will extend our study to understand the functional consequence of the FGF12 duplication on the Nav1.6 channel, and to find a precise therapeutic intervention.

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Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.09/B68

Topic: B.10. Epilepsy

Support: AES Predoctoral Fellowship

Title: lncRNAs modulate MAPK signaling in human neocortical epilepsy

Authors: *A. KIRCHNER[1], S. ZARINEBEF[1], F. DACHET[1], L. LIPOVICH[2], J. A. LOEB[1]; [1]Univ. of Illinois at Chicago Col. of Med., Chicago, IL; [2]Wayne State Univ., Detroit, MI

Abstract: BACKGROUND: One third of all epileptic patients are resistant to current drug treatments. To develop improved therapeutics, the molecular basis of epilepsy needs to be better understood. Previous studies from our group have compared high versus low spiking from human epileptic neocortical regions as an unbiased approach to identify new molecular targets. We previously identified the Mitogen Activated Protein Kinase (MAPK) pathway as highly upregulated in epileptic brain and showed in parallel rodent studies that MAPK inhibition prevented the development of epileptic spiking.Given the ubiquitous presence of MAPK signaling, a brain specific regulator of MAPK signaling could be a useful therapeutic avenue that would limit peripheral side effects. In addition to looking at genes that code for proteins, we examined both the differential expression and potential regulatory roles of non-protein coding, long non-coding RNAs (lncRNAs) in the human brain. **OBJECTIVE**: To identify lncRNAs that are either regulated by or regulate MAPK signaling in human epilepsy and could be translated into targeted epilepsy therapeutics by reducing MAPK selectively in the brain. **METHODS**:Differentially expressed MAPK genes and lncRNA genes were identified using genome-wide protein coding as well as customized lncRNA microarrays by comparing high versus low spiking regions of human neocortex from 8 epilepsy patients studied with long-term intracranial recordings. Clustering of the differentially expressed genes was used to identify lncRNAs that demonstrated the same expression patterns as the differentially expressed MAPK genes. Mechanistic studies were performed using Sh-SY5Y cells following depolarization and using specific MAPK signaling modulators. Human epileptic tissues were used to localize lncRNAs in the neocortex. **RESULTS**: Clustering of the transcriptomic data comparing the differentially expressed lncRNA and MAPK genes showed that 4 lncRNAs were significantly co-expressed with MAPK genes. Expression of 2 of these lncRNAs were activity dependent and induced with depolarization of the Sh-SY5Y cells that could then be blocked with MAPK inhibitors, suggesting these lncRNAs are regulated by activity and MAPK signaling. lncRNAs were spatially located nearby MAPK genes, suggesting that they may regulate the expression of MAPK genes at the transcriptional level and dependent on their relative genomic positions.

CONCLUSION: lncRNAs spatially positioned near MAPK genes could play an important regulatory role of MAPK induced synaptic plasticity needed to promote epileptic circuits in the epileptic brain and could have potential uses as targets to combat epilepsy.

Disclosures: A. Kirchner: None. **S. Zarinebef:** None. **F. Dachet:** None. **L. Lipovich:** None. **J.A. Loeb:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.10/B69

Topic: B.10. Epilepsy

Support: R01 NS095368
R01 NS084142

Title: Dynamics sustaining focal seizures: A dual function of inhibition and interaction across scales

Authors: *W. VAN DRONGELEN[1], A. K. TRYBA[2], T. PHAM[3], E. M. MERRICKS[4], A. BHANSALI[5], L. PESCE[3], S. CHO[3], S. LEE[3], T. EISSA[6], D. R. NORDLI, Jr.[3], C. A. SCHEVON[7];
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Abstract: Multi-electrode array recordings of human focal seizures showed distinct territories in a mm-sized cortical area: a core and penumbra, separated by a slowly propagating ictal wave(1. Correlation between spiking in this small area of hyperexcitation and the surrounding network's electrical activity was shown to be significant over a surprisingly extended, cm-sized area(2. Furthermore, while inhibition fails at the mesoscale, inhibitory function is crucial for the hypersynchronous oscillatory signal in the macroscopic recordings of brain electrical activity (2. Paroxysmal depolarization (PD) in the setting of inhibitory failure is a critical factor in sustaining focal seizures, and we describe supporting experimental, clinical, and modeling evidence. We present dual patch recordings in cortical cultures showing a reduction of synaptic transmission at presynaptic occurrence of PD (n=5). We find that PD is a cell-size related phenomenon causing smaller inhibitory cells to be more susceptible than larger excitatory ones (n=8). We further find that optically-evoked PD activity in parvalbumine neurons promotes propagation of activity in cortical networks. At the mesoscopic level, spike sorting results from microelectrode array measurements around ictal wave propagation in three patients with epilepsy demonstrate a strong

increase in putative inhibitory firing, followed by a sudden reduction of firing at wavefront passage. At the macroscopic level we summarize evidence that the excitatory ictal wave activity acts as a perturbator evoking oscillatory activity across a cm-sized cortical network, and that inhibitory function is a crucial modulator. Macroscopic oscillations are time-locked with the ictal wave's spikes but not necessarily with associated PDs. The above findings motivated us to simulate a network with neurons governed by a reduced version of the Hodgkin-Huxley formalism, to show how feed forward, feedback and local failure of inhibition contribute to the dynamics across network scales.
We conclude that the multidisciplinary data on the role of PD supports our hypothesis that it is not only a cellular marker of epileptiform activity, but actively contributes to the emergent seizure.
([1]Schevon et al (2012) *Nat Commun* **3**:1060.
([2]Eissa et al (2017) *PNAS* **114**:10761

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Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.11/B70

Topic: B.10. Epilepsy

Support: Austrian Science Fund (FWF) Grant P 26680

Title: Permanent functional silencing of hippocampal parvalbumin- or somatostatin-interneurons induces epilepsy

Authors: *M. DREXEL, A. BUKOVAC, R. O. TASAN, G. SPERK;
Dept. of Pharmacol., Med. Univ. of Innsbruck, Innsbruck, Austria

Abstract: Signal transduction in the hippocampus is based on excitatory neuronal pathways (trisynaptic circuitry of the hippocampus). Excitatory transmission is under the tight control of inhibitory interneurons. More than 20 subtypes of such interneurons have been characterized by their anatomy, neurochemical markers, and functional properties. Among these, (1) parvalbumin expressing basket cells exerting potent *feed-forward* inhibition upon pyramidal cell somata and (2) somatostatin-containing interneurons projecting from the stratum oriens to pyramidal cell dendrites in the stratum lacunosum moleculare (O-LM cells) and exerting potent *feed-back* inhibition are functionally especially prominent. Malfunctioning of either of these neurons has been proposed to cause epilepsy. In our experiments, we functionally silenced both subtypes of

interneurons and investigated the development of epileptic seizures. We used transgenic male mice expressing the enzyme Cre-recombinase either on the parvalbumin or somatostatin promoter. We locally injected a viral vector into the subiculum/CA1 of the mice expressing tetanus toxin light chain/GFP in a Cre-recombinase dependent manner. This treatment resulted in a selective functional silencing (inhibition of GABA release) of these neurons without damaging them. Control mice were injected with a viral vector expressing GFP only. We characterized the selectivity of tetanus toxin expression in the respective neurons and the functional impairment of GABA release by immunohistochemical and electrophysiological experiments. Both, silencing of parvalbumin-containing and somatostatin-containing GABA neurons resulted in epileptic discharges and spontaneous motor seizures in the mice and were monitored for up to three months by continuous telemetric EEG and video monitoring. The seizure threshold was already reduced before the first motor seizure (after about one week) was observed. Spontaneous seizures developed only after permanent silencing of parvalbumin or somatostatin interneurons but not after their selective transient inhibition. Furthermore, the frequency of spontaneous seizures declined after 4 to 6 weeks. At the same time, neuropeptide Y (NPY) expression increased and that of the cannabinoid receptor CB1 declined, indicating possible development of endogenous protective mechanisms.

Disclosures: **M. Drexel:** None. **A. Bukovac:** None. **R.O. Tasan:** None. **G. Sperk:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.12/B71

Topic: B.10. Epilepsy

Support: NIH R01NS069861 & R01NS097750

Title: Presynaptic GABA-B receptor modulation of hippocampal dentate network excitability during cortical UP states in an experimental model of epilepsy

Authors: *D. SUBRAMANIAN[1], S. RATNADURAI-GIRIDHARAN[2], V. SANTHAKUMAR[1];
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Abstract: Cortical UP/Down states (UDS) represent periods of highly synchronized epochs of excitability, followed by periods of neuronal quiescence occurring physiologically during NREM sleep and deep anesthesia (Steriade, 1993). While functionally UDS are thought to be critical for memory consolidation, the hippocampal subfields are barely influenced by UDS, most likely due to the strong gating function of the Dentate gyrus (DG) (Ouedraogo, 2016). However, this gating function of DG is severely compromised following status epilepticus (SE) mediated cell loss and

network reorganization leading to DG granule cell firing during UP states which could impact hippocampal information processing and development/propagation of seizure activity (Ouedraogo, 2016, Flynn 2015). GABA-B auto-receptors, known to augment DG-granule cell firing through disinhibition (Foster, 2014), are upregulated in epileptic animals (Straessele, 2003). Since disinhibition has the potential to compromise the dentate gate, we evaluated the role of presynaptic GABA-B receptors in modulating DG network excitability during cortical UDS. Evoked and spontaneous local field potentials (LFP) were recorded in the dentate and cortex of urethane anesthetized male Wistar rats 1 week, 4-8 weeks and 8-24 weeks after pilocarpine induced SE. Age matched, saline injected rats were used as controls. Labchart and custom Matlab codes were used for analysis. On stimulation of perforant path inputs, post-SE rats displayed enhanced population spike/slope ratio and reduced paired pulse ratio (to stimuli 20 ms apart) compared to controls ($p=<0.05$) indicating greater network excitability and robust feedback inhibition after SE. DG-LFP periodically switched between epochs of large-irregular activity and high amplitude slow wave oscillations with distinct UP/Down states during which dentate spikes were frequently observed during UP states. Evaluating the total number of dentate spikes within a 10 min period revealed a significant increase in post-SE rats compared to controls animals in all time points examined ($p= <0.001$). Selectively blocking presynaptic GABA-B receptors increased the total number of dentate spikes in controls ($p=0.011$), but failed to do so in post-SE rats ($p=0.851$). These data suggest changes in presynaptic GABA-B receptor mediated regulation of dentate network excitability after SE. Further understanding the cell and synapse specific effects of presynaptic GABA-B in dentate circuit would help resolve the role of GABA-B modulation of inhibition in DG network excitability in physiological and pathological conditions such as Epilepsy.

Disclosures: D. Subramanian: None. **S. Ratnadurai-Giridharan:** None. **V. Santhakumar:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

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Program #/Poster #: 370.13/B72

Topic: B.10. Epilepsy

Support: NIH 5R37NS077908
NIH 5R01NS034700

Title: Interneuron-driven interictal spikes in an *in vitro* model of post-traumatic epilepsy

Authors: *K. P. LILLIS, L. A. LAU, K. J. STALEY;
Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Interictal spikes are a diagnostic feature of EEG recordings from epileptic patients, but their role in ictogenesis and epileptogenesis remains elusive. Understanding the basic circuit and molecular mechanisms underlying interictal spikes will offer new insight into how the epileptic brain can transition to pathological activity but isolating key elements responsible for the generation of network rhythms is technically challenging. Mounting evidence from *in vivo* recordings, as well as chronic and acute brain slice recordings, suggest that interictal spikes may be *generated* primarily by synchronous interneuron activity. In this work, we imaged the earliest emergence of interictal spikes in an *in vitro* model of post-traumatic epileptogenesis: the organotypic hippocampal slice culture. Slices were prepared from P7 DLX-cre mice that were transduced intracerebroventricularly on P0 with two AAV vectors: FLEX-tdTomato and syn-GCaMP7f, producing pan-neuronal expression of a green calcium sensor and interneuron-specific expression of a red fluorescent protein. We then used a novel imaging system constructed inside of a tissue culture incubator, to image slices continuously beginning shortly after the injury and continuing through the onset of spontaneous recurrent seizures (after ~7 days *in vitro*). Every 4 hours during this latent period, a movie of calcium dynamics with ***cellular resolution*** and a ***field of view spanning the entire epileptic network*** was acquired. For each detected interictal spike, all imaged neurons (typically n~=1000) were quantified by onset time and amplitude of calcium transient (ΔF/F). Preliminary data suggest that there is a bimodal distribution of onset times and peak ΔF/F amplitude, with the earliest firing and highest amplitude activity occurring in interneurons. Next, we sought to test the hypothesis that synchronous interneuron activation is sufficient to trigger interictal spikes. We co-transfected DLX-cre animals with FLEX-CoChR and syn-jRCaMP1a, producing pan-neuronal expression of a red calcium indicator and interneuron-specific expression of a high-current channelrhodopsin variant. We then used a digital micromirror device to pattern light to selectively stimulate a selected groups of interneurons. Preliminary data indicate that the number of interneurons stimulated is directly proportional to the amplitude of the network-wide calcium response. Together, these results suggest that the earliest synchronous activity in post-traumatic epileptogenesis is interneuron-driven interictal spiking.

Disclosures: **K.P. Lillis:** None. **L.A. Lau:** None. **K.J. Staley:** None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.14/B73

Topic: B.10. Epilepsy

Title: Direct evidence for excitatory GABAergic transmission in acute and chronic mouse models of epilepsy *in vivo*

Authors: *A. FERREIRA GOMES DA SILVA[1], O. DUBANET[1], A. FRICK[1], H. HIRASE[2], A. BEYELER[1], X. LEINEKUGEL[1];
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Abstract: Numerous in vitro studies have reported high intracellular chloride levels, depolarizing and potentially excitatory actions of GABA in various pathological conditions such as epilepsy, autism spectrum disorders, or schizophrenia. It has therefore been recently proposed that drugs restoring low [Cl^-]i may provide novel therapies for a wide range of brain disorders. However, the relevance of this hypothesis in vivo remains largely speculative because of the technical difficulty to evaluate the polarity of GABAergic transmission in vivo. Combining non-invasive extracellular detection of unitary perisomatic inhibitory postsynaptic field-potentials (fIPSPs) with silicon probe recording of the firing activity of multiple individual neurons, we were able to probe the polarity of GABAergic transmission at the population and individual cell level in the hippocampal circuit in vivo. We now provide direct evidence for depolarizing actions of perisomatic GABAergic transmission and time-locked excitation of CA3 pyramidal neurons in acute and chronic mouse models of epilepsy. This approach will also prove useful to investigate alterations in the interplay between excitation and time-locked perisomatic inhibition in pathological conditions such as neurodegenerative and neurodevelopmental disorders.

Disclosures: A. Ferreira Gomes Da Silva: None.

Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

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Topic: B.10. Epilepsy

Support: NSERC Grant - RGPIN-2015-05333
CNPq - Scholarship
FRQS - Scholarship

Title: Sex and inhibition: Role of sex hormones during the development of hippocampal GABAergic network

Authors: *D. C. WOLF[1,2], N. T. SANON[1], A. O. CUNHA[3], T. SHAKER[1,2], A.-R. ELHASSAN[1], S. A. F. NASCIMENTO[1], L. CARMANT[1], G. DI CRISTO[1,2], A. G. WEIL[1,2];
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Abstract: Sexual differentiation of the brain is influenced by testosterone (T) and its metabolites during the perinatal period in males (♂) and females (♀). This period is also critical for GABAergic network maturation and may be involved in T♀ susceptibility to seizures. Similar happens to ♂ but it is unlikely in ♀, according to our epilepsy model. Since T (endo-or-exogenous) might be involved in brain excitability, we aim to understand the development of the GABAergic network in non-epileptic rats, not only in ♂ and ♀ rats, but also in T♀ and T-insensitive ♂ rats. Sexual morphological and hormonal parameters were accessed during development. Protein expression of the chloride co-transporter KCC2 was evaluated in the hippocampus at postnatal days (P) 3, 7 and 40, whereas its membrane protein fraction was analyzed at P7 and P40. Gene expression of GABA signaling components (chloride co-transporters, glutamic acid decarboxylase (GAD), GABAA receptor (GABAAR) subunits, and GABA transporter (GAT)) were analyzed by RT-qPCR at P40. Functional aspects were determined by spontaneous inhibitory postsynaptic currents (sIPSCs) recorded in CA1 pyramidal cells at P40. We found strong effects of circulating T levels on sexual phenotypes (absence of nipples and anogenital distance). Levels of T were significantly lower in ♀($P<0.0001$, n=15/group), whereas estradiol levels were similar between sexes. Total KCC2 protein expression was not significantly different between sexes, however ♀ have higher membrane KCC2 levels compared to ♂ ($P=0.0007$, n=8) and T♀ ($P=0.0071$, n=8) at P7. At P40, membrane KCC2 levels were similar between sexes. GABAergic spontaneous currents in CA1 pyramidal cells of the hippocampus were smaller ($P=0.0313$) and less frequent in ♂ ($P<0.0001$). Kinetic parameters of sIPSCs, such as rise-time, were different between ♂ and T-insensitive ♂ rats ($P= 0.0072$, n=5-7 cells/4 animals per group). However, no differences were found in the components of GABA signaling, such as GAD65/67, GABAAR subunits (α1, α5, β2, γ2), GAT-1, NKCC1 and KCC2 at P40. Thus far, phenotypical and physiological differences between sexes are observed, namely in KCC2 expression in a specific developmental time point and in the amplitude and frequency of GABAergic spontaneous currents in the hippocampus of adult animals, suggesting possible involvement of sex hormones in cell excitability. Further analysis of the GABAergic network in these sex conditions will help to understand the sex-specificity of epileptogenesis.

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Poster

370. Epilepsy: Genetic Mechanisms and Animal Models

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 370.16/B75

Topic: B.10. Epilepsy

Support: NIH Grant R01NS060757
NIH Grant R01NS032845

Title: Spontaneous seizures can cross a transection by electric field coupling *in-vitro* and *in-vivo*

Authors: *C.-C. CHIANG[1], R. SHIVACHARAN[2], N. H. COUTURIER[3], M. SUBRAMANIAN[1], D. M. DURAND[4];
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Abstract: In epilepsy, surgical transections are performed to isolate the epileptogenic zone while keeping brain function intact. However, this technique has a poor success-rate (30-40%) and the mechanism of how seizures in the epileptogenic zone expands beyond the transection is unclear. Several experimental results have shown that 4-AP-induced epileptiform activity in rodent hippocampi propagating at a speed of ~0.1 m/s independently of synaptic transmission and gap junctions is best explained by electric field coupling. Additional studies show that electric fields can activate neighboring neurons, thereby generating a self-propagating wave that could cross a transection. Using both *in-vitro* and *in-vivo* electrophysiology, we studied the mechanism driving activity through a physical cut or transection. We provide evidence that electric fields self-generate and recruit neighboring neurons to create a self-propagating wave through a transection/cut in the hippocampus. This self-generating seizure like activity can propagate through the cut ~0.11 m/sec, which was not significantly different if there was no cut present. We further show that these endogenous fields are self-sustaining through a cut with no significant difference in the field amplitude proximal to the cut, in the cut, and distal to the cut. Moreover, we show that in *in-vivo* 4-AP acute model seizures with larger amplitude and power have higher probability to cross a complete transection of the hippocampus. These results indicate that electric fields are sufficient for driving *in-vitro* and *in-vivo* epileptiform activity and could explain the low success rate of surgical transections.

Disclosures: C. Chiang: None. **R. Shivacharan:** None. **N.H. Couturier:** None. **M. Subramanian:** None. **D.M. Durand:** None.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.01/B76

Topic: B.10. Epilepsy

Support: NIH R15 NS072879-01A1

Challenge Grant from CURE Epilepsy Foundation
Connecticut Regenerative Medicine Fund
Wesleyan University Project Grant

Title: Fetal medial ganglionic eminence-derived GABAergic progenitors transplanted into the dentate gyrus of mice with temporal lobe epilepsy regulate adult neurogenesis

Authors: *M. N. ARSHAD[1], J. JEONG[1], B. BUYUKDEMIRTAS[1], M. ADLER-WACHTER[1], F. WICKHAM[1], S. MARRA[1], S. FOX[1], J. R. NAEGELE[2];
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Abstract: In the dentate gyrus of adult hippocampus, heterogeneous populations of GABAergic interneurons or their axons localize in the vicinity of the stem cell niche and both local and long-range GABAergic neurons regulate this niche as well as the maturation of adult-born hippocampal granule cells (GCs) (Wang et al., 2005; Masiulis et al., 2011; Bao et al., 2017). Partial loss and axonal sprouting of hippocampal GABAergic interneurons are two characteristic features of the pilocarpine (PILO) model of temporal lobe epilepsy (TLE) in rodents (Choi et al., 2007; Peng et al., 2013) and these changes may be linked to aberrant neurogenesis and seizure development in temporal lobe epilepsy (TLE) (Hester & Danzer, 2014). Hippocampal transplants of GABAergic progenitors from human PSCs or fetal medial ganglionic eminence (MGE) suppress seizures in rodents with PILO-induced TLE (Henderson et al., 2014; Hunt et al., 2013; Cunningham et al., 2014; Upadhya et al., 2019 but see Anderson et al., 2018). MGE-derived GABAergic interneuron transplants migrate in the adult brain and innervate host brain neurons, including adult-born GCs (Hsieh and Baraban 2017; Upadhya et al., 2019; Cunningham et al., 2014; Henderson et al., 2014; Gupta et al., 2019). Recent work shows that transplanted MGE-derived GABAergic progenitors not only innervate adult-born GCs in TLE mice but also restrict the growth of their dendritic arbors (Gupta et al., 2019). However, the effects of MGE transplants on adult-neurogenesis have not been directly examined. We investigated the neurogenic effects of transplanting MGE progenitors harvested from ChR2 -EYFP transgenic mouse embryos (embryonic day 13.5) into the dentate gyrus of epileptic adult C57Bl/6N mice (Envigo, males 6-8 weeks of age). The number of adult-born doublecortin (DCX) neurons was quantified in the hilus, subgranular zone (SGZ), and granule cell layer (GCL). We found significant reductions in DCX-positive cells in mice with MGE transplants, consistent with the hypothesis that maturation of adult-born GCs may be inhibited by MGE transplants. To determine whether MGE transplantation impacts earlier stages of adult neurogenesis, we made systemic injections of either BrdU or EdU, thymidine analogues incorporated into DNA during S-phase of the cell cycle (Mandyam et al., 2007). In agreement with previous studies, mice with TLE show increased numbers of BrdU-labeled cells (Parent et al., 1997). However, MGE transplantation resulted in further increases in BrdU-labeling. Together, these findings suggest that MGE progenitors increase the proliferation of neural stem cells but diminish the survival or maturation of post-mitotic granule cells.

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Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.02/B77

Topic: B.10. Epilepsy

Support: CT Regenerative Medicine Fund (GA, JRN, LBG)

Title: Development of electrophysiological properties and dendritic morphologies in human pluripotent stem cell-derived GABAergic interneurons transplanted into the hippocampus of adult mice with pilocarpine-induced temporal lobe epilepsy

Authors: *S. SHRESTHA, N. ANDERSON, J. NAEGELE, L. GRABEL, G. AARON; Wesleyan Univ., Middletown, CT

Abstract: Human embryonic stem cell (hESC)-derived GABAergic interneurons are being studied for cell replacement therapies directed toward replacing damaged GABAergic interneurons in neurological disorders. Human neurons show protracted differentiation over periods of months (Cunningham *et al.,* 2014, Anderson *et al.,* 2018 Upadhya et al., 2019), but little is known about how their dendritic development is linked to electrophysiological maturation. To examine this question, we derived GABAergic progenitors from the hES3 NKX2.1: GFP/Ubi:mCherry cell line and transplanted them into the hippocampus of NOD-SCID immunodeficient mice (JAX labs) with pilocarpine-induced temporal lobe epilepsy. Four months after post-transplantation, *ex vivo* whole-cell patch clamp recordings were performed in hippocampal slices and the recorded cells were filled with biocytin for morphological analyses. Dendritic morphology was studied with IMARIS 9.2 (Bitplane) by making 3-dimensional (3D) computer-based reconstructions of 75 biocytin-filled neurons that were characterized by electrophysiology. Of these, 32 were completely reconstructed and morphological features were quantified including: soma volume, primary dendrites, total dendritic length, dendritic terminal points and dendritic complexity. We performed hierarchical clustering analyses and identified four morphologically unique clusters (Type A-D). Based on electrophysiological firing responses to current injections, five functional groups were identified (Type I-V, Anderson *et al.,* 2018). We found significant differences in action potential (AP) firing rates between groups: type I cells had immature firing patterns and type V cells had more mature firing patterns. Interestingly, 75% of the type I cells were also categorized as type C (simple morphologies) cells. Likewise, 40% of type V cells fell into the type D cluster, with complex dendritic morphologies. Six neurons had spinous dendrites and 4 of these were type D cells, consistent with this group's more mature electrophysiological and morphological properties. These findings suggest that hESC-derived interneuron progenitors differentiate at different rates following transplantation and by 4 months, some have developed complex dendritic morphology and mature firing patterns. Our

classification scheme linking different structural and functional maturation of hESC-derived GABAergic neurons provides a foundation for work aimed at identifying how neural activity, genetic programs, and neurological disorders regulate interneuron development.

Disclosures: S. Shrestha: None. **N. Anderson:** None. **J. Naegele:** None. **L. Grabel:** None. **G. Aaron:** None.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.03/B78

Topic: B.10. Epilepsy

Support: Arnold and Mable Beckman Foundation Undergraduate Fellowship (NHC)
CT Regenerative Medicine Fund
Randy and Lisa Siegel Summer Research Fellowship (DBL, ARH)

Title: Patterns of limbic seizures induced by chemogenetic silencing of GABAergic neurons in the adult mouse hippocampus and entorhinal cortex

Authors: *N. H. CIMINO, M. N. ARSHAD, M. A. VAN ZANDT, D. B. LAWRENCE, K. T. COBBOL, A. R. HOOKER, J. R. NAEGELE;
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Abstract: Functionally diverse types of γ-aminobutyric acid-releasing (GABAergic) interneurons in the hippocampus and entorhinal cortex regulate patterns of neuronal activity and play key roles in learning and memory. Dysfunction within these populations is implicated in some neurological disorders including epilepsy, schizophrenia, and autism. Experimentally manipulating GABAergic circuits in these brain regions by means of optogenetics and chemogenetics is providing new insights into the functional roles of limbic GABAergic interneurons. We expressed a floxed ivermectin-gated glycine receptor (iGlyR3-GFP) (kindly provided by Anthony van den Pol, Yale Univ. Sch. of Med. New Haven, CT) in a cell-type and region-specific manner to examine the role of silencing hippocampal and entorhinal cortical GABAergic neurons in limbic seizures. Adult male and female mice expressing Cre-recombinase under control of the vesicular GABA transporter (VGAT-ires-Cre; B6J.129S6(FVB)-Slc32a1$^{tm2(cre)Lowl/}$MwarJ) received stereotaxic injections of an AAV-DJ vector containing the iGlyR3-GFP construct. Hippocampal GABAergic interneuron expression occurred 2-5 weeks following stereotaxic delivery of this AAV-DJ vector. Video-electroencephalographic recordings (V-EEGs) were carried out for ~12 hours to obtain baseline EEGs prior to injecting ivermectin (5mg/kg, i.p.) or vehicle (i.p.) into the mice. V-EEGs were continued for ~7 hours post-injection. In ivermectin-treated mice, we observed bursts of high amplitude activity that typically

developed into non-convulsive and convulsive seizures with corresponding hypersynchronous waveforms. On average, iGlyR-expressing mice injected with ivermectin developed ~8 seizures within 7 hours (durations ~20-60 sec), whereas none of the vehicle-injected mice showed abnormal EEGs. These experiments demonstrate that acute silencing of hippocampal GABAergic circuits promotes seizure activity. In mice with longer periods of AAV-DJ-mediated iGlyR expression, the expression spread to GABAergic cells in the entorhinal cortex, including some parvalbumin-positive neurons. We are examining whether the expression is specific to the long-range GABAergic neurons that comprise bidirectional hippocampal-entorhinal inhibitory connections and preferentially target GABAergic interneurons of the hippocampus, and how expression of iGlyR in these pathways influences seizure phenotypes. Additional studies are underway in brain slices and in parvalbumin (PV)-expressing GABAergic interneurons in PV-ires-Cre transgenic mice to study the effects of silencing distinct types of GABAergic neurons on seizure activity.

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Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.04/B79

Topic: B.10. Epilepsy

Title: *In vitro* anticonvulsant activity of pterolobium stellatum extracts

Authors: *S. S. SALILE[1,2], T. ABULA[1], J. H. LEE[2], J. V. RAIMONDO[2];
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Abstract: Though there are conventional antiepileptic drugs in use ,one third of cases are refractory to treatment which underscores the need for new anticonvulsant agent. While four-fifths of the potential market for antiepileptic drugs is in the developing world, up to 90% of people with epilepsy in developing countries receive no treatment at all. In Ethiopia, many diseases are treated using traditional medicines. Pterolobium stellatum is often used to treat epilepsy. The whole plant juice is given orally for one month. The aim of this study is to investigate the anticonvulsant activity of P. stellatum extracts using the *in vitro* 0 Mg2+ model of seizures in mouse hippocampal brain slices. Methods Plant material was collected and extracted using standard methods. The crude hydroalcholic extracts of P. stelatum: petether, chloroform, butanol and water extracts with 0.7 mg/ml concentration were tested for anticonvulsant activity. Extracellular field potential recordings were performed in coronal hippocampal slices from P14-P21 of C57BL16 mice.The 0Mg2+model of seizures was utilized. Baseline recordings were

made for 600s with normal artificial cerebrospinal fluid(aCSF) before 0Mg2+aCSF was washed in for 3000s in order to induce seizure-like activity. The 0 Mg2+ solution either contained plant extract or solvent as a control. The presence of seizure-like events was compared in treated versus untreated control. The Chi square test with $P<0.05$ was used to determine statistical difference between groups. Results The crude extract had a statistically significant anticonvulsant activity compared to control($P=0.0153$). The chloroform and water extracts were also shown to have significant anticonvulsant activity as compared to control ($P=0.0008$ and $P= 0.0001$ respectively). The petether and buthanol extract activity was not statistically significant compared to control ($P=0.4760$ and $P=0.4637$ respectively). A positive control using the known anticonvulsant diazepam(3µM), showed significant anticonvulsant activity ($P= 0.0118$). Discussion and recommendations Our results demonstrate that P. stellatum has anticonvulsant activity. Active compounds are likely in both the water fraction and the chloroform fraction of the extracts as these both demonstrated good anticonvulsant activity. Further chemical studies are required to isolate the active compounds from these fractions. The mechanism of action of the active compounds in terms of their targets will also require further elucidation. This work demonstrates the utility of harnessing Africa's indigenous knowledge and rich biodiversity to identify novel anticonvulsant therapies based on natural compounds.

Disclosures: **S.S. Salile:** None. **T. Abula:** None. **J.H. Lee:** None. **J.V. Raimondo:** None.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.05/B80

Topic: B.10. Epilepsy

Title: Effects of hippocampal temporally irregular deep brain stimulation over PTZ-induced seizures in rats

Authors: *D. MARTINEZ-VARGAS[1], E. URBINA-TREJO[1], F. SANTOS-VALENCIA[1], S. ALMAZÁN-ALVARADO[2], A. RUBIO-LUVIANO[1];
[1]Lab. de Neurofisiología del Control y la Regulación, [2]Lab. de Bioelectrónica, Inst. Nacional de Psiquiatria, Mexico City, Mexico

Abstract: Deep brain electrical stimulation (DBS) is an alternative therapy used to treat pharmacoresistant epilepsy; however, the optimal parameters of stimulation are undetermined. The variation of temporal patterns of electrical pulses in stimulation trains is an emerging proposal that has opened a wide new field of opportunity to improve the effectiveness of the stimulation and minimize its side effects. It has been described that temporally irregular DBS (TiDBS) applied to amygdala reduces the severity and number of seizures induced by pentylenetetrazole (PTZ) and pilocarpine in rats. Thus, TiDBS could be an alternative approach

against epilepsy in other therapeutic targets involved in the generation of seizures like the hippocampus. The aim of this work was to analyze the effects of unilateral responsive TiDBS in hippocampus over PTZ-induced seizures and compare their efficacy with low-frequency stimulation (LFS) and high-frequency stimulation (HFS) in rats. Twenty-four adult male rats were implanted in the right dentate gyrus (DG) (AP -4 mm; ML 2.4 mm; V -3.6 mm from bregma) and both motor cortices for EEG recording. The implanted electrode in DG was used for stimulation and recording. Rats were randomly assigned to 4 groups; control, stimulation at 4 Hz (LFS), 130 Hz (HFS) or TiDBS (pseudo-random intervals inter-stimulus). The convulsive agent PTZ (60 mg/kg, i.p.) was applied to induce generalized tonic-clonic seizures (GTCS). LFS, HFS or TiDBS was applied for 30 min with a continuous train of biphasic square-wave pulses, onset immediately after of PTZ-injection. The latency to neck jerks (NJ), GTCS number, seizure score, and survival percent were analyzed. In addition, the effects over EEG seizures were evaluated. Obtained results showed a decrease of GTCS number [$F(3,20)=6.667$, $p=0.003$] with LFS ($p=0.013$), HFS ($p=0.013$) and TiDBS ($p=0.005$) compared to control. On the other hand, the seizure score decrease [$H=0.007$] with HFS ($p=0.05$) and TiDBS ($P=0.025$) compared to control. The latency to NJ increase [$F(3,23)=6.151$, $p=0.004$] with TiDBS ($p=0.004$) compared to control. The percent of survival after the PTZ administration was increased in rats treated with LFS (50%), HFS (83.3 %) and TiDBS (83.3 %), which were superior to the control group (0%). The EEG analyses showed a stronger effect of TiDBS in comparison with LFS and HFS. The animals treated with TiDBS only showed spike-wave discharges while animals treated with LFS or HFS showed a great number of spikes or recurrent electrographic seizures. The data suggest that TiDBS in DG can be an effective anticonvulsant method and could be lead an alternative treatment against the progress and the pathophysiology of epilepsy.

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Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.06/B81

Topic: A.04. Transplantation and Regeneration

Support: VA Merit Award (5I01BX002351-04 to A.K.S.)
Department of Defense (W81XWH-14-1-0558 to A.K.S.)

Title: Combined neural stem cell grafting and ganaxolone therapy greatly eases spontaneous seizures and co-morbidities in a model of chronic TLE

Authors: *R. UPADHYA[1,2], M. KODALI[1,2], B. SHUAI[1], J. XU[1,2], D. UPADHYA[1], A. K. SHETTY[1,2];

[1]Inst. For Regen Med, Texas A&M Univ. Coll Med., College Station, TX; [2]Olin E. Teague Veterans Med. Center, CTVHCS, Temple, TX

Abstract: Epilepsy, typified by spontaneous recurrent seizures (SRS), affects ~60 million people worldwide. Temporal lobe epilepsy (TLE), seen in ~30% of epilepsy patients, is a condition where seizures originate from the hippocampus. Memory and mood impairments are co-morbidities of chronic TLE. Since >35% of TLE patients acquire intractable epilepsy, alternative approaches such as cell therapy, either alone or in combination with drugs, have received much interest. We tested the hypothesis that combined intrahippocampal grafting of neural stem cells (NSCs) with short-term oral administration of ganaxolone (GAN, a synthetic analog of the neurosteroid allopregnanolone), facilitates better seizure control and improved cognitive and mood function than NSC grafting alone in rats afflicted with chronic TLE. We induced status epilepticus (SE) in young adult F344 rats via graded intraperitoneal injections of Kainic acid. After 2 hours of SE, the seizures were terminated through a dose of diazepam, and the frequency of behavioral SRS was quantified at 2-4 months after SE. Chronically epileptic rats (CERs) exhibiting comparable frequency of SRS were next randomly assigned to one of the five groups: CERs receiving sham surgery, CERs receiving oral GAN treatment for 14 days, CERs receiving intrahippocampal NSC grafting, and CERs receiving NSC grafting plus two-weeks of GAN. The donor NSCs were expanded from the subventricular zone of postnatal F344 rats expressing the green fluorescent protein in all cells. The frequency of SRS or Stage V-SRS, measured through continuous video-EEG recordings for 14-21 days at 4-5 months after treatment, was reduced in all treated groups. Furthermore, grafting of NSCs with or without GAN treatment reduced neuroinflammation, enhanced neurogenesis, and improved cognitive function. However, the overall efficacy for suppressing SRS was much higher in CERs receiving combined NSC and GAN treatment (63-65% reduction), than CERs receiving GAN (20-29% reduction) or NSC grafts alone (37-38% reduction). Besides, unlike with NSC or GAN treatment alone, combined NSC and GAN therapy improved pattern separation function and reversed anhedonia. Combined therapy also mediated superior enhancement of hippocampal neurogenesis and repression of activated microglia without altering the survival and differentiation of graft-derived cells. The results underscore that combined NSC and GAN therapy is superior to NSC or GAN treatment alone for alleviating seizures and easing the co-morbidities of TLE.

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Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

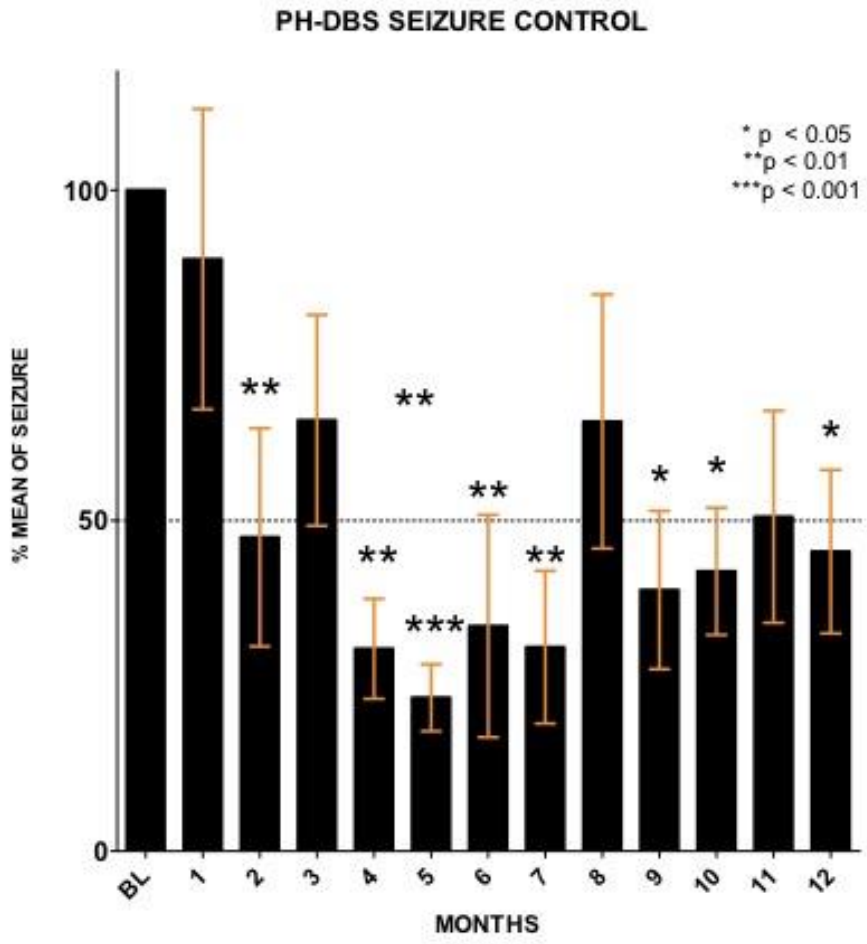
Program #/Poster #: 371.07/B82

Topic: B.10. Epilepsy

Title: Deep brain stimulation in the parahippocampus as an alternative target for the treatment of mesial temporal lobe epilepsy in patients with hippocampal sclerosis

Authors: *P. E. SAUCEDO-ALVARADO[1], F. J. VELASCO[2], A. VELASCO[2], M. A. AVILA-RODRIGUEZ[1], A. AVENDAÑO-ESTRADA[1], M. CUELLAR-HERRERA[2], R. MARQUEZ-FRANCO[3];
[1]UNAM, Mexico City, Mexico; [2]Hosp. Gen. de Mexico, Mexico City, Mexico; [3]UAG, Guadalajara, Mexico

Abstract: Objectives:Evaluate the response of seizure control in drug-resistant Mesial Temporal Lobe Epilepsy (MTLE) patients with Hippocampal Sclerosis (HS) after neuromodulation in the parahippocampal cortex (PH-DBS). Methods:A group of 6 MLTE-HS patients were implanted in the parahippocampal cortex and neuromodulated with High-Frequency Stimulation (HFS). Stimulation parameters were the same in all patients (2.5 V, 130 Hz, 450 μs, Open Loop 1 min ON/4min OFF), and they were kept in anticonvulsive medication that had worked best in pre-operative evaluation. The evaluation included: number of seizures measured as percentage decrement from the previous 3 months baseline (BL), neuropsychological performance and 18FDG interictal PET-CT before and at the end of one-year follow-up.Results:Plotting the active contacts of DBS electrodes in post-operative MRI confirmed the correct placement of electrodes in all cases. The number of seizures decreased significantly since the second month ON stimulation to reach decrements from 50% to 80% in subsequent months. Secondary tonic-clonic seizures and Focal seizures with impairment awareness decreased significantly but not focal seizures. Neuropsychological performance remained unchanged for the two groups with an improvement in those patients that had best seizure control. 18FDG interictal PET-CT values increased after twelve months of therapy of those hypometabolic regions before PH-DBS. The obtained data were compared with two more targets results, Hippocampal and Subiculum neuromodulation, from previous studies. In contrast with these two other targets, in patients with HS, PH-DBS has better results than the previous studies.Conclusions:PH-DBS is a great alternative on seizure control in MTLE-HS, this could be related to changes in the metabolism of the epileptogenic zone as a result of DBS therapy.



Disclosures: **P.E. Saucedo-Alvarado:** None. **F.J. Velasco:** None. **A. Velasco:** None. **M.A. Avila-Rodriguez:** None. **A. Avendaño-Estrada:** None. **M. Cuellar-Herrera:** None. **R. Marquez-Franco:** None.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.08/B83

Topic: B.10. Epilepsy

Support: INPRF Grant NC123240.1

Title: Changes of the electrical activity of the thalamic reticular nucleus by the electric stimulation

Authors: *V. M. MAGDALENO-MADRIGAL[1], J. VALERIO-MUÑOZ[2], M. I. ORTIZ-ZARATE[2], G. CONTRERAS-MURILLO[1], S. ALMAZÁN-ALVARADO[1];
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Abstract: The high-frequency deep brain stimulation (DBS) is used for the control of refractory epilepsy. In our laboratory, we have reported that DBS in the thalamic reticular nucleus (TRN) protects against generalized seizures provoked by Pentylenetetrazol (PTZ). The TRN contains inhibitory neurons that release GABA and is involved in the generation and control of spike-wave discharges (SWD) and tonic-clonic generalized seizures (TCGS). To explore the mechanism underlying TRN-DBS we recorded the multiunit activity of TRN in response to DBS-TRN and PTZ. In the acute protocol, under deep anesthesia induced by intraperitoneal injection of urethane (1.5 g/1000 g) the rats were prepared with a stainless-steel bipolar electrode directed to TRN and standard extracellular recordings were performed using glass microelectrodes (4–8 MΩ) filled with 3M KCl solution. Two nail-shaped were implanted in the frontal bone to EEG recording and a jewelry screw as a reference was implanted in the occipital bone. TRN recording tracks were aimed vertically at the stereotaxic coordinates. The PTZ caused a significant decrease in the firing rate of TRN cells, in 15 neurons their firing from tonic mode to burst mode was modified, while in five neurons an increase in the firing rate was induced. The DBS-TRN decreased the firing rate compared to the baseline and the second dose of PTZ, and changes in the type of firing in three neurons did not observe. The results suggest that the NRT participates in the maintenance of the SWD characteristics of the absence seizures, and the DBS in the NRT may induce the inhibition of the oscillatory activity synchronization.

Disclosures: V.M. Magdaleno-Madrigal: None. **J. Valerio-Muñoz:** None. **M.I. Ortiz-Zarate:** None. **G. Contreras-Murillo:** None. **S. Almazán-Alvarado:** None.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.09/B84

Topic: B.10. Epilepsy

Title: Focal suppression of epileptiform activity in the hippocampus by a time-varying magnetic field

Authors: H. YE, V. CHEN, J. HELON, N. APOSTOLOPOULOS;
Loyola Univ. Chicago, Chicago, IL

Abstract: Electric current has been used to suppress seizure activity clinically by targeting specific neural circuity. When electric current is delivered by implanted electrodes, the direct

contact between electrodes and tissue causes many side effects such as pain, inflammation, and other adverse biological reactions. An alternative method to generate electric current inside the brain, is via electromagnetic induction through a magnetic coil. In comparison to electrical stimulation, magnetic stimulation offers several advantages in biocompatibility and operational feasibility. However, further development of this technology for clinical practice is hindered by the lack of complete understanding of the cellular mechanism of magnetic stimulation, particularly, in the context of hyperexcitable neural activity (seizure) in epilepsy. In this paper, we report that epileptiform activity in the CA3 area of the hippocampus could be locally suppressed by a mini size coil in an *in vitro* low-Mg^{2+} /high K^{+} model. The inhibition effect is dependent on the magnitude, frequency, and duration of the magnetic field delivered by the coil. This work provides a platform for further investigation of cellular/molecular mechanisms underlying seizure inhibition by time varying magnetic field, and insights on the clinical TMS treatment for epilepsy.

Disclosures: H. Ye: None. **V. Chen:** None. **J. Helon:** None. **N. Apostolopoulos:** None.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.10/B85

Topic: B.10. Epilepsy

Support: Momentum II program of the Hungarian Academy of Sciences

Title: Closed-loop implementation of intersectional short-pulse (ISP) stimulation to stop temporal lobe seizures

Authors: M. HARANGOZO, **T. FOLDI**, G. KOZAK, A. J. NAGY, T. GYURKOVICS, M. VOROSLAKOS, *A. BERENYI;
Univ. of Szeged, Szeged, Hungary

Abstract: Transcutaneous electric stimulation (TES) using weak currents has been used extensively in attempts to influence brain activity. *In vitro* and *in vivo* experiments in rodents and computational modeling suggest that the magnitude of voltage gradient of the induced electric field should exceed 1 mV/mm to instantaneously and reproducibly alter neuronal spiking and consequent brain network patterns. Evidence for immediate and unconditional neuronal effects of TES in the human brain is still lacking, mainly due to the saturation of the recording amplifiers by the large induced electromagnetic fields. For many therapeutic applications, it is desirable to affect neurons in a regionally constrained manner to reach maximum on-target effects and reduce side effects on unintended brain networks.
Here, we determine the needed TES currents in human cadavers to achieve 1 mV/mm fields.

Scalp stimulation greatly reduced the generated intracerebral electric fields (>50% in cadavers) and these measurements predicted that ~5 mA is needed to achieve 1mV/mm electric field gradient via scalp stimulation. To reach the desired intracerebral field strength without the adverse peripheral effects of >5 mA currents, we introduce a spatially focused multiple site, Intersectional Short-Pulse (ISP) stimulation. We demonstrate the instantaneous entraining effect of ISP on EEG waves in human subjects and on neuronal spiking in rats. Immediate effects of TES can be best utilized in disorders with sudden, major electrographic changes such as epileptic seizures. We showed earlier that thalamocortical seizures can be quickly terminated by temporally targeted, diffuse transcranial stimulation, however secondarily generalized temporal lobe seizures are more resistant to these diffuse interference interventions.
ISP also has the capacity to spatially focus its effect, thus it is capable to overcome the unwanted mirror effect (anodal vs cathodal) of the traditional TES protocols. We report here a novel stimulation pattern, that can simultaneously entrain both hippocampi. To evaluate its utility, temporal lobe seizures were induced in rats by electrical kindling, and each electrically kindled seizures were automatically detected and silenced by a closed loop ISP stimulation. By comparing to closed-loop diffuse TES, we found that ISP with bilateral foci is more effective in early seizure termination.
Lastly, we introduce our prototyping efforts to implement an implantable, minimal-invasive, transcranial closed-loop seizure termination device, aiming for human clinical applications.

Disclosures: **M. Harangozo:** None. **T. Foldi:** A. Employment/Salary (full or part-time):; Neunos Ltd. **G. Kozak:** None. **A.J. Nagy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amplipex Ltd. **T. Gyurkovics:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neunos Ltd. **M. Voroslakos:** None. **A. Berenyi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neunos Ltd.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.11/B86

Topic: B.10. Epilepsy

Support: NIH Grant NS047557
Student Fellowship from Office for People with Developmental Disabilities

Title: Dynamic volume changes of the brain’s extracellular space as a target for eliminating epileptiform seizure activity

Authors: R. COLBOURN[1,2], J. H. GOODMAN[3,4], *S. HRABETOVA[2];
[1]Sch. of Grad. Studies, SUNY Downstate Med. Ctr., Brooklyn, NY; [2]Dept. of Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY; [3]Inst. for Basic Res. in Developmental Disabilities, Staten Island, NY; [4]Dept. of Physiol. and Pharmacol. SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: Chronic seizure syndromes are notoriously difficult to treat, with between 20-40% of cases being refractory to anti-seizure medications. The identification of therapeutic targets that promote seizure generation are critical in the development of new treatments for these patients. The volume of the brain's extracellular space (ECS) has long been recognized as having an important role in seizure generation, but how it exerts its influence is still up for debate. The brain's ECS represents a large, interconnected compartment that is a reservoir of ions and a communication channel for neurotransmitters, which allows any changes in volume of the ECS to modulate the effects those functions might have on net neuronal excitability and synchrony. To investigate how ECS volume changes during epileptiform activity, we utilized a technique called relative volume monitoring (RVM), which continuously tracks the concentration of an ECS probe to calculate changes in its volume. By using RVM in the neocortex of brain slices from C57BL/6 mice undergoing 4-aminopyridine (4AP, 100 μM) induced epileptiform activity (n = 40 slices), we have discovered that the ECS transiently shrinks up to 13% during each synchronous epileptiform discharge. Because ECS shrinkage is an important pro-excitatory force in the brain, these dynamic volume changes (DVCs) of the ECS may represent a therapeutic target for inhibiting seizures. To determine this, we decided to pharmacologically block channels and transporters that were likely necessary to allow for these DVCs to occur. Blockade of sodium-bicarbonate cotransporter 1 (NBCe1) with 4,4'-Diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS, 300 μM) led to the elimination of both DVCs and epileptiform activity (n = 5 slices). Based on the effect of blockade, we argue that it was because of the elimination of DVCs that the epileptiform activity was halted. In summary, we conclude that DVCs represent a mechanism that helps promote excitability and synchrony and therefore may serve as a possible target for seizure treatment.

Disclosures: R. Colbourn: None. **J.H. Goodman:** None. **S. Hrabetova:** None.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.12/B87

Topic: B.10. Epilepsy

Support: RO1HD091994-01A1

Title: Transcriptomic alterations following early life hypoxia-induced seizures and anti-seizure drug exposure

Authors: *S. M. M. QUINLAN, P. A. FORCELLI;
Georgetown Univ., Washington DC, DC

Abstract: Hypoxic-ischemic encephalopathy (HIE) is the main precipitator of neonatal seizures and a leading cause of long-term disabilities in infants (Soul, 2018). Survivors are at increased risk of autism, epilepsy, cognitive and learning difficulties and psychiatric conditions (Glass et al., 2016; Soul, 2018). Common clinical practice is to suppress neonatal seizures using pharmacotherapies such as phenobarbital [PB] or levetiracetam [LEV]. However, treatment of neonatal seizures poses a particular challenge: both seizures and anti-seizure drugs (ASDs) can result in long-term adverse effects on brain development. While there is growing evidence for the adverse impact of both seizures and medication alone, very little is known about how these insults interact. Identifying early mechanistic targets triggered by seizures and ASDs will be crucial for understanding how HS and ASDs affect the developing brain, and how we can improve the long-term negative neurological outcomes.
Postnatal day (P)7 Sprague-Dawley rat pups were exposed to graded global hypoxia-induced seizures (HS) with or without anti-seizure drug treatment (PB 75 mg/kg; LEV 200 mg/kg). Hippocampal transcriptome was assessed 72 h post-HS by RNAseq. Animals subjected to HS had 280 significantly dysregulated transcripts, including a cluster involved in glutamate transmission. Using 4-channel multiplex qPCR we validated the changes seen with RNAseq, using pre-designed primers. We observed a 25% decreased expression of genes coding for ionotropic glutamate receptor subunits when compared to control animals. Levels of transcripts coding for AMPA receptor subunits *GRIA1, GRIA2, and GRIA3* were all significantly decreased by HS and LEV alone, but not by PB, however levels of *GRIA4* were significantly decreased by PB. *GRIN1, GRIN2A, GRIN2B,* and *GRIN3A* transcripts, which code for NMDA receptor subunits, were all significantly decreased by treatments HS, LEV, and combinations, apart from PB treatment alone. Validation of other transcripts is ongoing, as is validation at the level of protein. While alterations in glutamatergic transmission have previously been reported after HS, these data raise the possibility that ASDs, when given during a critical developmental period, may also dysregulate glutamatergic transmission.

Disclosures: S.M.M. Quinlan: None. **P.A. Forcelli:** None.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.13/B88

Topic: B.10. Epilepsy

Support: CSIR Fellowship
Science and Engineering Research Board; Grant Number: SB/S5/AB/05/2016

Title: Deciphering the neuroprotective mechanism of ayurvedic formulations using mouse model of epilepsy

Authors: *B. VERMA, P. SINHA, S. GANESH;
Biol. Sci. and Bioengineering, Indian Inst. of Technol. Kanpur, Kalyanpur, Kanpur, India

Abstract: Epilepsy affects approximately 50 million people worldwide and continues to remain as one of the leading causes of disability. Much progress has been made in understanding the etiology and pathogenesis of epilepsy, and several anti-epileptic drugs (AED) have been developed. Nonetheless, the AEDs are often associated with adverse effects, and over one-third of the patients with epilepsy fail to respond to the AEDs after prolonged usage. Therefore, the use of AEDs is often considered as a symptomatic treatment, and there is a genuine need to explore the alternative therapeutic approaches with fewer side effects when administered for the long term. *Ayurveda*, the traditional Indian medicinal system, describes complex formulations that aim to bring about healthy aging and thereby prevent and/or delay the neurological dysfunctions with no/minimal adverse effects. However, their mode of action and physiological basis have not yet been well characterized. The current study aimed at investigating the physiological basis of neuroprotection conferred two formulations, namely the *Amalaki Rasayana* and *Rasa sindoor*. For this is we used a mouse of Lafora disease - a progressive and fatal form of epilepsy in humans - known to show increased susceptibility to induced seizure, neurodegeneration, and deficit in neurocognitive functions. The animals were fed with the formulations for a period for six months, and at the end of the treatment, the animals were tested for their susceptibility to pentylenetetrazole-induced seizure, neuroinflammation, and cognitive functions. We found a significant reduction in the seizure susceptibility in the groups that received *Ayurvedic* formulations as compared to the controls, and this correlated with a significant reduction in the neuroinflammation in the treated group. In addition, the treated animals showed a significant improvement in learning & memory, anxiety and depression. These findings indicate that the *Ayurvedic* formulations confer neuroprotection and are attractive alternative modes of treatment for some of the epileptic disorders.

Disclosures: B. Verma: None. **P. Sinha:** None. **S. Ganesh:** A. Employment/Salary (full or part-time):; Indian Institute of Technology Kanpur. 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 and Engineering Research Board.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.14/B89

Topic: B.10. Epilepsy

Support: NIH Grant MH105527

Title: Drug repositioning in epilepsy using the zebrafish model system

Authors: *M. STURGEON[1], L. BRUEGGEMAN[2], R. MARTIN[2], A. GROSSBACH[2], Y. NAGAHAMA[2], A. ZHANG[3], M. HOWARD, III[2], H. KAWASAKI[2], S. WU[2], R. CORNELL[2], J. MICHAELSON[2], A. BASSUK[2];
[1]Interdisciplinary Grad. Program in Mol. Med., [2]Univ. of Iowa, Iowa City, IA; [3]Univ. of Washington, Seattle, WA

Abstract: Roughly 30% of epileptic patients do not respond to available anti-epileptic drugs, often leading to surgeries which can be dangerous and costly. Finding novel drugs for these patients is costly and time-consuming. Therefore, repurposing compounds approved by the FDA to treat other diseases is appealing for use in treating epilepsy. To identify candidate compounds for this effort, we harvested seizing and non-seizing brain tissue that had been clinically resected from patients suffering intractable epilepsy. We generated RNA expression profiles of both tissues, and identified a list of differentially expressed genes, i.e., an epileptic gene signature. We compared this list to previously-published expression profiles of cells exposed to various FDA-approved compounds. Those that induced changes in gene expression that were anti-correlated with the epileptic gene signature were candidate therapeutics for epilepsy. Next, we screened a subset of such candidates in an animal model of epilepsy: zebrafish larvae treated with the GABAergic inhibitor, PTZ. We found that metformin, nifedipine, and pyrantel tartrate were able to partially suppress convulsions in PTZ-treated larvae. Currently, we are testing these drugs in zebrafish *scn1a* mutants, which are a model of genetic epilepsy. These findings illustrate a pipeline for identifying drugs that are candidates for re-purposing to anti-epileptic therapy.

Disclosures: M. Sturgeon: None. **L. Brueggeman:** None. **R. Martin:** None. **A. Grossbach:** None. **Y. Nagahama:** None. **A. Zhang:** None. **M. Howard:** None. **H. Kawasaki:** None. **S. Wu:** None. **R. Cornell:** None. **J. Michaelson:** None. **A. Bassuk:** None.

Poster

371. Antiepileptic Therapies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 371.15/B90

Topic: B.10. Epilepsy

Support: Interagency Agreement AOD18013-001-00000 between the NIH Office of the Director and the U.S. Army Medical Research Institute of Chemical Defense under the oversight of the Chemical Countermeasures Research Program (CCRP) within NIAID/NIH

Title: Evaluating in-hospital care for the treatment of nerve agent-induced status epilepticus in rats

Authors: J. E. MORGAN, S. C. WILSON, J. M. COPPOLA, C. JACKSON PIERCY, H. M. BELSKI, K. A. HUNDERTMARK, S. T. MILLS, M. R. EISEN, D. L. NGUYEN, *H. S. MCCARREN;
US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: Nerve agent (NA) poisoning initiates a catastrophic cholinergic crisis that can induce *status epilepticus* (SE) if left untreated. Survivors of NA exposure will likely require treatment in a hospital intensive care setting. Guidelines currently exist for pre-hospital management of NA-induced SE, which includes up to two doses of a benzodiazepine. However, previous research in rodent models has indicated that doses of diazepam equivalent to 3-12 convulsive antidote for nerve agent (CANA) auto-injectors are insufficient to stop SE. There is currently no standard therapy regimen for definitive termination of NA-induced SE. Our goal is to determine a treatment strategy to completely resolve NA-induced SE in a hospital setting using benzodiazepines as a first-line therapy, followed by antiepileptic drugs and anesthetic agents as second- and third-line therapies respectively, if benzodiazepines prove insufficient. Male Sprague Dawley rats were surgically implanted with jugular vein catheters and tethered EEG headpieces. Twenty minutes after soman (GD) exposure they received human equivalent doses of standard pre-hospital countermeasures for NA exposure, followed ten minutes later by initiation of in-hospital treatments. When compared head-to-head as first-line treatments, midazolam and lorazepam were equally ineffective at terminating SE ($p = 0.56$), with respective seizure termination rates of 7% ($n = 15$) and 18% ($n = 11$). The second-line treatments valproic acid and phenobarbital also showed equivalent efficacy ($p = 0.68$), with respective seizure termination rates of 43% ($n = 14$) and 60% ($n = 10$). The third-line treatments ketamine ($n = 6$) and propofol ($n = 8$) were dosed until SE terminated in all subjects, but 24-hour survival rates in animals that required these drugs were very low (14%). Still, animals that were successfully treated in the ICU and returned to their home cages after 24 hours of seizure freedom showed a

return to baseline body weight by 6 days post-exposure, while animals that only received pre-hospital treatment and did not demonstrate SE termination never recovered to baseline before the end of the study (10 days post-exposure). Work to identify impacts of treatment regimen on behavioral task performance, as well as neuropathology analysis, is ongoing.

Disclosures: **J.E. Morgan:** None. **S.C. Wilson:** None. **J.M. Coppola:** None. **C. Jackson Piercy:** None. **H.M. Belski:** None. **K.A. Hundertmark:** None. **S.T. Mills:** None. **M.R. Eisen:** None. **D.L. Nguyen:** None. **H.S. McCarren:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.01/B91

Topic: B.11. Glial Mechanisms

Title: The direct contact of astrocytes to neuron is critical for lowering excitatory synaptic transmission after the exposure of amyloid β-protein 25-35 onto astrocytes

Authors: *K. OYABU[1], H. KIYOTA[1], K. KUBOTA[1,2], T. WATANABE[1,2], S. KATSURABAYASHI[1], K. IWASAKI[1,2];
[1]Fukuoka Univ., Fukuoka, Japan; [2]Collaborative Res. Inst. for Aging and Brain Sci., Fukuoka, Japan

Abstract: Alzheimer's disease is a progressive neurodegenerative disease that presents with cognitive and behavioral disorders. Because Aβ25-35 cause synaptic dysfunction and synapses loss, Aβ25-35 are regarded as an initiator of the pathogenesis of AD. Giving that astrocytes play important roles, such as modulation of neurotransmission, synaptogenesis, in information processing of the central nervous system, the close relationship between astrocytes and synapses suggests that astrocyte dysfunction by Aβ25-35 affects synaptic abnormalities in AD. However, their relationship is not well understood so far. In this study, the synaptic transmission and synaptic number were assessed in single neuron cultures, where the neuron was co-cultured with astrocytes exposed Aβ25-35 3 days beforehand, namely, the neuron had not undergone the exposure of Aβ25-35. Neurons in direct contact with astrocytes showed a significant decrease in excitatory postsynaptic current together with a significant decrease in the number of glutamatergic synapses. However, the vesicular release probability was unchanged. On the other hand, the number of synapses was not changed in neurons that did not contact astrocytes that exposed Aβ25-35. Taken together, the direct contact of astrocytes to neurons is important for the synaptic abnormality by the astrocyte-mediated exposure of Aβ25-35. This study further implies that not only the direct neuronal effect of Aβ25-35 but also the astrocyte-mediated effect is severe in the pathogenesis of AD.

Disclosures: **K. Oyabu:** None. **H. Kiyota:** None. **K. Kubota:** None. **T. Watanabe:** None. **S. Katsurabayashi:** None. **K. Iwasaki:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.02/B92

Topic: B.11. Glial Mechanisms

Support: NIH Grant NS088058

Title: Neuron-derived estrogen is critical for neuroprotective astrocyte activation after ischemic injury to the brain

Authors: ***Y. LU**[1], G. R. SAREDDY[2], J. WANG[1], Q. ZHANG[1], F.-L. TANG[1], U. PRATAP[2], R. K. VADLAMUDI[2], D. W. BRANN[1];
[1]Augusta Univ., Augusta, GA; [2]Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: 17β-estradiol (E2) is produced from androgens via the action of the enzyme aromatase. E2 is known to be made in neurons in the brain, but its precise functions in the brain are unclear. Here, we used a forebrain-neuron-specific aromatase knockout (FBN-ARO-KO) mouse model to deplete neuron-derived E2 in the forebrain of adult female ovariectomized mice and determine its roles in the hippocampus after global cerebral ischemia (GCI). The results revealed that FBN-ARO-KO mice had significantly reduced hippocampal E2 levels, as well as a significant attenuation of astrocyte activation and increased neuronal damage after GCI. Behavioral tests demonstrated that FBN-ARO-KO mice had worse cognitive outcome compared to control mice, and *in vivo* E2 replacement was able to rescue the cognitive deficits. RNA-seq analysis revealed alterations in pathways and genes associated with astrocyte activation, neuroinflammation and oxidative stress in FBN-ARO-KO mice. Double immunohistochemistry analysis revealed that the compromised astrocyte activation in FBN-ARO-KO mice was associated with robust downregulation of the astrocyte-derived neurotrophic factors, like BDNF and IGF-1, as well as glutamate transporter levels in astrocytes. Moreover, neuronal FGF2 that acts in a paracrine manner to suppress astrocyte activation, was found to be dramatically increased in FBN-ARO-KO neurons. Interestingly, blocking FGF2 signaling in astrocytes by central injection of an FGFR3 antibody was able to reverse the decrease in neuroprotective astrocyte reactivity, as well as attenuate neuronal damage in FBN-ARO-KO mice. Collectively, our data provides novel genetic evidence for an essential role of neuron-derived E2 in neuroprotection and cognitive preservation by maintaining a beneficial neuroprotective astrocyte reactive status following ischemic injury to the brain.

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Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.03/B93

Topic: B.11. Glial Mechanisms

Support: National Research Foundation of Korea (NRF) grant, funded by the Korean Government 800-20190161

Title: Disruption in the formation of tripartite synapses by reactive astrocytes is responsible for the learning and memory impairments in Alzheimer's disease

Authors: ***M. CHOI**[1], H. KIM[2], E. YANG[1], H.-S. KIM[3];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Seoul Natl. Univ., Yeongeon-Dong, Jongno-Gu, Seoul, Korea, Republic of; [3]Seoul Natl. Univ. Col. Med., Seoul, Korea, Republic of

Abstract: Astrocytes are the most abundant cell type in the central nervous system, and various roles of the astrocytes have been well known from the formation of blood brain barrier to the involvement in the memory formation. These cells have a potential of dynamic morphological changes according to the status of the cells. Astrocytes closely interact with neurons, making structures called as tripartite synapses. However, the pattern of dynamic morphological changes in memory induction status of astrocytes and/or tripartite synapses in Alzheimer's disease (AD) have been not clarified yet. In the present study, we investigated the changes in morphology of astrocytes and the number of tripartite synapses during learning and memory processes using AD model, 5XFAD mouse. The hippocampus dependent long-term spatial memory was induced with the contextual fear conditioning test . 1 hour after the induction of memory formation, the number of processes of astrocytes was increased in both wild type (WT) and 5XFAD mice group. 24 hour after memory induction, the number of processes of astrocytes was increased in WT but not in 5XFAD mice group. In addition, the number of tripartite synapses was increased in both WT and 5XFAD group after 1 hour after the induction of memory formation. Taken together, our results suggest that disruption in the formation of tripartite synapses is responsible for the learning and memory impairments in AD.

Disclosures: **M. Choi:** None. **H. Kim:** None. **E. Yang:** None. **H. Kim:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.04/B94

Topic: B.11. Glial Mechanisms

Support: JSPS KAKENHI

Title: Phosphatidylserine recognition and rac activation link proliferation, gliosis and phagocytic activity of muller glia in the rat retinal injury model

Authors: *K. NOMURA-KOMOIKE, F. SAITOH, H. FUJIEDA;
Dept. of Anat., Tokyo Women's Med. Univ., Tokyo, Japan

Abstract: Müller glia, the principal glial cell type in the retina, have the potential to reenter the cell cycle after retinal damage. In mammals, proliferation of Müller glia is followed by gliosis, but not neuronal regeneration. Retinal damage is also followed by phagocytic removal of degenerated cells. Here we investigated the possibility that proliferation of Müller glia, gliosis, and phagocytosis of degenerated cells may be regulated by the same molecular pathways. N-methyl-N–nitrosourea was intraperitoneally injected into five-week-old male Wistar rats (MNU, 70mg/kg BW) to induce photoreceptor damage, and the retinas were subjected to immunohistochemistry for cell cycle and cell type-specific markers. Retinas were also cultured as explants after MNU treatment. All experiments were repeated at least 3 times and at least 3 animals were used per stage or condition. Degeneration of photoreceptors was observed by day 1 after MNU treatment, followed by cell cycle reentry of Müller glia by day 2. Notably, most degenerated photoreceptors were eliminated by day 2.5 prior to the infiltration of microglia/macrophages into the outer nuclear layer and inhibition of microglia/macrophages by minocycline did not affect the elimination of degenerated photoreceptors. Besides, accumulation of lysosomes and rhodopsin-positive photoreceptor debris were found within the cytoplasm of Müller glia. These data indicate that Müller glia, rather than microglia/macrophages, play a major role in the phagocytic clearance of degenerated photoreceptors. We treated retinal explants with *O*-phospho-L-serine (L-SOP), which inhibits the recognition of phosphatidylserine (PS), the key “eat me” signal exposed by apoptotic cells. Cell cycle reentry, GFAP upregulation, and phagocytic activity of Müller glia were significantly inhibited by L-SOP treatment, while all these responses were observed in the control explants. We also tested the involvement of Rac, a member of the Rho family small GTPases, known to promote phagocytic engulfment by regulating cytoskeletal rearrangement and found that Rac1 expression was upregulated in Müller glia after MNU treatment. Treatment of retinal explants with NSC23766, a specific inhibitor of Rac1 activation, significantly inhibited the proliferative, gliotic, and phagocytic responses of Müller glia. These data provide evidence that Müller glia play a major role in phagocytic

clearance of degenerated photoreceptors and that PS recognition and Rac1 activation are required not only for phagocytic activity but also for proliferation and gliosis of Müller glia in the mammalian retina.

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Poster

372. Glia-Neuron Interactions in Diseased Brain

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Program #/Poster #: 372.05/B95

Topic: B.11. Glial Mechanisms

Support: H2020 Flagship Graphene Core2 n.785219

Title: Synaptic and glial cell responses to neuroinflammation in spinal organotypic slices

Authors: *G. PANATTONI[1], V. GIACCO[3], C. BALLERINI[4], L. BALLERINI[2];
[2]Neurosci., [1]SISSA-ISAS, Trieste, Italy; [3]SISSA-ISAS Trieste, Trieste, Italy; [4]Univ. di Firenze, Firenze, Italy

Abstract: Neuroinflammation is a characterizing trait of various central nervous system (CNS) pathologies, from neurodegenerative diseases to neuropsychiatric disorders.In the effort of dissecting the impact of immune status alterations on neural circuit function, we focused our study on the effects of local inflammation in a controlled micro-environment where neurons and neuroglial cells maintain their appropriate organization: the organotypic spinal cord slices. These cultures, developed from the spinal cord of mouse embryos, represent a complex in vitro model where sensory-motor cytoarchitecture, synaptic properties and spinal cord resident cells, encompassing heterogeneous neuronal phenotypes and neuroglia, are retained in a 3D fashion.Organotypic spinal cord slices are cultured for two weeks in vitro. Then, they are exposed for 4 and 6 hours to a cocktail of cytokines (CKs, 10 ng/mL), composed by tumor necrosis factor alpha (TNF alpha), interleukin-1 beta (IL-1 beta) and granulocyte macrophage-colony stimulating factor (GM-CSF), or to lipopolysaccharide (LPS, 1 µg/mL). We use single cell electrophysiology, live cell calcium imaging, immunocytochemistry and confocal microscopy to investigate and compare the spinal tissue responses to neuroinflammation induced by CKs and LPS. We focus first on the synaptic level, describing the shortening of GABAergic current due to CKs incubations, absent in LPS treated ones, despite the overall increased network activity. We further explore by immunofluorescence and confocal microscopy, resident neuroglia reactivity and by calcium live imaging we document an increase in the occurrence of calcium waves displayed by the glial cells located in the ventral horn, differently tuned by the diverse threats.

Disclosures: **G. Panattoni:** None. **V. Giacco:** None. **C. Ballerini:** None. **L. Ballerini:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.06/B96

Topic: B.11. Glial Mechanisms

Support: European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant Agreement No 764860

Title: Calcitonin gene-related peptide (CGRP) influences cortical spreading depolarization (CSD) in adult rats - Could this be important in brain pathophysiology?

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Abstract: It is known from the literature that CGRP plays an important role in migraine, and antagonizing CGRP is an effective treatment against migraine pain. CSD, the correlate of the migraine aura, is able to release CGRP in rat neocortical slices. CGRP may increase neuronal excitability, but a possible interference with CSD has not been investigated yet. To test this, we applied CGRP at different concentrations topically to a restricted part of the cortical surface and compared the electrocorticogram and CSD there with the untreated brain area. In spontaneously breathing anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.) CSDs were recorded in cerebral cortex with two pairs of glass micropipettes (distance 5-6 mm) at depths of 400 and 1200 µm in two areas of the cortex, separated by a wall. In the untreated area, CSD was elicited by a microinjection of 1 M KCl (100 kPa, 300 ms up to 1 s) into the grey matter at intervals of 30 min. In the remote area 100 µl of CGRP at concentrations from 10^{-8} M to 10^{-5} M were applied topically and left there for three hours. In the treated area additionally changes in extracellular potassium concentration and in both areas regional cortical blood flow were measured. In all rats tested, a pulse of KCl elicited a single propagating CSD. The topical application of CGRP to the brain surface reduced the amplitudes of CSD in the treated area (10^{-5} M to 60 % of controls; 10^{-8} M to 70 % of controls; untreated to 85-90 % of controls) and slowed the propagation velocity (10^{-5} M from 3.0 to 2.6 cm/s; 10^{-8} M from 2.4 to 2.2 cm/s). Rarely spontaneous CSDs were observed originating from the CGRP treated area. In another few rats CGRP induced focal ictal activity after 2-3 hours of application that did not spread into the untreated cortex. This activity was accompanied by increases in extracellular potassium concentration and occurred at intervals of 8-10 min. Our results identify the neuropeptide CGRP as a candidate that could interfere with CSD by changing neuronal excitability. In contrast to other previously investigated

neuropeptides, the capability of CGRP to ignite CSD at the same concentrations varies markedly between the animals. This has to be taken into account when using CGRP as a model for neuroinflammation or disturbed brain homeostasis.

Disclosures: F. Gimeno-Ferrer: None. **F. Richter:** None. **R. Bauer:** None. **A. Lehmenkuhler:** None. **H. Schaible:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.07/B97

Topic: B.11. Glial Mechanisms

Support: NIH-NINDS Grant R01NS097312

Title: The effect of a high fat diet on endocannabinoid-mediated astrocyte-neuron signaling in the arcuate nucleus

Authors: *A. K. SCOTT[1], C. D. MURAT[2], P. KOFUJI[1], A. ARAQUE[1];
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Abstract: Astrocyte-neuron interactions in the arcuate nucleus (ARC) of the hypothalamus are emerging as important elements of regulating energy homeostasis. The dysregulation of this astrocyte-neuron interaction may contribute to the imbalance in energy homeostasis that leads to obesity. An acute high fat diet (HFD) is known to induce astrogliosis in the ARC. However, the functional effect of this astrogliosis has seldom been explored. Here we examined the effects of HFD on astrocyte-neuron signaling in the ARC of adult, male and female mice. Using calcium indicators in acute coronal brain slices, we found that spontaneous calcium activity in ARC astrocytes was increased in mice fed with HFD compared to animals fed with normal diet. Using whole-cell patch clamp electrophysiology, we also found an increase in the frequency of slow inward currents (SICs), which are known to be mediated by astrocytic glutamate release and consequential activation of neuronal extrasynaptic NMDA receptors, in ARC neurons from animals fed a HFD. No HFD-induced changes in astrocyte calcium or SICs were observed in $IP_3R2^{-/-}$ mice. Due to the increased levels of endocannabinoids (eCBs) within the arcuate of obese mice and patients, we investigated the effects of a HFD on eCB-mediated astrocyte-neuron signaling. Using transgenic and pharmacological tools, we found that eCBs regulate the frequency of basal astrocyte calcium oscillations and SICs in control and HFD animals. These findings indicate the existence of astrocyte-neuron communication in the ARC, its regulation by eCB signaling, and its dysregulation in HFD.

Disclosures: **A.K. Scott:** None. **C.D. Murat:** None. **P. Kofuji:** None. **A. Araque:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.08/B98

Topic: B.11. Glial Mechanisms

Title: Glial cells induced restructuring of corpus callosum and pattern of constancy in fractional anisotropy in high-grade glioma

Authors: *V. PAREEK[1], P. K. ROY[2];
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Abstract: Introduction High-Grade Gliomas (HGG) with poor prognosis is the fatal gliomata condition. In the current study, we endeavor to explore the restructuring/remodeling induced in the corpus callosum (CC) structure evaluated using diffusion tensor imaging (DTI) application. **Materials and Methods** The unilateral HGG subjects are exclusively recruited for this study having normal appearing CC in the T1 weighted structural image and the FA map of the subject's diffusion space. The total of 8 glioma and control subjects are scanned for T1-weighted images and DTI acquisitions using 3 Tesla magnetic resonance scanner. Turbo field echo (TFE SENSE) sequence and pulse sequence with 32 gradient directions with a gradient of 1000s/mm2 are used for TI-weighted and DTI acquisitions respectively. FDT (FMRIB's Diffusion Toolbox) a software tool from FSL (FMRIB's Software Library) is used for the analysis of diffusion-weighted images evaluating Fiber density, Radial Diffusivity (RD), Mean Diffusivity (MD), Fractional Anisotropy (FA), and Lattice Index (LI). **Results** No differences ($p>0.05$) observed for Fiber density, RD, FA, and LI. Mild Significant Difference ($p=0.028$) for MD is observed. **Discussion** With the glioma cell infiltration in the CC, the axonal loss is evident because of malignant glioma-induced neuronal cell death because of glutamate excitotoxicity primarily, the increased cellular count of glioma cells may induce the CC to undergo remodeling whereby the fiber density does not change, even though the CC dystrophies. The constancy of FA and LI are representative of in-variant fiber density. The mild increase in MD and no difference in RD represents slight edema and myelin intactness respectively in the glioma condition.

Fiber Density and Diffusivity Indices/	t	df	*p*-Value	*p*-Value Summary	R squared (eta squared)	Glioma Effect ($p < 0.05$)
Fiber Density	0.3013	14	0.7675	ns	0.006444	x
Radial Diffusivity	2.009	14	0.0643	ns	0.2237	x
Mean Diffusivity	2.435	14	0.0289	*	0.2975	⬆
Fractional Anisotropy	0.88173	14	0.4274	ns	0.04555	x
Lattice Index	0.7929	14	0.4411	ns	0.04297	x

Disclosures: **V. Pareek:** None. **P.K. Roy:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.09/B99

Topic: B.11. Glial Mechanisms

Support: AHA 19POST34380079 [Martinez]
NIH RO1 HL128454 (DDK)

Title: Excitatory amino acid transporters (EAATs) and presynaptic afferent metabotropic glutamate Receptors (mGluRs) differentially limit synaptic currents via control of presynaptic calcium and extracellular glutamate kinetics in normoxia and chronic intermittent hypoxia (CIH)

Authors: *D. MARTINEZ[1], G. E. HERMANN[2], E. M. HASSER[3], R. C. ROGERS[2], D. D. KLINE[4];
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Abstract: The nucleus tractus solitarii (nTS) is the first central integration site for visceral reflexes including the chemoreflex. Sensory afferent signals are transmitted to the nTS via release of glutamate (Glu). Extracellular Glu is removed from the synaptic cleft via EAATs, limiting Glu receptor activation. We have shown that general EAAT block with DL-TBOA reduced the amplitude of afferent (TS)-evoked EPSC (TS-EPSC). Interestingly, both activation of Group II/III mGluRs and CIH, a model of obstructive sleep apnea, decrease TS-EPSC amplitude. Here we sought to identify the contribution of presynaptic mGluR-II/III receptors, which attenuate TS-EPSCs in naïve rats, to the decrease in TS-EPSC amplitude following EAAT

block and CIH. We used a combination of electrophysiology, live cell imaging with the fluorescent Glu reporter (iGluSnFR), and presynaptic afferent Ca^{2+} imaging. Male Sprague-Dawley 3-8 week old rats underwent 10 day normoxia (Norm, 21% O_2) or CIH (alternating 21% O_2 and 6% O_2, 8h/day) followed by brain slice generation. Prior to slice generation, a subset of rats received either nTS microinjection of AAV-hSyn-iGluSnFR followed by 4 weeks of expression, or injection into the nodose ganglia of the Ca^{2+} tracer Cal-520 Dextran and allowed 5 days for anterograde nTS transport. TS-EPSC amplitude in monosynaptic nTS neurons, extracellular Glu concentration $[Glu]_e$, or presynaptic Ca^{2+} [(ΔF/F)%] was monitored during 20 Hz stimulation in aCSF, EAAT block (TFB-TBOA, 500 nM) alone, and during block of EAAT and mGluR-II/III receptors (eGlu & MSOP 200 μM). In Norm, EAAT block reduced TS-EPSC amplitude, and mGluR-II/III block limited the EAAT-induced depression. To confirm a presynaptic mechanism, presynaptic Ca^{2+} was measured; Ca^{2+} entry decreased after EAAT block but was restored to control after EAAT-mGluR-II/III block. To examine the consequence of reduced afferent Ca^{2+}, which is responsible for attenuated TS-EPSCs, we examined $[Glu]_e$. EAAT block increased $[Glu]_e$ relative to aCSF; additional mGluR-II/III block further enhanced $[Glu]_e$ and dramatically slowed its decay. In CIH-exposed rats, TS-EPSC amplitude was smaller compared to Norm. However, in CIH EAAT block increased TS-EPSC amplitude differing from the decrease seen in Norm. Following CIH, in aCSF $[Glu]_e$ during 20 Hz stimulation was reduced relative to Norm. EAAT block produced only a modest increase in $[Glu]_e$, further mGluR-II/III block dramatically enhanced $[Glu]_e$. These data suggest that EAATs regulate $[Glu]_e$, Glu kinetics and the amplitude of TS-EPSCs through mGluR Group II/III. CIH reduces EAAT influence on synaptic activity and mGluRs contribute to the reduced TS-EPSC.

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Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.10/B100

Topic: B.11. Glial Mechanisms

Support: NIH RF1AG051495

Title: Impact of a TREM2 variant on neuronal pathology in a mouse model of tauopathy

Authors: *V. JADHAV[1], G. XU[3], S. PUNTAMBEKAR[3], S. BISSEL[3], P. LIN[4], S. PHILTJENS[5], M. MOUTINHO[3], A. OBLAK[2], B. T. LAMB[3];
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Abstract: Genome-Wide Association Studies have identified variants of Triggering Receptor Expressed on Myeloid cells-2 (TREM2) as risk factors for different types of dementia. The TREM2-R47H variant confers increased risk for developing Alzheimer's disease, while the TREM2-Y38C mutation is associated with a Frontotemporal Dementia-like phenotype. Hyperphosphorylated tau (pTau) is the key similarity in these diseases, however, the impact of microglial TREM2 on tau pathology remains understudied. We hypothesized that TREM2 mutations will change microglial homeostasis early in the disease, leading to altered tau pathology and neuronal functions.
To test this, we generated mice expressing human tau (htau) with the TREM2-Y38C mutation (htau;Trem2$^{Y38C/+}$). We found that 6-month-old htau;TREM2$^{Y38C/+}$ mice showed reduced intraneuronal pTau as compared to controls. There was a nonsignificant trend of reduced cortical neuronal counts in htau;TREM2$^{Y38C/+}$ mice, suggesting neuronal death did not significantly account for the reduced tau pTau. Next, we analyzed protein levels of PSD95 and synaptophysin in cortical brain lysates and found a reduction in PSD95, but not in synaptophysin, suggesting a loss of post-synaptic elements. These results suggest that reduced intraneuronal tau is not protective. For an unbiased look at pathology, we performed gene expression analysis, using the mouse Neuroinflammatory panel from NanoString Technologies on cortical lysates. Endothelial cells showed the most gene changes in htau;TREM2$^{Y38C/+}$ mice, therefore, we quantified cortical cytokines using Meso Scale Discovery to recognize the factors that impact endothelial cells. Increased IL12, IL6 and decreased IL2 in htau;TREM2$^{Y38C/+}$ mice were observed. These cytokines have been reported to reduce vascular permeability.
Vascular permeability alters neuronal functions and excitability which potentially explains the significant alterations observed in neuronal genes in our Nanostring analysis. We plan to study the underlying mechanisms of reduced intraneuronal pTau and altered neuronal functions elicited by TREM2-Y38C mutation by deciphering the molecular links between microglial TREM2 and endothelial cell hemostasis.

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Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.11/B101

Topic: B.11. Glial Mechanisms

Title: Signaling through the C3a receptor modulates peri-infarct reactive gliosis after ischemic stroke

Authors: A. STOKOWSKA[1], Y. LI[1], J. MORAN SUAREZ[1], M. PEKNY[2], *M. PEKNA[1]; [1]Lab. of Regenerative Neuroimmunology, Dept. of Clin. Neurosci., [2]Lab. of Astrocyte Biol. and CNS Regeneration, Dept. of Clin. Neurosci., Sahlgrenska Acad. at the Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Ischemic brain injury is a powerful inducer of reactive gliosis that serves to demarcate the lesion and restore tissue homeostasis. Prolonged reactive gliosis, can, however, inhibit ischemia-induced plasticity and functional recovery. Complement is a component of the innate immune system that has emerged as a regulator of multiple neural processes such as developmental neuronal migration, synaptic pruning and neuroprotection.
We previously showed that signaling through the receptor for complement-derived peptide C3a (C3aR) stimulates neural plasticity and intranasal treatment with C3a facilitates recovery of motor function after ischemic stroke (Stokowska et al., Brain, 2017). In an *in vitro* ischemia model, C3a increased the survival of astrocytes and reduced their expression of GFAP (Shinjyo et al, Mol Neurobiol, 2016). Here we used the photothrombotic stroke model in mice to investigate the role of C3a-C3aR axis in the regulation of glial responses to brain ischemia. We observed that transgenic overexpression of C3a in reactive astrocytes reduced the immunoreactivity of GFAP in the peri-infarct cortex 3 weeks after ischemic stroke; the absence of C3aR had the opposite effect. Overexpression of C3a increased whereas the absence of C3aR reduced the density of microglia and infiltrating blood-derived monocytes / macrophages (Iba-1 positive cells). Daily intranasal treatment of wild-type mice with C3a for 2-3 weeks starting 7 days after stroke induction reduced the expression of GFAP but did not affect the density of Iba-1 positive cells in the peri-infarct cortex assessed 3 and 8 weeks after ischemic stroke. Together these results show that the C3a-C3aR axis regulates reactive gliosis in the peri-infarct region exerting opposite effects on astrocytes and microglia / macrophages. Modulation of peri-infarct reactive astrogliosis may contribute to the positive effect of intranasal treatment with C3a on neural plasticity and functional recovery.

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Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.12/B102

Topic: B.11. Glial Mechanisms

Support: ARSEP project R18126KK

Title: Actomyosin inhibition in astrocytes leads to cytoskeleton remodeling and to an enhanced neuronal regenerative profile

Authors: *S. C. LEITE[1], R. SANTOS[2], A. MEI[3], M. C. MARCHETTO[3], F. H. GAGE[3], Z. LENKEI[2];
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Abstract: Cytoskeleton remodeling is fundamental for neuronal development and function and its impairments often featured in neurodegenerative diseases. A major key regulator of actin cytoskeleton is the RhoA/ROCK pathway. RhoA/ROCK pathway is a negative regulator of axonal regeneration and its role has been confirmed in *in vivo* models of central nervous system (CNS) injury. However, transition to clinics has been hindered by many systemic side effects of inhibitors of the RhoA/ROCK pathway. An interesting alternative is the targeted inhibition of a key individual downstream effector that would ideally result in a similar effect with decreased side effects. Here, we present actomyosin inhibition as an alternative to target to RhoA/ROCK pathway.
Actomyosin is a negative regulator of axonal growth by direct reorganization of the axonal growth cones. However, it's *in vivo* use has been limited because blebbistatin, the commonly used inhibitor, has low aqueous solubility and high toxicity. Recently, new blebbistatin-derived molecules such as para-amino-blebbistatin and para-nitro-blebbistatinhave been developed, conjugating decreased toxicity with increase solubility and photo-stability, while conserving the growth-enhancing effect of blebbistatin in neurons. Importantly, actomyosin inhibition by these new tools in astrocytes leads to a fast morphological reorganization, leading to the reactive astrocyte stellate shape. According to recent literature, stellate reactive astrocytes can either be of a protective or of a pro-inflammatory subtype. Our data suggests that actomyosin-inhibited astrocytes are not pro-inflammatory, as shown by their interleukin secretion profile. Moreover, the secretome of actomyosin inhibited astrocytes has a pro-regenerative effect in neurons, further suggesting that actomyosin inhibition drives astrocyte maturation into a protective subtype.
Our data suggests that the regulation of the CNS into a more permissive environment through actomyosin inhibition opens new solutions to enhance CNS regeneration in neurodegenerative conditions.

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Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

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Program #/Poster #: 372.13/C1

Topic: B.11. Glial Mechanisms

Support: T32 GM07628

Title: Lipolysaccharide attenuates NMDAR dependent LTD in the nucleus accumbens

Authors: *B. C. COLEMAN, D. KASHIMA, B. GRUETER;
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Abstract: The nucleus accumbens (NAc) is a key node within the mesolimbic reward network implicated in maladaptive motivational states including addiction. Adaptations at glutamatergic synapses in the NAc critically contribute to behavioral states associated with drugs of abuse. Recent work from our lab and others suggests a contribution from the innate immune system in synaptic function. Particularly, toll-like receptor 4 (TLR4), a pattern recognition receptor known to activate the innate immune response, has been implicated in NAc circuit development and synaptic plasticity. While synaptic plasticity mechanisms in the NAc are increasingly well-characterized, little is known about how the innate immune system regulates synaptic strength. We hypothesized that microglia, the brain's resident macrophage which express TLR4, modulate synaptic plasticity within the NAc. Utilizing *ex vivo* slice electrophysiology and D1 dopamine receptor reporter mice, we report that lipopolysaccharide, an immunogenic TLR4 agonist, attenuates NMDAR-dependent long-term depression in the NAc core. Further studies are ongoing to elucidate the mechanisms by which activated microglia can alter synaptic function in the NAc, and may ultimately contribute to maladaptive motivational states.

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Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.14/C2

Topic: B.11. Glial Mechanisms

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

Title: The role of axons in glioblastoma migration

Authors: *G. VELEZMORO JAUREGUI, V. M. POZO DEVOTO, M. NOVAKOVÁ, M. FEOLE, V. LACOVICH, G. B. STOKIN;
Translational Neurosci. and Aging Program (TAP), FNUSA-ICRC, Brno, Czech Republic

Abstract: Derived from glial cells, glioblastoma (GBM) is the most common and highly aggressive malignant brain tumors affecting adults. Currently, the standard treatment combines surgery with chemo- and radio-therapy. Despite enormous advances on the therapeutic field, the median overall survival is of only 12-18 months mainly because the GBM property to infiltrate

over a significant distance in the brain parenchyma; commonly along nerve fiber tracts followed by extracellular matrices of blood vessels according to clinic-pathological observations. Studies on GBM migration are hindered by the lack of efficient *in vitro* or *in vivo* migration models that describe their real-time interaction with axons. In this study, we postulate that glioblastoma cells exploit axons as physical tracks for their migration. We plan to test this hypothesis by co-culturing neurons and glioblastoma cell lines on microfluidic devices. In order to achieve this goal, a well characterized neuronal culture differentiated from human Inducible Pluripotent Stem Cells (iPSC) was used to be co-cultured with Glioblastoma line A172 (ATCC® CRL1620™) in compartmentalized microfluidic devices that allow specific interaction between GBM and isolated axons. The number of GBM able to migrate through the channels in presence and absence of axons was recorded every 24 hours using time lapse imaging. Our preliminary data show out an interesting close interaction between GBM and axonal structures in terms of migration.Further experiments already planned will shed light on understanding the genes and molecular pathways involved in the migration of GBM on axonal fibers.

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Poster

372. Glia-Neuron Interactions in Diseased Brain

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Program #/Poster #: 372.15/C3

Topic: B.11. Glial Mechanisms

Support: MOST 107-2321-B-010-009-MY3
MOST 107-2811-B-010-010

Title: The reduction of astrocytic Epm2a induces GLT1 process withdrawal accompanied by neuronal damage in a neuron-glia mixed culture system

Authors: *C.-C. HUNG[1], S.-P. HSU[1], Y.-H. LEE[1,2];
[1]Physiol., Natl. Yang-Ming Univ., Taipai, Taiwan; [2]Natl. Yang-Ming Univ., Brain Research Center, Taiwan

Abstract: Laforin, encoded by the *Epm2a* (epilepsy of progressive myoclonus type 2 A), is a dual specificity phosphatase with a carbohydrate-binding module that prevents glycogen hyperphosphorylation leading excessive glycogen deposition, and maintains normal glycogen metabolism through forming a complex with an E3 ligase, malin. Astrocytes are the main glycogen storage cells that provide energy to neurons in the brain for survival and function. *Epm2a* deficiency increases the abnormal glycogen inclusions knowns as Lafora bodies, and recently reported to decrease the expression of surface glutamate transporter 1 (GLT1) in

astrocytes. In this study, we investigated the effects of specific knockdown of *Epm2a* in astrocytes on glutamate homeostasis, neuronal survival, and neuron-astrocyte interaction in a neuron-glia culture system. We used GFAP promoter-driven shRNA to specific knockdown *Epm2a* expression in astrocytes. On immunostaining with GLT1 and MAP2, we found that astrocytic *Epm2a* knockdown significantly caused GLT1 process withdrawal and decreased the number of neuronal dendrites in this mixed cultured cell; moreover, western blot analysis showed the induction of GLT1 protein expression in the neuron-glia culture. Further, the survival pathway of AKT phosphorylation was decreased after astrocytic *Epm2a* knockdown. We also found that *Epm2a* knockdown in pure astrocytes with stellate-like morphology revealed shorter GLT1-labeled process and decreased GAP43 phosphorylation, which mediates astrocyte plasticity. Thus, astrocytic *Epm2a* may mediate astrocyte plasticity and GLT1 function to support neuronal survival. Importantly, *Epm2a* increases GLT1 expression on the astrocytic process to maintain glutamate homeostasis and prevent excitotoxicity leading to neuron damage.

Disclosures: C. Hung: None. **S. Hsu:** None. **Y. Lee:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

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Support: This work was partially supported by Conacyt grant 239192 (SLC) and scholarship 338376 (CJCA).

Title: Repeated morphine or fentanyl administration in rats induces glial and neuronal NLRP3 inflammasome activation on the dorsal raphe nucleus

Authors: *C. J. CARRANZA-AGUILAR, C. GONZÁLEZ-ESPINOSA, S. L. CRUZ; Dept. de Farmacobiología, Ctr. de Investigación y de Estudios Avanzados del IPN, Mexico City, Mexico

Abstract: Morphine and fentanyl are effective μ-opioid receptor (MOR) agonists, but tolerance to their analgesic effects develops after repeated use. Several opioids induce neuroinflammation by nuclear transcription factor (NF)-κB-dependent pro-inflammatory cytokine release, but the participation of intracellular innate immune receptors in the phenomenon remains as an important question to be solved. Here, we studied the effects of repeated administration of morphine or fentanyl on NOD-like receptor protein 3 (NLRP3) activation in glial and neuronal cells in the dorsal raphe nucleus (DRN), which exerts a regulatory role in pain. Male Wistar rats (250 g) were i.p. injected with morphine (10 mg/kg) or fentanyl (0.1 mg/kg) three times daily, for 6 days. Antinociception was measured by the tail-flick test after the 1st, 7th, and 19th

administration. Activation of caspase-1 and NLRP3 was analyzed by immunofluorescence. For specific cell staining, the following antibodies were used: anti CD11b (microglia), anti-Glial Fibrillary Acidic Protein (GFAP) for astrocytes and anti-NeuN for neurons. As expected, repeated morphine or fentanyl administration produced analgesic tolerance to different extents. Rats develop hyperalgesia two hours after the 19th fentanyl (but not morphine) administration. Co-treatment with any of the used opioids and the NF-κB inhibitor minocycline delayed tolerance development. Both morphine and fentanyl increased the immunoreactivity to NLRP3 and caspase-1 in astrocytes and neurons (but not in microglia), whereas fentanyl produced greater effects. NLRP3 activation was mediated by opioid receptors because naloxone co-treatment prevented it. Differences between both opioids were also observed in their ability to induce NLRP3 activation in the DRN. In conclusion, repeated morphine and fentanyl induces NLRP3 activation in DRN and drugs targeting NF-kB or NLRP3 could be used as adjuvant therapy to prevent opioid tolerance.

Disclosures: C.J. Carranza-Aguilar: None. **C. González-Espinosa:** None. **S.L. Cruz:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.17/C5

Topic: B.11. Glial Mechanisms

Support: Faculty of Medicine, University of Cologne, Germany (#/2017, #345/2018)

Title: Crosstalk between stressed brain-cells: Direct and indirect effects of ischemia and aglycemia in microglia

Authors: *M. RABENSTEIN[1], S. U. VAY[1], S. BLASCHKE[2,3], H. L. WALTER[1], A. LADWIG[1], G. R. FINK[1,3], M. SCHROETER[1,3], M. A. RUEGER[1,3];
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Abstract: *Background:* In cerebral ischemia, microglia have a dichotomous role in keeping the balance between pro- and anti-inflammatory mediators to avoid deleterious chronic inflammation and to leverage repair processes. *Methods:* We examined functional and inflammatory markers in primary rat microglia *in vitro* after oxygen-glucose-deprivation (OGD) or glucose-deprivation (aglycemia). We then investigated the preconditioning effect of OGD or aglycemic preconditioning to a subsequent strong inflammatory stimulus, namely lipopolysaccharides (LPS). Moreover, an "*in vitro* brain model" of neurons and glia, differentiated from primary rat neural stem cells, was exposed to OGD or aglycemia. Conditioned medium (CM) of this neuronal/glial co-culture was then used to pre-condition microglia, followed by LPS as a "second

hit". *Results:* OGD or aglycemia at sublethal doses did not significantly affect microglia function including the expression of inflammatory markers. However, preconditioning with either OGD or aglycemia led to a decreased pro-inflammatory response to a subsequent stimulus with LPS. Interestingly, the anti-inflammatory markers IGF-1 and IL-10 were additionally reduced after such preconditioning, while expression of CD206 remained unaffected. Treatment with CM from the neuronal/glial co-culture alone did not affect the expression of inflammatory markers. In contrast, preconditioning with CM increased the expression of both pro- and anti-inflammatory markers from microglia upon a second hit with LPS. *Conclusions:* Data suggest specific and distinct microglia signatures in response to metabolic stress: While metabolic stress directly applied to microglia mitigated their subsequent response to inflammation, indirect metabolic stress experienced by neighboring cells such as neurons and astroglia induced microglia alertness, with subsequent increase of both pro- and inflammatory microglia markers.

Disclosures: M. Rabenstein: None. **S.U. Vay:** None. **M.A. Rueger:** None. **M. Schroeter:** None. **G.R. Fink:** None. **S. Blaschke:** None. **A. Ladwig:** None. **H.L. Walter:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.18/C6

Topic: B.11. Glial Mechanisms

Support: Veteran Affairs Career Development Award
Brain and Behavior Research Foundation NARSAD Young Investigator Award

Title: The effects of adolescent intermittent ethanol exposure on astrocyte-synaptic coupling and contact mediated signaling

Authors: *C. D. WALKER[1], H. G. SEXTON[1,2], M.-L. RISHER[1,3,2];
[1]Joan C. Edwards Sch. of Medicine, Marshall Univ., Huntington, WV; [2]Huntington Veteran Affairs Med. Ctr., Huntington, WV; [3]Duke Univ. Med. Ctr., Durham, NC

Abstract: INTRODUCTION: Adolescence is a critical time of brain development, characterized by neuronal maturation and refinement of synaptic circuitry. During this period, increased independence is at odds with delayed development of inhibitory control, contributing to impulsive, risky behavior including binge drinking. Animal models of binge drinking have shown that ethanol (EtOH) engenders long-term deficits in cognition associated with changes in neuron structure and function, but the mechanisms underlying these changes are not well understood. While progress has been made to understand the consequences of binge drinking on neuronal function, little is known about the effects of EtOH on glia. Astrocytes couple with synapses and through secreted and contact mediated signaling, play a critical role in regulating

synaptic structure and function. Therefore, we hypothesize that adolescent EtOH exposure disrupts astrocyte-synapse coupling to drive changes in neuronal circuitry that persist into adulthood.
METHODS: Male Sprague Dawley rats received intracranial injections of an astrocyte-specific adeno-associated GFP virus in medial prefrontal cortex (mPFC) and dorsal hippocampus (dHIPP). Beginning at PND30, animals received intermittent EtOH (5g/kg i.g.) or water for 16 days. Brains were collected 24 hours after the 10^{th} dose and 26 days later (PND70). Co-localization of postsynaptic marker PSD95 with fine astrocyte processes was quantified from 3D reconstructions generated via IMARIS analysis of confocal microscopy-imaged astrocytes. Western blotting was performed to investigate the expression of proteins involved in astrocyte-synaptic contact mediated signaling.
RESULTS: 24 hours after the 10^{th} dose of EtOH there was a significant decrease in the co-localization of astrocytic processes with PSD95 in dHIPP, indicative of astrocyte-synaptic decoupling. Data from mPFC will also be presented. At PND70 there is a significant decrease in astrocyte volume and a decrease in astrocyte-synaptic coupling following EtOH in both the dHIPP and mPFC. Changes in protein expression will also be presented.
CONCLUSION: Appropriate communication between astrocytes and neurons via secreted and contact mediated signaling require astrocytes to be in close proximity to synapses. The decoupling of astrocyte-synaptic partners may play a critical role in the disruption of normal synaptic structure and function that we have previously observed following adolescent intermittent EtOH exposure.

Disclosures: C.D. Walker: None. **H.G. Sexton:** None. **M. Risher:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.19/C7

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01AG041944

Title: An analysis of hippocampal microglia and astrocytes in a rat model with features of delirium reveals possible mechanisms behind memory dysfunction

Authors: *L. G. RODRIGUES[1], N. SALLA[2], A. S. ARNOLD[1], J. WEIR[3], S. L. PATTERSON[1];
[1]Biol., [2]Dept. of Biol., [3]Temple Univ., Philadelphia, PA

Abstract: Older individuals are more likely to experience an abrupt decline in mental function, termed delirium, after events (e.g. infection, injury, or surgery) that trigger activation of the

peripheral immune system. Although the decline is often temporary, it is associated with increased risk for dementia and can exacerbate existing neurocognitive dysfunction. Little is known about the underlying mechanisms of this decline, but a rodent model may provide some clues. Aging (24 month old) F344xBN rats are generally healthy with no major physical or cognitive deficits. However, after a peripheral immune challenge (i.p. injection of *E. coli*), the brains of aged rats mount an exaggerated inflammatory response (producing more pro-inflammatory cytokines, e.g. IL-1β) compared to those of younger (3 month old) counterparts. The aberrantly elevated levels of these cytokines are associated with deficits in memory and synaptic plasticity.
Memory-related plasticity is dependent on complex, highly regulated interactions between neurons, astrocytes, and microglia. Microglia phagocytize foreign invaders and clear debris from damaged CNS cells; they also interact with neurons by removing synapses marked by "eat-me" signals (C1q and C3). We have shown that microglia display a more amoeboid morphology, consistent with a more activated state in aged vs young, and *E. coli*- vs saline-injected. The combination of age and *E. coli*-injection exacerbated these differences. Similarly, we are investigating the distribution and morphological profiles of astrocytes to better understand their role in the immune response in the aging brain. Astrocytes may interact with microglia to regulate neuroinflammation and maintain neuronal health and integrity.
During development, astrocytes and microglia contribute to the elimination of inappropriate synapses and integration of new neurons into existing circuits, essential processes for establishing appropriate connectivity in a healthy brain. Increasing evidence suggests that adult neurogenesis is regulated by similar neuron-glia interactions involved in memory formation. We will investigate how glial responses to acute immune challenge may impact the process of neurogenesis. Immunohistochemistry (IBA1, GFAP, DCX) will be used to examine differences in microglia, astrocytes, and newly differentiated neurons between young and aged rats. A more complete understanding of interactions between these cells following an immune challenge could provide insights into the etiology of delirium.

Disclosures: L.G. Rodrigues: None. **N. Salla:** None. **A.S. Arnold:** None. **J. Weir:** None. **S.L. Patterson:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.20/C8

Topic: B.11. Glial Mechanisms

Support: 2018R1A2A1A05077118
2016M3C7A1904148
NRF-2017R1A5A2015391

Title: Pyruvate dehydrogenase kinase governs hypothalamic inflammation in mouse models of diabetes and obesity

Authors: *M. H. RAHMAN[1], A. BHUSAL[1], J.-H. KIM[1], M. K. JHA[2], Y. GO[3], I.-S. JANG[4,5], I.-K. LEE[6], K. SUK[1,4];
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Abstract: Chronic inflammation in the hypothalamus has been proposed as a key pathological factor that alters feeding behaviors and energy homeostasis associated with obesity and diabetes. Emerging evidence suggests that metabolic shift from oxidative phosphorylation to glycolysis contributes to neuroinflammatory responses and pathophysiology of diverse neurological disorders. We report that streptozotocin- or high fat diet-induced diabetes enhanced hypothalamic expression and activity of pyruvate dehydrogenase kinase (PDK), a key regulatory enzyme in mitochondria, that caused glycolytic metabolic shift along with substantial inflammatory activation in the hypothalamus. Genetic ablation or inhibition of hypothalamic PDK attenuated diabetes/obesity-induced neuroinflammatory hallmarks in the hypothalamus and food/calorie intake. Moreover, dysregulation of hypothalamic neuropeptidergic circuitry involved in the regulation of feeding behavior was improved by deficiency or inhibition of hypothalamic PDK. Studies using primary astrocytes revealed that PDK plays a critical role in altered glycolytic metabolism and inflammatory activation of glial cells, which favor hypothalamic inflammation *in vivo*. Collectively, these findings unveil a novel role of PDK in regulating metabolic and inflammatory pathways that contribute to hypothalamic manifestations of diabetes and obesity.

Disclosures: **M.H. Rahman:** None. **A. Bhusal:** None. **J. Kim:** None. **M.K. Jha:** None. **Y. Go:** None. **I. Jang:** None. **I. Lee:** None. **K. Suk:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.21/C9

Topic: E.04. Voluntary Movements

Support: Canadian Institute of Health Research
American Association of Endodontists

Canadian Academy of Endodontics
Alpha Omega
Faculty of Dentistry Bertha Rosenstadt Endowment Fund
International College of Prosthodontics

Title: Are astrocytes the stars controlling orofacial cortical neuroplasticity?

Authors: *L. AVIVI-ARBER;
Univ. of Toronto Dent., Toronto, ON, Canada

Abstract: In the healthy brain, star-shape astroglial cells (i.e., astrocytes) play an important role in driving, mediating and modulating neuronal function and neuroplasticity. Similar to neurons, astroglial cells have a remarkable capacity for structural as well a functional plasticity that underlies their diverse functions. Little is known of their structural characteristics within the orofacial sensorimotor cortex and their involvement and roles in orofacial sensorimotor functions in health and after injury or disease. In this presentation, findings from our recent studies will be presented. We have shown that: **(1)** extraction of 3 maxillary molar teeth produces sex-dependent changes in peri-oral mechanosensitivity of BXA24 mice; **(2)** extraction of 3 maxillary molar teeth induces, 1-8 weeks later, **(a)** functional neuroplasticity in Sprague-Dawley rats and C57BL/6J male mice manifested as decreased jaw and tongue motor representations and sensorimotor cortex excitability; **(b)** structural plasticity in BXA24 female mice manifested as decreased volume of MRI-defined gray and white matter of the sensorimotor cortex; **(c)** sex-dependent structural plasticity in BXA24 mice manifested in differential morphological changes in astroglial processes; **(3)** acute noxious stimulation of the tooth pulp in Sprague-Dawley male rats produces decreased sensorimotor cortex neuroplasticity manifested as decreased excitability that can be modulated by inhibition of the astroglial enzyme glutamine synthetase (GS) within the sensorimotor cortex. GS is a specific astroglial enzyme that converts the astroglial glutamate to glutamine which is a major precursor for the excitatory (glutamate) and inhibitory (gamma aminobutyric acid, GABA) neurotransmitters in neurons; **(4)** tooth extraction in Sprague-Dawley rats and BXA24 mice can produce sex-dependent plasticity in the morphological features of astroglial cells. Acute tooth stimulation can also produce astroglial plasticity in Sprague-Dawley male rats. Better understanding of neuro-astroglial processes within the sensorimotor cortex is an important step towards the development of improved prevention and management of impaired oral sensorimotor functions.

Disclosures: L. Avivi-Arber: None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.22/C10

Topic: C.03. Parkinson's Disease

Title: Human astrocytoma line 1321N1 spontaneously drives SH-SY5Y neuronal differentiation in the absence of traditional differentiation factors in coculture

Authors: A. F. HOFFMAN, P. NUTHULAGANTI, A. S. GOETZ, ***J. C. CLEMENS**; GlaxoSmithKline, Collegeville, PA

Abstract: As the incidence of neurodegenerative diseases continues to rise, it is becoming increasingly clear that these diseases will represent the top unmet medical and public health needs in the coming decades. We are developing neuron-based 384 well assay platforms for target and phenotypic based drug discovery approaches. The platform utilizes the SH-SY5Y neuroblastoma cell line that can be differentiated into a dopaminergic neuron phenotype. Traditionally, SH-SY5Y differentiation protocols utilized retinoic acid, dibutyryl cyclic AMP, and brain derived neurotrophic factor to achieve differentiation and support cell survival. We have found that these factors could be removed from the differentiation protocol if the SH-SY5Y cells were grown in coculture with the human astrocytoma line called 1321N1 in the presence of Laminin 521. SH-SY5Y cells elaborate robust neurite processes in the coculture by day ten, which is similar to what we typically see in SH-SY5Y monoculture with differentiation factors at three weeks. Once differentiation has occurred, the elaborated neuron networks can be maintained in culture for several weeks allowing for the analysis of both short and long-term assay modalities. Coculture of SH-SY5Y cells not only reduces reagent costs and time to produce the assay platform, it also is more representative of the *in vivo* environment and a better experimental system for drug discovery. Combined with high content image analysis and a battery of cellular and mitochondrial health reporters, we anticipate that this platform will better enable and accelerate drug discovery and our understanding of the etiology of neurodegenerative diseases.

Disclosures: A.F. Hoffman: None. **P. Nuthulaganti:** None. **A.S. Goetz:** None. **J.C. Clemens:** None.

Poster

372. Glia-Neuron Interactions in Diseased Brain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 372.23/C11

Topic: B.11. Glial Mechanisms

Support: NIH NIA AG048232
NIH NIA AG058047
Paul & Daisy Soros Fellowship
Gerald J. Lieberman Fellowship

Title: Inhibition of IDO1 metabolically reprograms the neuronal milieu to rescue energetic and cognitive deficits in Alzheimer's disease

Authors: *P. S. MINHAS[1], A. L. HERNANDEZ[1], M. MCREYNOLDS[2], L. LIU[2], E. GAUBA[1], S. D. MHATRE[3], J. RABINOWITZ[2], F. LONGO[1], K. ANDREASSON[1];
[1]Dept. of Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA; [2]Lewis-Sigler Inst. for Integrative Genomics, Princeton Univ., Princeton, NJ; [3]AMES Res. Ctr., NASA, Mountain View, CA

Abstract: Background: Metabolic dysfunction is a well characterized feature of Alzheimer's disease (AD) that often precedes symptomatic AD. Indoleamine-2,3-dioxygenase 1 (Ido1) is the rate-limiting step of de novo nicotinamide adenine dinucleotide (NAD+) synthesis from tryptophan, an enzymatic cascade that is expressed in the glia of the brain. In this study, we sought to determine whether glial de novo NAD+ synthesis through Ido1 impacts cognition in AD. Method: APP PS1;IDO1-/- mice were subject to electrophysiologic, metabolomic, and behavioral studies. Genetics studies were complemented with pharmacological administration via oral gavage of Ido1 inhibitor EOS200271/PF-0684003 (15mg/kg) in APP-PS1 and 5xFAD mice for 10d. Metabolomics was performed using a Q-trap LC-MS/MS. Result: IDO1 inhibition increased the glial NADH/NAD+ ratio within the hippocampus of transgenic or treated mice, favoring the production of lactate. *In vivo* isotope metabolite tracing revealed a decrease in lactate shuttling from the astrocyte to the neuron through the monocarboxylate transporter 4 (MCT) in AD mice. Specifically, decreased U-13C-Glucose incorporation into the TCA cycle and oxidative phosphorylation resulted in decreased LTP and spatial memory deficits. APP-PS1;IDO1-/- mice as well as AD mice treated with the IDO1 inhibitor exhibited a rescue in metabolomic, LTP, and cognitive deficits. Blockage of MCT4, the transporter responsible for translocation of lactate from the astrocyte to the neuron, recapitulated cognitive deficits seen in AD even when the Ido1 enzyme was inhibited. Conclusion: Ido1 plays a pivotal role in the bioenergetic phenotype of glia and glial production of lactate may serve as a novel therapeutic target for normalization of bioenergetic and cognitive phenotypes associated with AD.

Disclosures: P.S. Minhas: None. **A.L. Hernandez:** None. **M. McReynolds:** None. **L. Liu:** None. **E. Gauba:** None. **S.D. Mhatre:** None. **J. Rabinowitz:** None. **F. Longo:** None. **K. Andreasson:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.01/C12

Topic: B.11. Glial Mechanisms

Support: R21HD091512

R01NS102382

Title: Xenotransplantation of human PSC derived microglia creates a chimeric mouse brian model that recapitulates features of human adult microglia

Authors: *R. XU, P. JIANG;
Rutger Univ., Piscataway, NJ

Abstract: Microglia, the brain-resident macrophages, exhibit highly dynamic functions in neurodevelopment and neurodegeneration. Human microglia possess unique features as compared to mouse microglia, but our understanding of human microglial functions is largely limited by an inability to obtain human microglia under resting, homeostatic states. We developed a human pluripotent stem cell (hPSC)-based microglial chimeric mouse brain model by transplanting hPSC-derived primitive macrophage precursors into neonatal mouse brains. The engrafted human microglia widely disperse in the brain and replace mouse microglia in corpus callosum at 6 months post-transplantation. Single-cell RNA-sequencing of the hPSC microglial chimeric mouse brains reveals that xenografted hPSC-derived microglia largely retain human microglial identity, as they exhibit signature gene expression patterns consistent with physiological human microglia and recapitulate heterogeneity of adult human microglia. Importantly, the chimeric mouse brain also models species-specific transcriptomic differences in the expression of neurological disease-risk genes in microglia. This model will serve as a novel tool to study the role of human microglia in brain development and degeneration.

Disclosures: R. Xu: None. **P. Jiang:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.02/C13

Topic: B.11. Glial Mechanisms

Support: NSFC Grant 81790642

Title: P2X7 receptor-mediated reactivation of retinal microglia in a mouse chronic ocular hypertension model

Authors: *X. HU, G.-L. ZHAO, N. YIN, F. LI, Y. MIAO, X.-L. YANG, Z. WANG;
Inst. of Brain Science, State Key Lab. of Med. Neurobio. and MOE Frontiers Ctr. for Brain Science, Fudan Univ., Shanghai, China

Abstract: In the present study, we investigated the mechanisms underlying retinal microglia reactivation in a mouse chronic ocular hypertension (COH) model. Our results showed that the

branched microglia in normal retina was changed to the ramified and amoeboid-like one and further agminated during 4 day to 4 weeks (G4d to G4w) after the elevation of intraocular pressure (IOP). The rod-like microglia in morphology was observed during G4w to G6w. Meanwhile, microglia was translocated from the inner/outer plexiform layer to the ganglion cell layer in COH retina. The expression of translocator protein (TSPO), a marker of the reactivated microglia, was increased in COH retinas, which could be reversed by intravitreal/intraperitoneal injections of the P2X7 receptor (P2X7R) inhibitor brilliant blue G (BBG), suggestive of P2X7R-mediated reactivation of microglia. Calcium imaging showed that the P2X7R agonist BzATP-induced elevation in intracellular Ca2+ level was quicker and higher in acutely isolated microglia from COH retinas, as compared with control. Moreover, in primary cultured mouse microglia, BzATP treatment increased the TSPO protein level, and induced the release of inflammatory factors (IL-6 and TNF-α). These results suggest that P2X7R mediates retinal microglia reactivation in experimental glaucoma model.

Disclosures: **X. Hu:** None. **G. Zhao:** None. **N. Yin:** None. **F. Li:** None. **Y. Miao:** None. **X. Yang:** None. **Z. Wang:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.03/C14

Topic: B.11. Glial Mechanisms

Title: Gene-wide screen and validation of micriglia pro-inflammatory mediators in stroke

Authors: *Y. YI[1], **Y. LU**[2];
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Abstract: Stroke activates microglia pro-inflammatory response that not only induces the early neuronal injuries but also causes the secondary brain infarction. Yet, the underlying mechanisms for how microglia becomes activated in stroke are still unknown. Here, using the next-generation of RNA sequencing we find a total of 778 genes increasingly expressed in brain of stroke mice. Of these, Hmgb2 is validated as a microglia activator and pro-inflammatory mediator. Hmgb2 binds to a promoter region of Ctss and activates Ctss transcription. Inhibition of either Hmgb2 or Ctss blocks microglia pro-inflammatory response and protects against brain damages and improves the neurological functions of stroke mice. The same neuronal protective effects against stroke injuries are achieved by knocking down C3ar1 gene, a complement 3a receptor 1 that modulates cytotoxic neuronal immune reactions in stroke. This study uncovers Hmgb2, Ctss and C3ar1 as the major microglia inflammatory and immune response mediators in stroke and hence warrants the promising targets for stroke therapies.

Disclosures: **Y. Yi:** None. **Y. Lu:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.04/C15

Topic: B.11. Glial Mechanisms

Support: JSPS Grant 17H03988
JST Grant JPMJPR18H4
JST Grant JPMJER1801

Title: Microglial networks in response to exogenously delivered particles in the brain

Authors: *A. OGAKI, Y. IKEGAYA, R. KOYAMA;
Grad. Sch. of Pharmaceut. Sciences, The Univ. of Tokyo, Tokyo, Japan, Tokyo, Japan

Abstract: Immune response mechanisms to exogenously delivered factors in the brain remain unclear. Accumulating evidence suggests that exogenous particles such as PM2.5 in the environment, especially those particles with a diameter less than a micron, i.e., nanoparticles, have been found in the human brain. Further, it has been suggested that the early-life exposure to nanoparticles can be a cause of the development of psychiatric disorders such as cognitive dysfunction, attention-deficit hyperactivity disorder (ADHD), or depression. However, cellular and molecular mechanisms how nanoparticles affect the structure and function of brain remain unknown. Here we examined the possible involvement of microglia, the resident major immune cells in the brain, in nanoparticle-mediated brain malfunction. We administered silica nanoparticles, major components of yellow dust in the atmosphere, to postnatal mice. Three days after the administration, we found the invasion of silica nanoparticles in the brain parenchyma. The nanoparticles were frequently found around the brain vessels and were surrounded and engulfed by gathering microglia. Further, the process of microglia that engulfed the nanoparticles often interacted each other. Real-time imaging of microglia in vitro revealed that silica nanoparticles engulfed by one microglia were frequently passed to another microglia in a form incorporated in microglia-associated vesicles. Finally, the nanoparticle-injected mouse pups exhibited aggressive and anxiety-like behaviors and these behavioral abnormalities were correlated to the activation of microglia around silica nanoparticles. Thus, it is possible that activated microglia configure microglial networks in response to silica nanoparticles for the protection of brain structure and function.

Disclosures: **A. Ogaki:** None. **Y. Ikegaya:** None. **R. Koyama:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.05/C16

Topic: B.11. Glial Mechanisms

Title: Dual effect of microglia on blood brain barrier permeability induced by systemic inflammation

Authors: *K. HARUWAKA[1,2], A. IKEGAMI[1], Y. TACHIBANA[1], N. OHNO[3], A. HASHIMOTO[1], D. KATO[1], R. ONO[1], A. J. MOORHOUSE[4], J. NABEKURA[2], H. WAKE[1]; [1]Div. of Syst. Neurosci., Kobe Univ. Grad. Sch. of Med., Kobe, Japan; [2]Natl. Inst. For Physiol Sci., Okazaki-Shi, Japan; [3]Dept. of Anatomy, Div. of Histology and Cell Biol., Jichi Med. University, Sch. of Med., Shimotsuke, Japan; [4]Sch. of Med. Sci., UNSW Australia, Sydney, Australia

Abstract: Microglia are sole immune cells in the central nervous system (CNS). Microglia are activated in neurological diseases such as multiple sclerosis, Alzheimer's disease and epilepsy, and release cytokines and neuro-trophic factors, which act on neurons and vessels to affect disease pathologies. The inflammatory cytokines released from the activated microglia enhance the permeability of the blood-brain barrier (BBB), which causes infiltration of immune cells and worsens intracerebral inflammation. On the other hand, microglia are also activated even during systemic inflammation such as severe infections or auto-immune diseases (e.g., systemic lupus erythematosus: SLE) that increase BBB permeability. However, it is not clear how systemic inflammation causes microglia activation and brain pathologies. Since microglia are highly motile cells and frequently contact vessels, this study focused on microglia, and investigated their dynamics and change of BBB permeability during systemic inflammation. Toward this goal, this study used SLE model mice and systemic inflammation induced by lipopolysaccharide (LPS) intraperitoneal injection. Using two-photon *in vivo* imaging, we showed that microglia migrated to the vessel in response to systemic inflammation which was associated with increased BBB permeability. Following genetic ablation of microglia, BBB permeability was increased in the early phase and reduced in the late phase of systemic inflammation. Those results indicated that microglia prevent BBB leakage in the early phase of inflammation, but exacerbate the leakage under prolonged inflammation. Further details shown by immunostaining revealed that vessel-associated microglia expressed Cldn5, a tight junction-related molecule, in the early phase and a phagocytosis marker, CD68, in the late phase. The CD68 immunoreactivity co-localized with that of a BBB component, aquaporin-4, suggesting that microglial phagocytosis of BBB component impairs BBB functions. In addition, inhibition of microglial activation with minocycline suppressed the increase of BBB permeability in the late phase. In summary, these

results showed that microglia have a dual function onto the BBB, and suggest that microglia can be the therapeutic target for the brain pathologies induced by systemic inflammation.

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Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.06/C17

Topic: B.11. Glial Mechanisms

Support: AA020023
NIAAA Grant AA020022
NIAA GRANT AA11605

Title: Dreadded microglia in ethanol-induced neuroimmune signaling and epigenetic modification

Authors: *J. Y. ZOU, L. G. COLEMAN, F. T. CREWS;
Ctr. for Alcohol Studies, Univ. North Carolina, Chapel Hill, Chapel Hill, NC

Abstract: Microglia play a critical role in ethanol-induced persistent brain neuroimmune signaling. To dissect the role of microglia in neuroimmune signaling during ethanol exposure, the present study used a novel Designer Receptor Exclusively Activated by Designer Drugs (DREADD) to selectively inhibit microglia in an organotypic hippocampal-entorhinal cortex (HEC) slice culture model. DREADDs under a CD68 (microglia/macrophage) promoter were transfected via an AAV9 vector. HEC slices were transfected with inhibitory Gi-coupled DREADDs for 24hrs and then treated with ethanol (100mM) for 4 days in the absence or presence of the DREADD-selective ligand clozapine-N-oxide (CNO). RT-PCR analysis indicates that ethanol-treated Gi-DREADD slices without CNO stimulation had significant higher levels of proinflammatory mediator mRNAs such as IL-1β, TNFα, MCP-1 and Trail comparing those activated by CNO stimulation. Similarly, activation of Gi-DREADD slices with CNO significantly reduced proinflammatory cytokine induction stimulated by TLR4/7 agonist LPS (100ng/ml) and Imiquimod (5ug/ml) respectively. These data suggest an essential role of microglia in neuroimmune signaling in response to ethanol and TLR activation. Furthermore, intracellular epigenetic molecules including G9a, KMD6B, DNMT1, DNMT3a and 3b were monitored. Our data indicate that ethanol exposure of Gi-DREADD slices significantly increased G9a, KMD6B and DNMT3a mRNA levels, which were further potentiated by CNO activation of Gi-DREADD slices. However, CNO activation of Gi-DREADD slices resulted in significant

reduction of DNMT1 and DNMT3b mRNA expression altered by ethanol exposure. We also tested the effects of CNO-alone on the expression of neuroimmune genes and epigenetic molecules in this model. Treatments of HEC slices with CNO alone for 4 days dose-dependently increased proinflammatory cytokine gene expression but significantly down-regulated epigenetic molecules, for example: TNFα.(Control:100±1; CNO-500nM:70±6; CNO-1uM:215±11; CNO-10uM:1346±19); G9a (Control:101±12; CNO-500nM:68±7; CNO-1uM:59±2; CNO-10uM:44±7). Together, these results reveal the important role of microglia in ethanol-induced neuroimmune signaling and epigenetic modification. Our results also suggest that cell type-specific DREADD-based approach may be a unique tool to selectively explore the physiological and pathological role of neurons or glia during ethanol exposure. (Supported by NIAAA)

Disclosures: **J.Y. Zou:** None. **L.G. Coleman:** None. **F.T. Crews:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.07/C18

Topic: B.11. Glial Mechanisms

Support: Grant-in-Aid for Scientific Research (B) (17H03988)
JST PRESTO (JPMJPR18H4)
JST ERATO (JPMJER1801)

Title: Microglia collect maternally delivered exogenous nanoparticles in the postnatal brain

Authors: *R. KONO, Y. IKEGAYA, R. KOYAMA;
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Abstract: Growing attentions have been paid to the influence of exogenous nanoparticles, including $PM_{2.5}$ in air pollution, on the brain. Particularly, embryonic brains are considered to be fragile to the invasion of exogenous nanoparticles because of the immaturity of blood-brain barrier. In addition, epidemiological studies have suggested that the exposure to concentrated exogenous nanoparticles during prenatal life correlates with the increased possibility to exhibit attention-deficit hyperactivity disorder (ADHD) later in life, but the underlying cellular mechanisms left undiscovered. Here, we examined the possible involvement of the brain resident immune cell microglia in the regulation of exogenously delivered nanoparticles during pregnancy in the brain of offspring. Silica nanoparticles, major components of yellow dust in atmosphere, were intranasally administered to pregnant mice. The brains of offspring during postnatal days were subjected to immunohistochemical analysis to examine the localization of fluorescently-labeled silica nanoparticles. We often encountered localized silica nanoparticles in cerebral cortex and meninges. Further, we found that localized nanoparticles were engulfed by

accumulating microglia. Because the processes of microglia that incorporated nanoparticles were extended toward the brain surface, it is possible that microglia play a role to remove maternally delivered nanoparticles from the postnatal brain.

Disclosures: R. Kono: None. **Y. Ikegaya:** None. **R. Koyama:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: B.11. Glial Mechanisms

Support: NRF-2016M3C7A1905098

Title: Chemokine production by microglia mediates blood derived-monocytes trafficking in neuroinflammation

Authors: M. HUANG[1,2], J. KIM[1], J. PARK[1,2], W. LEE[1], *J. LEE[1,2,3];
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Abstract: Microglia and recruited macrophages play major roles in neuroinflammation. We explored how these cells affect counterpart's polarization and infiltration and revealed some chemokines and receptors can be important modulators of the interaction. We set up a undirect co-culture system of BV2 (microglia cell line) and THP-1 (monocyte cell line). BV2 or THP-1 cultured in M1, M2 conditioned media. Transwell migration was used for assessment of THP-1 infiltration and BV2 migration. M1/M2 conditioned media of BV2 and THP-1 were assessed by proteome profiler array to find target cytokine and chemokine. The expression of chemokine receptors in THP-1 was confirmed by western blotting. 8 weeks old male, CCR2::RFP C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME) and followed by LPS injection (i.c.v). All animal procedures were following National Institutes of Health guidelines. Mice were anesthetized with an intraperitoneal (i.p.) injection of a mixture of zoletil (100 mg/kg) and xylazine (rompun, 10 mg/kg). THP-1 expressed CD206 when cultured in M2 conditioned media of BV2, and also got increased the infiltratory ability. Several chemokines such as CCL2, CCL3, CCL4 and CCL5 were significantly increased in conditioned media of BV2 while their receptor CCR4, CCR5 shows high expression in THP-1. Antagonists of CCR2, CCR4 and CCR5 significantly reduced the infiltration ability of THP-1 to BV2 M2 conditioned media. CCR2 positive cells in mouse cortex were significantly increased after LPS injection, when CCR4, CCR5 antagonist co-injection can reduce the number of infiltrated CCR2 positive cells.

Chemokine receptors CCR4, CCR5 can be strong candidates of target protein in new therapeutic strategies to acute brain inflammation by modulating the functions of microglia and monocytes.

Disclosures: **M. Huang:** None. **J. Kim:** None. **J. Park:** None. **W. Lee:** None. **J. Lee:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

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Program #/Poster #: 373.09/C20

Topic: B.11. Glial Mechanisms

Title: Targeting neuregulin-mediated microglial activation in Alzheimer's disease

Authors: *F. SONG, J. LIU, S. SCHRAM, S. MARTIN, J. A. LOEB;
Univ. of Illinois at Chicago, Chicago, IL

Abstract: The gliotrophic factor neuregulin1 (NRG1) is a key neuronal communication signal critical for normal peripheral and central nervous system development. However, in the mature nervous system NRG1 is reactivated in response to injury and leads to abnormal, pathogenic microglial activation. This occurs in the spinal cord after peripheral nerve injury in a model of chronic pain and in the SOD1 model of amyotrophic lateral sclerosis (ALS) where NRG1 promotes disease progression through microglial activation and neuronal death. We have developed a novel way to block NRG1 signaling using a humanized fusion protein that targets heparin-rich surfaces using NRG1's heparin-binding domain fused to a decoy NRG1 receptor called HBD-S-H4. We have previously shown that HBD-S-H4 effectively blocks microglia-induced inflammation after nerve injury preventing chronic pain and slows ALS disease onset and progression by blocking inflammatory microglia and preventing motor neuron loss in SOD1 mice.

In Alzheimer's Disease (AD), microglia have mixed roles in disease progression by both clearing Aβ deposits and releasing cytotoxic mediators. Aβ has been shown to activate microglia through innate immune receptors. Here, we hypothesize that NRG1 signaling from neurons to surrounding microglia promotes the local spread of AD pathology through microglial activation that could lead to synaptic loss and neurodegeneration. We found that intraventricular NRG1 augments microglial activation and Aβ plaque formation in early-stage 5XFAD mice. Blocking endogenous NRG1 activity with HBD-S-H4 prevents microglial activation and Aβ plaque formation in early-stage and reduces microglial activation and Aβ plaque formation in later stage disease. Mechanistically, NRG1 induces pro-inflammatory cytokine expression in cultured microglia and decreases $A\beta_{42}$ and the $A\beta_{42}/A\beta_{40}$ ratio in 5XFAD mice. Consistently, we have previously found that human cerebrospinal fluid from AD patients has increased NRG1 activity compared to normal controls or patients with other neurodegenerative diseases. Our results

suggest that blocking NRG1 signaling prevents and reduces AD pathology and supports the future use of our targeted therapeutic to slow disease progression in AD.

Disclosures: F. Song: None. **J. Liu:** None. **S. Schram:** None. **S. Martin:** None. **J.A. Loeb:** None.

Poster

373. Microglial Activation in Disease States

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Program #/Poster #: 373.10/C21

Topic: B.11. Glial Mechanisms

Support: Landesforschungsförderung Hamburg

Title: Optogenetic manipulation of microglia inhibits fundamental response properties

Authors: *L. LAPRELL, T. G. OERTNER;
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Abstract: Microglia are the only resident immune cells in the brain. Under physiological conditions microglia are highly ramified; with their thin processes they surveil the surrounding area and detect changes in the extracellular space, such as dying neurons, invading organisms, or alterations in neuronal activity. Under physiological conditions microglia exhibit a hyperpolarized membrane potential around -40 mV. Hyperpolarization together with low cAMP levels result in high microglial ramification and baseline surveillance. However, hyperpolarization has also been linked to pathophysiological microglia activation states, such as ageing and local tissue damage. Surprisingly, this damage-induced hyperpolarization could not be directly linked to the chemotactic response of microglia. In contrast, depolarization of microglia by pharmacological blockade or genetic deletion of potassium channels as well as raising extracellular K^+ leads to retraction of processes and decrease in microglia surveillance activity, also inducing an activated microglia phenotype. As pharmacological manipulations may affect glia, microglia and neurons, a direct causal link between microglia membrane potential, chemotaxis and activation state has been difficult to establish. By selectively manipulating microglia membrane potential using optogenetic tools we are able to gain a better understanding of the relationship between membrane potential and microglia activation state. Activation of channelrhodopsin with blue light allows us to depolarize microglia robustly. Repetitive light stimulation at 1 Hz can be applied for at least 45-60 minutes without adverse effects. In our experiments, we tested whether optogenetic depolarization before and during the local tissue damage affects the chemotactic response towards the damaged area. Activation of channelrhodopsin reduced the amplitude of damage-induced hyperpolarization and more importantly, increased the response time towards the damaged site. This attenuation in

chemotactic motility establishes neuronal damage-induced membrane hyperpolarization as a causal factor in microglia motility. We hypothesize that optogenetic depolarization reduces the driving force for Ca^{2+}, a very tightly controlled second messenger in microglia which has been shown to play a role in the induction of chemotaxis. In future experiments, we would like to use optogenetic microglia manipulation not only to investigate intracellular signaling pathways, but also to study the role of microglia in synaptic physiology with high temporal and spatial precision.

Disclosures: L. Laprell: None. **T.G. Oertner:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.11/C22

Topic: B.11. Glial Mechanisms

Title: Restraint stress suppresses microglia activation and production of pro-inflammatory cytokines

Authors: *Z. YU[1], M. SAKAI[4], Y. TAKAHASHI[2], C. ONO[3], H. TOMITA[3];
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Abstract: Altered inflammatory cytokine profiles are observed in the peripheral blood of patients with major depression and the brain of depression mice models, which suggest that depression is accompanied by immune dysregulation and activation of the inflammatory response system. Microglia are the primary immune cells of the central nervous system, which form a major component of the brain immune system and release pro- and anti-inflammatory cytokines in neurodegenerative diseases. However, microglial activation response to chronic stress such as chronic restraint stress had not been extensively explored. In this study, we subjected mice to 3 weeks restraint stress and allowed to recover for 3 weeks to investigate the stress and recovery impact on microglia activation. We found that restraint stress induced depression-like behaviors that were maintained 2 weeks after cessation of restraint stress. Following the restraint stress, microglia activation was significantly suppressed with decreased *Tnf, Il1b, Il10* and *Tgfb1* mRNA expression compared with controls. During the first week or two of recovery period, microglia activation and its M2 phenotype marker *Cd86* mRNA expression displayed a significant up-regulation along with significantly increased *Tnf, Il1b,Il10* and *Tgfb1* mRNA expression. Furthermore, *Il10* and *Tgfb1* mRNA expression were significantly increased throughout the 3 weeks after cessation of chronic restraint stress. These findings demonstrated that a chronic stressful event could produce delayed immune response in the brain,

may help us understand the microglia reversibility of the effects of chronic stress, which could also be used for further studies on anti-depression drug development.

Disclosures: Z. Yu: None. **M. Sakai:** None. **Y. Takahashi:** None. **C. Ono:** None. **H. Tomita:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

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Topic: B.11. Glial Mechanisms

Support: KGM4621922
KGM5281921
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NRF-2016M3A9B6903268

Title: Increased CD68 microglia/macrophages after transientmiddle cerebral artery occlusion in the rhesus monkey

Authors: *H.-G. YEO[1,3], J. HONG[2], Y. LEE[2,3], K. YI[4], C.-Y. JEON[2], J. PARK[2], J. WON[2], J. SEO[2], K. KIM[2], Y.-J. AHN[2,3], S. BAEK[2], Y. JIN[2], K.-J. JEONG[2], C.-H. CHOI[4], S.-H. CHA[4], S.-R. LEE[2,3];
[1]Natl. Primate Res. Ctr., Cheonju-si, Korea, Republic of; [2]Natl. Primate Res. Ctr., Cheongju, Korea, Republic of; [3]Korea Univ. of Sci. and Technol., Daejeon, Korea, Republic of; [4]Chungbuk Natl. Univ. Hosp., Cheongju, Korea, Republic of

Abstract: The role of microglia/macrophages after ischemic stroke is poorly understood. This study is to examine the function of microglia/macrophages in the focal infarct area after transient middle cerebral artery occlusion (MCAO) in rhesus monkeys. We evaluated infarct volume with MRI and neurological function to assess subsequent temporal changes. The post-mortem brains (n = 8) were harvested and analyzed immunohistochemically including two controls and at 3 and 24 hours; 2 and 4 weeks; 4, and 20 months respectively after MCAO by examining activated phagocytic microglia/macrophages. We found that the infarct volume progressively decreased from 1 to 4 weeks after MCAO, along with the neurological recovery. Cluster of differentiation (CD) 68-expressing microglia/macrophages in the infarct lesion were identified more in the chronic stage (2 weeks-20 months) than in the acute stage (3-24 hours). Surprisingly, 96-99 % of transforming growth factor beta (TGFβ) was co-localized in CD68-expressing cells. In addition, Ki67 was found in neurons and microglia in the infarct area. Collectively, microglia/ macrophages in the chronic stage, may exert anti-inflammatory effects by expressing TGFβ, which can promote neurogenesis and functional recovery after ischemic stroke.

Disclosures: H. Yeo: None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.13/C24

Topic: B.11. Glial Mechanisms

Title: Effects of α7 nicotinic receptor positive allosteric modulator PNU120596 on expression of PPAR-α and NF-κB following LPS-induced depressive-like behavior in mice

Authors: *S. RAHMAN[1], S. ALZAREA[2];
[2]Pharmaceut. Sci., [1]South Dakota State Univ., Brookings, SD

Abstract: Previous evidence indicates that brain α7 nicotinic acetylcholine receptor (α7 nAChR) has a critical role in regulating neuroinflammation implicated in the pathophysiology of major depressive disorder (MDD). The α7 nAChR stimulation was found to modulate the anti-inflammatory activity of nuclear peroxisome proliferator-activated receptor-α (PPAR-α) via its endogenous ligands in the brain. The objective of the present study was to determine the effects of PNU120596 on expressions of PPAR-α and nuclear factor-κB (NF-κB) in the hippocampus and prefrontal cortex, MDD-relevant brain regions in an inflammatory mouse model of MDD. We also examined the combination effects of PNU120596 and GW6471, a PPAR-α antagonist, against lipopolysaccharide (LPS)-induced depressive or cognitive deficit-like behavior using tail suspension test (TST), forced swim test (FST) and Y-maze test. Systemic administration of LPS (1 mg/kg, i.p.) resulted in downregulation of PPAR-α and upregulation of phosphorylated-NF-κB p^{65} in the dentate gyrus and CA1 regions of the hippocampus, and prefrontal cortex. The PNU120596 pretreatment (4 mg/kg, i.p.) significantly prevented LPS-induced dysregulation of PPAR-α and p-NF-κB p^{65} in these specific brain regions. The PNU120596 showed antidepressant-like activity against LPS-induced depressive-like behavior by decreasing immobility time during TST and FST. LPS-induced cognitive deficit-like behavior was also attenuated by the effects of PNU120596 due to increasing spontaneous alternations during Y-maze test. Coadministration of PNU120596 and GW6471(2 mg/kg) resulted in the disappearance of antidepressant and pro-cognitive-like effects of PNU120596. Overall, these results suggest that PPAR-α plays a key role in regulating antidepressant-like effects of PNU involving α7 nAChR signaling pathway in MDD. (*Supported in part by grant from SACM*)

Disclosures: S. Rahman: None. **S. Alzarea:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

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Topic: B.11. Glial Mechanisms

Support: NIH Grant NS060017
NMSS RG1507

Title: Disruption of microglial Stat3 signaling during early postnatal development has long-lasting effects on synaptic plasticity and social behaviors

Authors: H. C. LU, B. BARTH, S. KIM, K. KONGANTI, J. CAI, W. H. GRIFFITH, ***J. LI**; Texas A&M Univ., College Station, TX

Abstract: Microglia play critical roles in maturation and refinement of neural networks during postnatal brain development. Although microglia have been shown to arise from the yolk sac and colonize the CNS during early embryogenesis and undergo expansion during the first two postnatal weeks, it remains unclear what intrinsic signals regulate early postnatal microglia and how disruption of microglia at this stage affects brain function. Here we report that signal transducer and activator of transcription 3 (Stat3) regulates microglial survival and activation in the developing brain and that neonatal disruption of Stat3 signaling in microglia results in impaired hippocampal synapse plasticity in adult mice. *Cx3cr1*$^{CreERT2/+}$*:Stat3*^{loxP} mice were generated to achieve inducible deletion of exon 22 of the *Stat3* gene in microglia, which results in truncated Stat3 protein incapable of nuclear translocation. Neonatal *Stat3* ablation caused spontaneous microglial over-activation and transient loss of microglial cell population at postnatal day 10 (P10) that was recovered by P28. The transient decrease of microglial density was due to increased DNA damage and cell cycle arrest, and was associated with increased immune responses including type I interferon signaling, complement pathways and cytokine/chemokine production as determined by transcriptome analysis of acutely isolated microglia. Although adult microglia were morphologically indistinguishable between Stat3 mutant and littermate controls, hippocampal long-term potentiation was significantly impaired in the mutant mice. Similarly, the mutant mice exhibited spatial and social memory deficits. Together, our data demonstrate that Stat3 signaling plays a critical role in normal postnatal microglial development, and highlight that early-life events disrupting microglial regulatory networks at critical developmental stages could have long-term neurological consequences.

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Poster

373. Microglial Activation in Disease States

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Topic: B.11. Glial Mechanisms

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Title: Acute effects of α7nAChR agonists on pro-inflammatory cytokine release in primary microglia

Authors: *M. E. HAMMARLUND, S. HUA, F. ALBABILY, M. E. JOHANSSON;
Dept. of Neurosci. and Physiol., Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Background: Inflammation is a key mediator in ischemic brain injury, and microglia, the "macrophage of the brain" plays a central role in providing immunosurveillance. The alpha 7 acetylcholine nicotinic receptor (α7nAChR), present on peripheral macrophages are involved in the cholinergic anti-inflammatory pathway. The α7nAChRs are also expressed in the brain were they contribute to regulation of neuronal plasticity and neuroprotection. The aim of this project was to investigate the effect of two different agonists specific for the α7nAChR on pro-inflammatory cytokine release in microglia. **Methods:** Primary microglia was isolated from C57BL6Jrj mice pups, 0-3 days old. Microglia was stimulated for 4 hours with LPS (10ng/ml) with or without AR-R17779 (1-15 μM) or for 24 hours with LPS (10ng/ml) with or without PHA 568487 (0.5 μM). Cytokine release was measured in the supernatants using TNFα and IL-6 ELISA. **Results:** Stimulation with the specific α7nAChR agonist AR-R17779 at 5, 10 and 15 μM significantly decreased the release of TNFα after 4 hours incubation with LPS ($p < 0.05$). There was no significant difference in TNFα or IL-6 release between cells stimulated with PHA or LPS + PHA for 24 hours. **Conclusion:** The specific α7nAChR agonist AR-R17779 exhibits anti-inflammatory effects in microglia after 4 hour activation with LPS, while no statistical difference was seen after stimulation with PHA 568487 for 24 hours. However, it is possible that a higher concentration and different time point would result in a different outcome.

Disclosures: M.E. Hammarlund: None. **S. Hua:** None. **F. Albabily:** None. **M.E. Johansson:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.16/C27

Topic: B.11. Glial Mechanisms

Support: R01 AA025591

Title: Increased number and altered morphology of hippocampal microglia in a female rat model of alcohol use disorder

Authors: *J. K. MELBOURNE[1], H. PENG[2], C. ANASOOYA SHAJI[3], K. NIXON[3]; [1]Pharmacol. & Toxicology, Univ. of Texas at Austin, Austin, TX; [2]Pharmaceut. Sci., Univ. of Kentucky, Lexington, KY; [3]Pharmacol. & Toxicology, The Univ. of Texas at Austin, Austin, TX

Abstract: Neuroimmune activation is a common feature of neurodegeneration, including in alcohol use disorders (AUDs). Microglia are one of the main drivers of a neuroimmune response in male rat models of AUDs; however, very little is known about microglial alterations following ethanol exposure in female rats. Microglia exhibit a range of contextually sensitive morphologies. They are highly ramified at “rest” and upon activation undergo cytoskeletal rearrangements spanning from hyper-ramification to a “fully activated” ameboid morphology. Therefore, we examined microglial morphology and number in a female rat model of an AUD. Rats were gavaged with 25% (w/v) ethanol (n=8) or isocaloric control diet (n=8) every 8 h for 4 days (modified Majchrowicz model), which resulted in 10.2 ± 0.9 g/kg/d ethanol and yielded 384.2 ± 19.4 mg/dl blood ethanol concentrations. Rats were perfused transcardially 7 days post alcohol, a time when glial activation remains high in male rats. Brains were harvested, sectioned on a vibrating microtome, and immunohistochemistry was performed for microglial marker, Iba-1. Pre-processing and quantification of microglial morphology was carried out on hippocampal (dentate gyrus) sections using ImageJ (FIJI). Intensity threshold and size filter parameters were adjusted to create two images displaying; 1) the entire cell body and processes, and 2) the cell body only. The ‘analyze particles’ function was used to quantify total cell size, cell body size and cell number for each image. Individual cells were selected for further analysis. Sholl analysis and fractal analysis (FracLac for FIJI) was carried out to determine the number of intersections per radius and to quantify the complexity (fractal dimension) and heterogeneity (lacunarity) for each cell. Binge ethanol increased microglia number ($p=.0001$) and average cell body to cell size ratio ($p<.0001$) in the female rat hippocampus compared to controls. Conversely, the average cell size ($p<.0001$), cell body size ($p=.02$) and cell process size ($p<.0001$) were decreased in ethanol animals. Sholl analysis of individual cells showed an overall decrease in the number of intersections ($p<.0001$) in microglia in ethanol rats. Further, the fractal dimension of the microglia was decreased ($p<.0001$), whereas lacunarity was increased ($p=.01$). These data

demonstrate that microglia in female rat hippocampus proliferate and decrease in size, primarily due to a decrease in ramification, which suggests a retraction of processes consistent with activation. Thus, we have demonstrated three complementary methodologies to quantify microglia morphological complexity at the multi- and individual-cell level.

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Poster

373. Microglial Activation in Disease States

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Topic: B.11. Glial Mechanisms

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Title: Single prolonged stress increases hippocampal inflammation and produces PTSD-related behaviors in rats

Authors: *O. I. TORRES-RODRÍGUEZ, Y. RIVERA-ESCOBALES, M. COLÓN-ROMERO, B. VELAZQUEZ-PEREZ, A. HERNANDEZ, J. T. PORTER;
Ponce Hlth. Sci. Univ., Ponce, Puerto Rico

Abstract: Post-traumatic stress disorder (PTSD) is a neuropsychiatric disorder often characterized by impaired discrimination between safe and harmful signals from the environment and increased peripheral inflammation. In addition, patients with PTSD may exhibit exaggerated anxiety and difficulty extinguishing fear-related memories. Although stress and inflammation are closely associated with PTSD, it is not well understood whether exposure to stress alters inflammatory signaling in the brain to cause the cognitive impairments seen in PTSD. We hypothesized that exposure to stress would impair cue discrimination, impair fear extinction and increase anxiety by increasing inflammatory microglial activity in the ventral hippocampus (vHPC). To test this, we exposed adult male and female rats to single prolonged stress (SPS) and then differential cue fear conditioning, extinction, and open field test 7 days later. Three days after SPS, we found increased expression of the pro-inflammatory genes high mobility group box 1 (HMGB1) and IL1b in the vHPC suggesting increased inflammation prior to behavioral treatment. Our behavioral results showed that both non-stressed (NS) and SPS-exposed animals were able to discriminate between auditory cues. However, SPS animals showed impaired fear extinction and increased anxiety. After behavioral analysis, we evaluated microglia activity in

the vHPC. Immunofluorescence staining showed increased Iba-1 expression in SPS-exposed animals, suggesting increased microglial activity. To further characterize vHPC microglial activity, we examined isolated hippocampal microglia with fluorescence-activated cell sorting (FACS) and found no differences in the expression of the pro-inflammatory marker, CD86. Our results suggest that exposure to SPS increases inflammatory processes and hippocampal microglia activity to impair fear extinction and increase anxiety.

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Poster

373. Microglial Activation in Disease States

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Program #/Poster #: 373.18/C29

Topic: B.11. Glial Mechanisms

Title: Increased amperage in electroconvulsive shock decreases microglia branch order

Authors: *E. A. AQUINO, R. M. HINES, D. J. HINES;
Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Damage to the central nervous system activates a series of structural and functional changes to microglia, collectively known as reactive microgliosis. Reactive microgliosis is implicated in both the symptomology and treatment of many neuropsychiatric disorders. Electroconvulsive therapy is an effective treatment for neuropsychiatric disorders, however, its typically only used on those who are treatment resistant. Although beneficial for treatment resistant, it has a potential to cause damage to the CNS due to the electroconvulsive shock itself. Weak stimulation paradigms with low amperage have low potential for damage but also reduced therapeutic potential. There is a need for an optimal stimulation paradigm that has a high therapeutic potential but also minimizes the potential for damage. This paradigm is possible but a better understanding of the mechanisms behind ECT is necessary. In addition to helping optimize optimal stimulation, examination of how ECT affects microglia may also provide insight into the therapeutic mechanism behind ECT. To determine the effects of ECT on microglia, transcranial ECT was administered at different amperages, followed by an immunohistochemical analysis of microglia and their structure. Results show that different amperages cause a differential extent of microglia cell activation and increased amperage causes a decrease in microglia branch order. Developing a better understanding of the mechanism behind ECT and how it affects glia cells will lead to the production of an optimal stimulation paradigm, and further refinement of the procedure to optimize beneficial aspects of microgliosis.

Disclosures: E.A. Aquino: None. **R.M. Hines:** None. **D.J. Hines:** None.

Poster

373. Microglial Activation in Disease States

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Support: NIH 8R24OD010947
NIH R01AI113883

Title: Persistent markers of neuroinflammation in Zika virus post infection stage of adult rhesus macaque

Authors: *K. M. SMITH[1], M. JACKSON[2], C. FONTENOT[3], E. L. V. SILVEIRA[4], K. A. ROGERS[5], M. M. TEIXEIRA[6], F. VILLINGER[7];
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Abstract: Zika virus is a member of the flavivirus family that gained world-wide notoriety as a central nervous system teratogen after an outbreak in Brazil was linked to an increased prevalence of microcephaly in infants exposed *in utero*. Zika virus has also been linked to both autoimmune destruction of myelin proteins and Guillain-Barré syndrome, as well as macular atrophy. Zika is primarily a mosquito borne disease, and pandemic outbreaks of Zika stand to infect a large portion of the population where the Aedes aegypti and Aedes albopictus mosquitos are prevalent. Transmission via sexual intercourse has also been reported. The long-term consequences infection in adults are not well understood. We have investigated neuroinflammation of the post-infected adult brain of 3 rhesus macaque monkeys compared to 5 control monkeys. We examined astrocytes (GFAP) and microglia (Iba1) in the hippocampus and lateral geniculate nucleus via immunohistochemistry. All three monkeys had been infected with a Brazilian isolate of the Zika virus (10\7 PFUs of a Brazilian viral strain HS-2015-BA-01), intravenously, with detectable viral loads in plasma up to 7 days and mucosal secretions up to 42 days post infection. No evidence of virus persistence was obtained thereafter and the monkeys were euthanized on day 142 after infection. We therefore examined whether persistent changes in astrocyte and microglial numbers were present in the brains of infected animals at necropsy. Results: Here, we show that the number of GFAP+ cells in the lateral geniculate nucleus was increased in monkeys infected with Zika virus ($p=0.017$). We also found an increased number of Iba1+ cells in the subgranular zone and hilus regions ($p=0.03$) of the hippocampus. We conclude that in spite of the resolution of viral replication *in vivo*, increases in astrocyte and microglia

populations indicate a potential for a sustained state of neuroinflammation not previously reported. Our results raise questions to the long-term effects of Zika virus and accompanying detrimental sequelae of infection.

Disclosures: K.M. Smith: None. **M. Jackson:** None. **C. Fontenot:** None. **E.L.V. Silveira:** None. **K.A. Rogers:** None. **M.M. Teixeira:** None. **F. Villinger:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.20/C31

Topic: B.11. Glial Mechanisms

Title: Modeling neuroinflammation using iPSC-derived engineered microglia

Authors: *B. C. FREITAS, M. MCLACHLAN, S. J. DICKERSON, C. A. MUNN, S. A. BURTON, A. MUSINSKY, M. GOEDLAND, D. RAJESH, S. HILCOVE, E. JONES; FUJIFILM Cell. Dynamics, Inc., Madison, WI

Abstract: Neurodegenerative and neurodevelopmental disorders can display neuroinflammation settings, having microglial cells as crucial players during this detrimental stage. Microglia, the resident immune cells of the central nervous system, is a necessary cell type for neuronal homeostasis also responsible for synaptic pruning and brain development. Human iPSC-derived microglia can serve as an authentic preclinical tool for understanding the pathobiology of neurodegenerative, neurodevelopmental diseases. The present study involves the generation and characterization of genome engineered iPSC-derived iCell Microglia to facilitate disease modeling for both neurodevelopmental (Rett Syndrome) and neurodegenerative disorder (Alzheimer's and Parkinson's disease). iCell Microglia generated from both the engineered and non-engineered clones offering a unique isogenic pair for research applications. Microglia-derived by differentiating cryopreserved and purified hematopoietic progenitor cells (HPCs) and further differentiating the HPCs to microglia using technology developed by the Blurton-Jones laboratory (Abud et al. Neuron 2017.) for which FujiFilm Cellular Dynamics Inc. has an exclusive license from the University of California-Irvine. End-stage Microglia were characterized by morphology, quantification of TREM2, P2RY12, CX3CR1, IBA1, CD33 and CD45 levels by flow cytometry, quantification of a phagocytic function using pHrodo BioParticles and aggregated amyloid beta, quantification of neuroinflammatory molecules by multiplex Luminex and quantification of soluble TREM levels and finally RNAseq analysis. Cryopreserved Microglia retained purity and function comparable to pre-cryopreserved end-stage Microglia. The results identified critical differences in survival, the kinetics of phagocytosis, and levels of molecules involved in neural inflammation between healthy control lines (Ctrl) and engineered microglia. Thus, iPSC-derived isogenic controls and engineered

microglia could serve as a powerful tool to gain insight into various physiological and pathological conditions associated with neurological disorders.

Disclosures: **B.C. Freitas:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **M. McLachlan:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **S.J. Dickerson:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **C.A. Munn:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **S.A. Burton:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **A. Musinsky:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **M. Goedland:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **D. Rajesh:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **S. Hilcove:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc. **E. Jones:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc..

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.21/C32

Topic: B.11. Glial Mechanisms

Support: NCCIH Grant P50 AT008661-01

Title: Microglia specific knockout of TLR4-mediated resiliency to chronic stress induced vulnerability to depression and anxiety phenotype

Authors: *U. H. IQBAL, T. FROLINGER, S. WESTFALL, G. PASINETTI;
Ichan Sch. of Med. At Mount Sinai, New York, NY

Abstract: Chronic stress-induced inflammatory responses occur in part via danger-associated molecular pattern (DAMP) molecules, such as high mobility group box 1 protein (HMGB1), but the receptor(s) underlying DAMP signaling have not been identified. Our recent findings suggested a major role for HMGB1/TLR4/NF-kB danger signaling in the microglia in stress-induced susceptibility to depression and anxiety phenotypes. Microglia morphology and DAMP signaling in enriched amygdala microglia were examined in a mouse model of microglial specific CRE-LOX knockout of TLR4 (TLR4$^{tm1.1Karp}$ Cx3cr1) during the development and expression of chronic unpredictable stress (CUS)-induced behavioral deficits, including long-term, persistent changes after CUS. Behavior and FACS enriched microglia were collected from vehicle or stressed mice exposed to CUS (CUS), followed by post-stress rest (post stress), followed by subsequent exposure to short term unpredictable stress (CUS+US), or from stressed mice exposed to short term unpredictable stress only (US) and compared to age matched non-

stressed, vehicle treated mice (Ctrl). The results show that microglial specific knockout of TLR4 prevents CUS-induced depression and anxiety phenotypes following CUS and CUS+US together with significant attenuation of CUS-induced microglia morphological changes and diminution of CUS-induced robust upregulation of cortical IL-1β protein expression. Our results provide further evidence for persistent DAMP signaling via microglia TLR4 that increases vulnerability to depressive-like behaviors long after chronic stress exposure.

Disclosures: **U.H. Iqbal:** None. **T. Frolinger:** None. **S. Westfall:** None. **G. Pasinetti:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.22/C33

Topic: B.11. Glial Mechanisms

Support: WMU Internal Funds
WMU GSRG

Title: *Ex vivo* examination of microglial proliferation patterns following a direct lesion to the olfactory bulb of the adult zebrafish brain

Authors: *S. R. VAR, C. A. BYRD-JACOBS;
Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: Plasticity of the zebrafish olfactory system makes it an ideal model for regeneration studies and for examining the immune cell response following injury. Microglia are the resident mononuclear phagocytes in the brain, with the potential to contribute to regeneration. Previously, we demonstrated the microglial response to a direct lesion to the olfactory bulb (OB), but it is unclear whether this is a contribution from local proliferation or peripheral migration. We hypothesize that, after damage, there will be a gradual increase in resident microglia, followed by an influx of macrophages from the periphery, rather than localized cellular proliferation. We compared a direct lesion injury to the right OB in the whole fish to direct lesion injury to the right OB in the ex vivo brain with afferent input removed. 4C4 antibody was used to label microglia. Proliferating cell nuclear antigen (PCNA) antibody was used to label cellular proliferation. Direct lesion injury in the whole fish showed at 1h after damage labeled microglia were scattered and ramified with few proliferating microglial profiles, comparable to controls. At 4h, there was a noticeable increase in transitioning and amoeboid microglia in both bulbs but a decrease in dividing microglial profiles compared to control. At 12h, there was an accumulation of transitioning microglia around the presumptive site of the wound in the right OB, with a noticeable increase in dividing microglia compared to controls. At 24h, there were fewer microglia compared to the previous time point but an additional increase in dividing microglia. A

direct lesion injury in the ex vivo brain showed that at 1h after damage both labeled microglia and proliferating microglia were similar to controls. At 4h, there was an increase in transitioning and amoeboid microglia around the periphery of both bulbs and a noticeable increase in dividing microglia compared to control. At 12h, there was a small decrease in amoeboid microglia around the periphery of both bulbs compared to the previous time point but levels of dividing microglia remained the same. At 24h, there was a slight decrease in transitioning microglia around the periphery and few to no dividing microglia. These results suggest that microglia can respond to damage effectively without afferent input or peripheral influence, with similar microglial response patterns as an intact brain. Comparisons between the intact and isolated brains demonstrate that further proliferation and potential contribution from peripheral leukocytes occurs at 12h and increases at 24h. Further work is required to explore the potential role of proliferating microglia in recovery and regeneration after injury.

Disclosures: S.R. Var: None. **C.A. Byrd-Jacobs:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.23/C34

Topic: B.11. Glial Mechanisms

Support: WMU Internal Funds
WMU GSRG

Title: Examination of the injury response to chemical ablation in the zebrafish olfactory epithelium

Authors: *M. M. ALI, C. A. BYRD-JACOBS;
Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: The ability of lower vertebrates to regenerate entire organs is an intriguing phenomenon with various beneficial implications for improving human health. Zebrafish have shared and conserved features with mammals, making them an ideal model to study regeneration. Intranasal irrigation with Triton X-100 produces severe degeneration of the olfactory epithelium, followed by rapid regeneration. We hypothesized that following chemical lesioning of the olfactory epithelium, there will be increased phagocytic activity by macrophages that leads to recovery of the epithelium. The right olfactory epithelium of adult zebrafish was damaged with infusion of 0.7% Triton X-100 into the olfactory cavity, and some fish also received an intraperitoneal injection of 50ug/ml body weight of 20mM bromodeoxyuridine (BrdU). Immunocytochemistry for anti-4C4 allowed identification of macrophages/microglia and anti-BrdU revealed cell proliferation. We determined infiltration of macrophages/microglia

into the olfactory organ and nerve at various survival times following damage.In control organs, scattered 4C4+ profiles were observed in the lamina propria of the lamellae, consistent with their role in surveying the epithelium for debris. Four hours after Triton X-100 treatment, there was obvious thinning of the olfactory epithelium from loss of olfactory sensory neurons, and numerous 4C4+ profiles were observed in the olfactory nerve and in the lamina propria beneath the olfactory epithelium of the troughs at the base of the lamellae, showing a macrophage/microglial response to the damage. At one day, enlarged oval ameboid 4C4+ profiles were present in the regions of thinned olfactory epithelium, possibly correlating with their role in phagocytizing injured neurons. At this time point, there was increased cell proliferation in the basal region of the olfactory epithelium. Additional studies will focus on determining if this degradation of damaged neurons via phagocytosis by 4C4+ cells is signaling basal cells to divide and replenish the olfactory epithelium. Further investigation will focus on understanding the mechanisms involved in the interaction between the phagocytic cells and regenerating neurons after damage, with an overall goal of facilitating recovery from neurodegenerative diseases and traumatic brain injuries.

Disclosures: **M.M. Ali:** None. **C.A. Byrd-Jacobs:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.24/C35

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01NS088627
NIH Grant R21DE025689

Title: Targeting microglial Gi DREADD for the inhibition of chronic pain

Authors: ***M.-H. YI**[1], Y. LIU[1], K. LIU[1,4], T. CHEN[1], D. BOSCO[1], J. ZHENG[1], M. XIE[1], L.-J. WU[1,2,3];
[1]Neurol., Mayo Clin., Rochester, MN; [2]Neurol., Mayo Clin., Jacksonville, FL; [3]Immunol., Mayo Clin., Rochester, MN; [4]Rutgers University, Robert Wood Johnson Med. Sch., New Brunswick, NJ

Abstract: Abstract
Microglia are known to be important for neuropathic pain, however, a causal relationship between microglia and development of neuropathic pain has yet to be directly tested in vivo. To address this question, we have developed CX3CR1^{creER}:LSL-hM4Di transgenic mice to enable selective expression of Gi DREADD and employed microglia-based chemogenetic techniques in a mouse model of neuropathic pain. We found that microglial Gi DREADD activation inhibited

spinal nerve transection (SNT)-induced microglial activation, chronic pain initiation and maintenance. Gi DREADD activation downregulated the transcription factor interferon regulatory factor 8 (IRF8) and its downstream proinflammatory cytokines including interleukin 1 beta (IL-1β). These findings deepen our understanding the causal role of microglia in neuropathic pain pathogenesis and suggest the potential therapeutic approach of targeting microglial Gi DREADD for neuropathic pain treatment.
This work is supported by National Institute of Health (R01NS088627 and R21DE025689)

Disclosures: **M. Yi:** None. **Y. Liu:** None. **K. Liu:** None. **T. Chen:** None. **D. Bosco:** None. **J. Zheng:** None. **M. Xie:** None. **L. Wu:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.25/C36

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01NS088627
NIH Grant R21DE025689

Title: TREM2 regulate microglia response to TDP43 pathology related neurodegeneration

Authors: *M. XIE[1], Y. LIU[1], M. P. MATTSON[2], L.-J. WU[1];
[1]Mayo Clin., Rochester, MN; [2]Lab. of Neurosciences, Natl. Inst. on Aging Intramural Res. Program, Baltimore, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, which is characterized by the progressive loss of both upper and lower motor neurons in the brain and spinal cord, leading to muscle weakness and paralysis. Although it is generally believed that the disease onset is initially derived from selective motor neuron degeneration, the non-cell-autonomous nature of this disease emphasizes the contribution of glia cells in disease progression. The TAR-DNA binding protein 43 kDa (TDP-43) is an RNA binding protein which was discovered to be the main component of intracellular, insoluble protein aggregates found within motor neurons in ALS pathology. Studies from rodent models of ALS demonstrate dynamic states of microglial morphology across different stages of ALS pathology, including a neuroprotective state in the early disease stage. However the underlying molecular mechanisms are still largely unknown. Triggering receptor expressed on myeloid cell 2 *(TREM2)* is a surface receptor that is exclusively expressed on microglia in the brain and plays a crucial role in microglial proliferation, migration and phagocytosis. Heterozygous expression of *TREM2* variants has been linked to increased risk for neurodegenerative diseases, including ALS. We recently generated an ALS-like motor neurodegenerative mouse model using viral

overexpression of hTDP-43 protein in neurons (AAV9-CAG-hTDP43) or control vector (AAV9-CAG-GFP). We found overexpression of hTDP-43 in WT mice resulted in progressive microglia activation characterized by larger soma size and shorter process. In TREM2 KO mice, the microglia exhibited less reactive phenotype. We further found that CD68 expression was significantly lower in TREM2 KO mice compared with WT mice, which indicated the microglia phagocytic function deficiency in TREM2 KO mice. To test this hypothesis, we did immunostaining for phosphorylated TDP43 and found that reactive microglia cleared pathology related phosphorylated TDP-43 only in WT mice. We further compared the TDP43 proteinopathy and found that at later stage, total hTDP-43 and phosphorylated TDP43 level were significant higher in TREM2KO mice compared with WT mice. Overall our results reveal a TREM2-mediated neuroprotective role for microglia in the hTDP-43 overexpression model of ALS-like motor neuron degeneration.

Disclosures: **M. Xie:** None. **Y. Liu:** None. **M.P. Mattson:** None. **L. Wu:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.26/C37

Topic: B.11. Glial Mechanisms

Support: R01NS088627
R21DE025689

Title: Microglia-astrocyte interaction drives neuromyelitis optica evolution

Authors: *T. C. CHEN, L. VANDA, Y. LIU, B. DALE, Y. LI, M.-H. YI, J. ZHU, L. WU; Neurol., Mayo Clin., Rochester, MN

Abstract: Neuromyelitis optica (NMO) is an inflammatory autoimmune CNS disorder triggered by binding of an IgG autoantibody to its antigen, the aquaporin 4 (AQP4) water channel on astrocytes. Microglia, as CNS sentinels, would be first responders to astrocytes being attacked by IgG. However, the role of microglia in the evolution of NMO is unknown. We developed an informative murine model in which NMO patient-derived IgGs are infused continuously into the spinal subarachnoid space. The outcome was that NMO-IgG induced motor impairment and classic NMO pathology in wild-type mice but not in AQP4-null mice. In vivo spinal cord imaging revealed that NMO-IgG induced microglia-astrocyte physical interaction. In mice depleted of microglia, both motor impairment and characteristic NMO immunohistopathology were significantly reduced. We further discovered that early microglial-astrocyte interaction requires astrocytic secretion of complement C3. Mice genetically lacking C3 or C3a receptor lacked motor deficits and NMO pathology after NMO-IgG infusion. Our study revealed that

early-activated complement components mediate astrocyte-microglia signaling in NMO initiation, and identifies microglia as a new target for NMO therapeutic interception.
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Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.27/C38

Topic: B.11. Glial Mechanisms

Support: NIH R01NS088627
NIH R21DE025689

Title: Neuronal network activity controls microglial process surveillance in awake mice via norepinephrine signaling

Authors: *Y. LIU, Y. LI, U. B. EYO, T. CHEN, A. D. UMPIERRE, J. ZHU, D. B. BOSCO, L.-J. WU;
Neurol., Mayo Clin., Rochester, MN

Abstract: Microglia are resident immune cells that dynamically survey the brain parenchyma. Microglial processes interact with neuronal elements, however, the role that neuronal network activity plays in regulating microglial dynamics is not entirely clear. Most studies of microglial dynamics have either utilized slice preparations or in vivo imaging in anesthetized mice. Here we demonstrate that microglia in awake mice have relatively reduced process area and surveillance territory. By contrast, reduced neuronal activity under general anesthesia increases microglial process velocity, extension and territory surveillance. Similarly, reductions in local neuronal activity via sensory deprivation or optogenetic inhibition increases microglial process surveillance. Using pharmacological and chemogenetic approaches, we demonstrate that reduced norepinephrine signaling is necessary for the observed increases in microglial process surveillance. Thus, we reveal that noradrenergic tone in awake mice normally suppresses microglial process surveillance under basal physiological conditions. Our results therefore unveil a novel mechanism for regulation of resting microglia dynamics.

Disclosures: Y. Liu: None. **Y. Li:** None. **U.B. Eyo:** None. **T. Chen:** None. **A.D. Umpierre:** None. **J. Zhu:** None. **D.B. Bosco:** None. **L. Wu:** None.

Poster

373. Microglial Activation in Disease States

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 373.28/C39

Topic: B.11. Glial Mechanisms

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NIH NINDS F32NS106732
VA Merit Review Grant I01-BX002949
DoD CDMRP W81XWH-18-1-0598

Title: Microglial modulation of hippocampal neurogenesis after global cerebral ischemia

Authors: *A. ISAKHAROV, C. BUTLER, W. ZHU, E. SCHNELL, I. KOERNER;
OHSU Dept of Anesthesiol. and Perioperative Medi, Portland, OR

Abstract: Hippocampal neurogenesis continues into adulthood and is modulated by numerous environmental factors, including neuronal injury. However, much remains unknown regarding the factors that influence proliferation, maturation, and integration of adult-born granule cells into the hippocampal circuit after injury. As prior studies have suggested that brain inflammation modulates neurogenesis after brain injury, we are investigating the role of microglia in sculpting hippocampal neurogenesis after global brain ischemia. We use a mouse model that allows selective ablation of microglia to determine how microglia modulate neurogenesis after cardiac arrest and resuscitation. In mice with intact microglia, this model of global ischemia robustly increases cell proliferation in the dentate gyrus granule cell layer, coincident with an increase in markers expressed in immature neurons. In the absence of microglia, our preliminary data suggest dramatic alterations in the early stages of post-ischemic neurogenesis. We are currently replicating and expanding these preliminary studies, with the hope of elucidating the rapidly expanding roles of microglia in the brain and the mechanisms underlying microglial modulation of post-injury neurogenesis.

Disclosures: A. Isakharov: None. **C. Butler:** None. **W. Zhu:** None. **E. Schnell:** None. **I. Koerner:** None.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.01/C40

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant R01AG033649
NIH Grant P30GM110787
NIH Grant T32AG057461

Title: Insulin and brain aging: Increasing signaling without the ligand to offset cognitive decline with aging

Authors: *K. L. ANDERSON[1], H. N. FRAZIER[1], R.-L. LIN[1], A. O. GHOWERI[1], G. J. POPA[2], M. D. MENDENHALL[2], O. THIBAULT[1];
[1]Dept. of Pharmacol. and Nutritional Sci., [2]Mol. and Cell. Biochem., Univ. of Kentucky, Lexington, KY

Abstract: Acute or short term intranasal delivery of different insulin formulations has been shown to enhance insulin signaling, alter cerebral blood flow, and enhance memory recall. Other approaches that do not increase ligand availability in the brain, but instead use molecular techniques to knockout the insulin receptor (IR), introduce an IR antisense sequence via a lentivirus, or introduce an inhibitory IR binding peptide have been employed to illustrate the impact of a loss-of-function of the IR on brain function. However, it has yet to be determined whether increasing IR activity without introducing the ligand can offset cognitive decline with age. Here, we tested whether increasing insulin signaling through *in vivo* expression of a constitutively active human insulin receptor beta subunit (IRβ) in the hippocampus of young and aged rats improves spatial memory and recall. We also studied the impact of IRβ manipulation on long-term potentiation induction and maintenance, and on key markers of the insulin signaling pathway.
Preliminary studies in primary hippocampal cell cultures optimized the construct of an IRβ based on prior work from Lebwohl et al. 1991, and subsequently confirmed the expression and constitutive activity of this receptor in neurons using lentiviral delivery. Following this work, we constructed an AAV for *in vivo* delivery of HA-tagged IRβ. The AAV was bilaterally injected into the hippocampus of young (2 mo) and aged (18 mo) F344 rats. Following three months of expression, animals were tested for learning and memory recall using the Morris water maze. The animals were then divided into two groups for analysis. The first group was used for electrophysiology measures (excitability, long-term potentiation), while the other was perfused with ice-cold saline. Hippocampal dorsal and ventral extremities, thalamus, and cortex were extracted from the electrophysiology group and frozen for later use in Western blots. Saline

perfused brains were split so that one hemisphere was post-fixed for immunohistochemistry against the HA tag on IRβ, while the other was divided into hippocampus, thalamus, and cortex sections and immediately frozen for Western blots against pAkt and Akt. Results from the Morris water maze were then analyzed in the animals showing successful HA-IRβ expression. These results provide new insights and test the efficacy of this ligand-less approach for offsetting cognitive decline with aging.

Disclosures: K.L. Anderson: None. **H.N. Frazier:** None. **R. Lin:** None. **A.O. Ghoweri:** None. **G.J. Popa:** None. **M.D. Mendenhall:** None. **O. Thibault:** None.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.02/C41

Topic: C.01. Brain Wellness and Aging

Support: AG0033649-S1

Title: *In vivo* measurements of cerebral blood flow and neuronal calcium in the F344 model of aging treated acutely with intranasal insulin

Authors: *R.-L. LIN, H. N. FRAZIER, A. O. GHOWERI, K. L. ANDERSON, O. THIBAULT; Dept. of Pharmacol. and Nutritional Sci., Univ. of Kentucky Med. Ctr., Lexington, KY

Abstract: Multiphoton calcium imaging techniques are quickly becoming favored approaches for investigations into cellular mechanisms of neurodegeneration as seen in aging and Alzheimer's disease. It is also well documented that intranasal insulin (IN) improves age-related cognitive deficits and can reverse some calcium electrophysiological correlates of brain aging. Previous work from our lab has also shown that IN can increase cerebral blood flow in aged animals, providing a novel mechanism for the role of insulin in improving memory function. Given the impact of insulin on neuronal calcium processes and on cerebral blood flow, we tested whether these processes we co-dependent in the brain and in response to IN *in vivo*.
We delivered calcium sensors to the somatosensory cortex of young and aged rats to characterized neuronal calcium changes, and also acutely injected rhodamine dextran to monitor hemodynamic changes in response to peripheral hind and fore limb activation prior to, and shortly after IN delivery. Bilateral acute cranial windows were created on the day of the experiment and two-photon microscopy was used to perform line scans and frame scans across different vascular beds and neuronal layers (depth) in anesthetized animals.
As previously reported, we show here a positive correlation between vessel diameter and velocity. We did note a significant reduction in velocity across all vessel sizes imaged in the aged, compared to the young group. Hindpaw and forepaw stimulation triggered neuronal

activation in the somatosensory cortex and also elicited rapid increases in vessel diameter in both young and aged groups, particularly in larger vessels (>30 μm). An interaction term was identified where IN did not alter velocity over a 30-minute period in the young group while an increase in velocity was seen across time in the aged animals. This result highlights greater insulin sensitivity may be present in the aged, compare to the young animals.
Our results align well with few studies that have used multiphoton imaging in the aged anesthetized animal and also reported decreases in blood flow. Here we provide evidence that IN increases vessel plasticity more in the aged compared to the young animal. This could highlight a potential mechanism of action for IN enhancing memory in clinical settings.
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Disclosures: R. Lin: None. **H.N. Frazier:** None. **A.O. Ghoweri:** None. **K.L. Anderson:** None. **O. Thibault:** None.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.03/C42

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01AG033649
NIH Grant T32DK007778
NIH Grant T32AG057461
University of Kentucky College of Medicine (fellowship to HF)
University of Kentucky Dept. of Pharmacology and Nutritional Sciences (Reinvestment Fund Award)

Title: Increasing insulin receptor signaling enhances glucose metabolism and elevates glucose-transporter 3 expression in primary hippocampal neurons

Authors: *H. N. FRAZIER[1], A. O. GHOWERI[1], K. L. ANDERSON[1], R.-L. LIN[1], G. J. POPA[2], M. D. MENDENHALL[2], R. J. CRAVEN[1], O. THIBAULT[1];
[1]Pharmacol. and Nutritional Sci., [2]Mol. and Cell. Biochem., Univ. of Kentucky, Lexington, KY

Abstract: Recent studies have reported that insulin signaling diminishes with aging, evidenced by decreased signaling markers, reduced insulin mRNA, and lower insulin receptor (IR) density. Similarly, many reports have also highlighted the role of insulin in normal brain function, with early stage clinical trials reporting a positive impact of intranasal insulin administration on memory recall in patients with mild cognitive decline or Alzheimer's disease. However, the specific pathways targeted by insulin signaling in the brain remain unclear. To address this, we conducted a series of experiments exploring the relationship between insulin signaling, glucose

metabolism and binding, and glucose transporter (GLUT) expression in hippocampal neurons. Using a lentiviral delivery system, we infected mixed, primary hippocampal cultures with either a control plasmid encoding only a red fluorescent marker (mCherry), or one encoding both mCherry as well as a constitutively active human IR (IRβ). A synapsin promoter was included in both plasmids to limit expression to neurons. 2-NBDG imaging was performed to assess the effect of increased IR signaling on glucose metabolism. Glucose uptake was obtained by measuring initial 2-NBDG fluorescence at the start of recording. The rate of fluorescent signal decay over time was calculated as an indirect measure of glucose utilization. Results were further corroborated using radiolabeled glucose uptake assays. To test if changes in glucose metabolism were related to GLUT density, immunocytochemistry and Western immunoblots against the neuron-specific GLUT3 and the insulin-sensitive GLUT4 were performed.
Visual observations suggested that expression of IRβ had no detrimental effects on neuronal survival, morphology, or density. 2-NBDG imaging revealed that IRβ-expressing neurons had elevated levels of glucose uptake and faster rates of glucose utilization compared to controls. No detectable differences in GLUT4 protein levels were noted between the two groups. However, Western immunoblots indicated that IRβ was associated with a significant increase in overall GLUT3 expression, as well as with alterations in this transporter's localization within the cell. The results presented here highlight the validity of using molecular approaches to study the effects of sustained IR activation without administration of exogenous insulin, and suggest that neuronal glucose metabolism, specifically GLUT3, may be an important downstream target of IR signaling in the hippocampus.

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Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.04/C43

Topic: C.01. Brain Wellness and Aging

Support: 17K00915

Title: Activated integrated stress response in obese diabetic mice may cause impairment of adult hippocampal neurogenesis and episodic-like memory

Authors: K. NAKAGAWA[1], S. ISLAM[2], M. UEDA[3], ***T. NAKAGAWA**[1];
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Abstract: The integrated stress response, eIF2α phosphorylation-ATF4 signaling, is activated in the brain of obese diabetic mice, leading to impairment of memory and adult hippocampal neurogenesis.
The eIF2α is phosphorylated at residue Ser51 by four protein kinases: general control nonderepressible-2 (GCN2) kinase, double-stranded RNA-activated protein kinase (PKR), PKR-endoplasmic reticulum (ER)-related kinase (PERK), and heme-regulated inhibitor kinase (HRI). These kinases are activated by amino acid starvation, viral infection, ER stress, and heme deficiency, respectively. The signaling cascade initiated by these kinases is known as the integrated stress response (ISR). In the ISR signaling, general protein synthesis is reduced, and the activating transcription factor 4 (ATF4) is paradoxically translated. Several studies, including ours, indicated that the levels of eIF2α phosphorylation and ATF4 expression are important for memory formation. Diabetes affects adult hippocampal neurogenesis through the increased levels of glucocorticoids and the hyperglycemia. However, whether the ISR affects adult hippocampal neurogenesis remains obscure. Therefore, this study aimed to determine whether ISR activation in the brain of mice with diabetes and obesity affects memory and neurogenesis. We used high fat diet (HFD)-induced diabetic C57BL/6, obese and diabetic *db/db*, and APP23 mouse model of Alzheimer's disease (AD) crossed with *db/db* (AD;*db/db*) mice. Bromodeoxyuridine (BrdU) at a dose of 150 mg/kg was administered intraperitoneally twice a day for three consecutive days. The eIF2α phosphorylation and ATF4 expression were increased in the brain of AD;*db/db* and *db/db* mice, indicating activation of the ISR signaling. The impairment of novel object localization, novel object recognition, and radial arm maze was exacerbated in obese mice with diabetes. Immunohistochemical analysis revealed a reduction in the number of bromodeoxyuridine-labeled cells expressing the neuronal migration protein doublecortin in *db/db* mice. In the hypothalamus of 45-week old *db/db* mice, brain-derived neurotrophic factor (BDNF) was increased during aging. These results suggested that the ISR may affect BDNF expression in the brain of mice with diabetes and obesity, impairing neurogenesis and memory.

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Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.05/C44

Topic: C.01. Brain Wellness and Aging

Support: Legacy Foundation Grant

Title: Long-term high-fat diet changes DNA methylation resulting in pro-neurodegenerative gene changes in the dorsal hippocampus

Authors: *D. M. OSBORNE[1], J. W. VANDER VELDEN[2];
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Abstract: Obesity and Type 2 Diabetes (T2D) are leading predisposing environmental factors for the development of neurodegenerative diseases. Prior research has established the hippocampus as particularly vulnerable to obesogenic lifestyles; as such, we investigated whether a long-term high-fat/high-sugar diet (HFD) would affect methylation and subsequent gene expression changes within the dorsal hippocampus of male mice. Male mice received either standard chow or a 60% HFD supplemented with 10% sucrose water beginning at six weeks of age and maintained until either six (young) or 14 months (old) of age when tissues were taken, following confirmation of T2D status. Bilateral hippocampi were submitted for Reduced Representation Bisulfite Sequencing (RRBS) to determine genome-wide methylation profiles at areas with high CpG content, while rt-PCR and Western blots were used to determine expression levels. Comparisons were made to determine effects of Chow vs HFD on both young and old mice. RRBS indicated 107 genes that were differentially methylated between the four groups. Several of these gene targets, with major roles in neuroprotection/neurodegeneration, had significantly altered methylation levels across multiple conditions. Both calsyntenin-1, an amyloid precursor protein transporter, and zinc finger homeobox-3 (related to neuroprotection and Alzheimer's Disease) had significantly increased methylation in old HFD mice, relative to young HFD and old Chow conditions. Young HFD mice also had significantly increased methylation of histone deacetylase-5 and the mitochondrial solute carrier Slc25a15, relative to young Chow, while methylation was further significantly increased in old HFD mice. Additionally, significant methylation changes were found in several genes related fibroblast growth factor signaling due to HFD. The net result of HFD exposure, in regard to methylation, is a pro-neurodegenerative state, with the potential "shutting off" of several different genes vital for neuroprotection.

Disclosures: D.M. Osborne: None. **J.W. Vander Velden:** None.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.06/C45

Topic: C.01. Brain Wellness and Aging

Title: Human plasma fractions as therapeutics to enhance cognitive function in aging by multiple modes of action

Authors: *D. D. LUU, I. GALLAGER, E. CZIRR, M. K. CAMPBELL, S. MINAMI, V. KHEIFETS, S. P. BRAITHWAITE, N. HUBER;
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Abstract: Cognitive impairment associated with neurodegenerative diseases of aging is a major unmet medical need. Multiple processes contribute to these medical conditions, such as decreased number of neuronal stem cells, reduced neurogenesis, decreased neuronal cell survival, and increased neuroinflammation. To combat these diseases and their symptoms, novel multimodal therapeutic strategies are urgently needed. Heterochronic parabiosis and the infusion of young plasma have demonstrated that age-related decline in neurogenesis and increase in neuroinflammation can be reversed, providing initial proof of concept for an approach to restore age-related impairment of processes in the CNS and to enhance cognition. To build on this concept and develop potential human therapeutics, we have characterized human plasma fractions with enhanced safety, tolerability and regenerative effects compared to whole human plasma. Administration of an active plasma fraction to neuronal cells promotes neurite outgrowth as well as neuronal activity and network formation in vitro. Infusion of this plasma fraction into aged mice improves cognitive behavior, which correlates with increased neurogenesis, neuronal survival, number of functional synapses and neuronal activity. These findings are deepening our mechanistic understanding of the multifactorial, regenerative properties mediated by circulating proteins in the blood. They also provide a rationale for the development of novel plasma fractions to enhance cognitive function in neurodegenerative diseases, which we are currently testing in clinical trials for Alzheimer's and Parkinson's diseases.

Disclosures: D.D. Luu: A. Employment/Salary (full or part-time):; Alkahest. **I. Gallager:** A. Employment/Salary (full or part-time):; Alkahest. **E. Czirr:** A. Employment/Salary (full or part-time):; Alkahest. **M.K. Campbell:** A. Employment/Salary (full or part-time):; Alkahest. **S. Minami:** A. Employment/Salary (full or part-time):; Alkahest. **V. Kheifets:** A. Employment/Salary (full or part-time):; Alkahest. **S.P. Braithwaite:** A. Employment/Salary (full or part-time):; Alkahest. **N. Huber:** A. Employment/Salary (full or part-time):; Alkahest.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.07/C46

Topic: C.01. Brain Wellness and Aging

Title: Transcriptomic characterization of therapeutic plasma fraction on mechanisms of neural progenitor self-renewal

Authors: *A. L. NGUYEN, N. HUBER, D. LUU, S. LOHR, V. KHEIFETS, S. BRAITHWAITE, D. P. LEONE;
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Abstract: Adult neurogenesis, the process of continuous generation of new neurons in discrete areas of the brain, decreases during aging and in neurodegenerative diseases. Modulation of

neurogenesis in rodent models is associated with changes in learning and memory leading to improvements in cognitive function. We have characterized a human plasma fraction that improves cognitive function when infused into aged mice. This observed regenerative effect correlates with increased neurogenesis and neuronal survival in the hippocampal region. Here we utilized RNAseq analysis of human neural progenitor cells as an in vitro model system to study the functional effect of transcriptional changes induced by this human plasma fraction. Gene ontology enrichment analysis reveals modulation of multiple pathways after plasma fraction treatment. Understanding the molecular response of neural progenitor cells to this therapeutically relevant plasma fraction will enable us to better understand molecular and cellular mechanisms that regulate adult neurogenesis during both healthy aging and in neurodegenerative diseases. These findings are deepening our mechanistic understanding of the multifactorial, regenerative properties mediated by circulating proteins in the blood. They also provide a rationale for the development of this novel plasma fraction as therapy for neurodegenerative diseases, currently being tested in clinical trials for Alzheimer's and Parkinson's diseases.

Disclosures: **A.L. Nguyen:** A. Employment/Salary (full or part-time):; Alkahest. **N. Huber:** A. Employment/Salary (full or part-time):; Alkahest. **D. Luu:** A. Employment/Salary (full or part-time):; Alkahest. **S. Lohr:** A. Employment/Salary (full or part-time):; Alkahest. **V. Kheifets:** A. Employment/Salary (full or part-time):; Alkahest. **S. Braithwaite:** A. Employment/Salary (full or part-time):; Alkahest. **D.P. Leone:** A. Employment/Salary (full or part-time):; Alkahest.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.08/C47

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant AG0336

Title: Estrogen regulation of miR-9-5p subcellular localization in neurons

Authors: *C. K. KIM[1], T. R. PAK[2];
[1]Loyola Univ. Chicago, Maywood, IL; [2]Dept Cell and Mol. Physiol., Loyola Univ. Chicago Stritch Sch. of Med., Maywood, IL

Abstract: Estrogens are a class of pleotropic steroid hormones crucial for the maintenance of normal physiology, especially in the central nervous system. Levels of estrogens fluctuate across the lifespan suggesting that they target intracellular mediators, such as microRNAs, to fine-tune homeostatic cellular processes. However, it is still relatively unknown how estrogens can regulate endogenous miRNAs, particularly in the context of the central nervous system. Previous work in our lab demonstrated that 17β-estradiol (E_2), the predominant circulating estrogen,

upregulated the total cellular expression of miR-9-5p, a brain-enriched miRNA that is necessary for neuronal differentiation; however, cytoplasmic levels of miR-9-5p remained unchanged with E_2 treatment. These results led to the hypothesis that E_2 might alter the subcellular localization of mature miRNA by increasing the nuclear levels of miR-9-5p. While the E_2-mediated nuclear import of miRNA is a novel phenomenon that has not been previously characterized, E_2 is a well-known regulator for the nuclear transport of estrogen receptors (ERs), raising the intriguing possibility of a nuclear import mechanism involving a direct ER-to-miRNA interaction. To test this hypothesis, IVB cells, derived from rat hypothalamus, were incubated with either E_2 or vehicle treatment for 2 HRs or 15 HRs. After E_2 treatment, nuclear and cytoplasmic compartments were separated using differential centrifugation. The purified RNA was reverse transcribed, and RT-qPCR was performed to measure levels of mature miR-9-5p in each fraction. Our results indicate that miR-9-5p subcellular localization could be altered with E_2, not only to the nucleus, but to other subcellular compartments such as polysomes. These data suggest a novel mechanism for hormonal regulation of miRNA stabilization in the brain, and future research will investigate the downstream cellular consequences of altered nuclear to cytosolic miR-9-5p localization.

Disclosures: C.K. Kim: None. **T.R. Pak:** None.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.09/C48

Topic: C.01. Brain Wellness and Aging

Support: NIH R01AG033605

Title: Phosphorylation and functional implications of estrogen receptor beta in the aging female brain

Authors: M. ZHANG, C. K. KIM, S. FLURY, ***T. R. PAK**;
Cell and Mol. Physiol., Loyola Univ. Chicago Stritch Sch. of Med., Chicago, IL

Abstract: Circulating estrogens dynamically fluctuate across the lifespan; they rapidly rise at puberty, peak during the reproductively competent years, and then sharply decline at menopause. The estrogen receptors (ERα/β) must adapt to this changing hormonal milieu to efficiently modulate the target genes of estrogens. We propose that there is a biological switch in ER action that occurs coincident with age and length of time after ovarian hormone depletion (i.e. menopause). Our data support the idea that phosphorylation of ERβ is a potential molecular mechanism mediating a menopausal “switch” in ER function. Phosphorylation of ERβ alters its protein conformation and interaction with other coregulatory proteins, thereby changing its

ability to transactivate target genes. In this study, we used a rat model of surgically-induced menopause to test the hypothesis that ERβ phosphorylation is differentially altered in the hippocampus dependent on length of time following deprivation of endogenous estrogens, and that these changes in phosphorylation dictate the functionality of the receptor on estrogen response element (ERE)-mediated target genes. We used a targeted quantitative mass spectrometry approach (absolute quantification (AQUA)) to precisely determine the phosphorylation status of multiple predicted serine and tyrosine residues on ERβ. In addition, we used ERE reporter gene assays to show that phosphorylation status of these sites altered ERE-mediated promoter activity. Together, our data revealed a novel molecular mechanism that could partly explain the discrepant clinical observations showing estrogens actions are different in recent (<5 yrs) compared to late (>10 yrs) post-menopausal women.

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Poster

374. Aging: Molecular Mechanisms II

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Topic: C.01. Brain Wellness and Aging

Support: Grant-in-Aid for Scientific Research A 15H02488
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Grant-in-Aid for Scientific Research on Innovative Areas 24116008
Grant-in-Aid for Scientific Research on Innovative Areas 24116001

Title: Vitamin B1 deficiency induces hippocampal dependent memory impairments via hippocampal degeneration and down-regulation of CREB signaling

Authors: *R. TSUJI[1,2], T. KISHIMOTO[1], K. NAGATA[1], T. WATANABE[1], S. KIDA[1,2];
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Abstract: Vitamin B1 (Thiamine) deficiency causes Wernicke-Korsakoff's syndrome in human that displays severe deficits in learning and memory. However, mechanism of Vitamin B1 deficiency-induced memory impairments remain unclear. Here we show that Vitamin B1 deficiency impairs hippocampus-dependent memory by degenerating hippocampus and imparting CREB-signaling pathway through neural inflammation. We showed that pyrithiamine-induced thiamine deficiency (PTD)-treated mice (PTD-recovery mice) that recovered from PTD display impairments of hippocampus-dependent memory. Importantly, we observed that PTD-recovery mice showed significant decreases in the number of NeuN positive cells in dentate gyrus (DG), CA1 and CA3 areas of the hippocampus and decreases in spine density in DG of the hippocampus. To further understand mechanisms of memory impairments and hippocampal

degeneration by PTD at the molecular level, we performed RNA-sequencing of hippocampus just (PTD mice) and 3 weeks (PTD-recovery mice) after PTD treatment. Interestingly, PTD mice showed significant increases in mRNA expressions of inflammatory related genes and decreases in mRNA expressions of synaptic signaling associated genes, whereas PTD-recovery mice showed decreases in mRNA expressions of immediate-early genes (IEGs). Since activity dependent expression of IEGs are regulated by CREB, we examined CREB mRNA level and found that PTD recovery mice show a significant decrease in expression of CREB mRNA in the hippocampus. This observation suggests that memory impairments observed in PTD-recovery mice is caused by dysfunction of CREB signaling pathway in the hippocampus. From these observations, we finally examined effects of PTD on transgenic mice expressing a constitutively active CREB mutant in the forebrain (DIEDML mice, Suzuki et al 2011) and found that PTD treatment failed to induce hippocampal degeneration and memory impairments in DIEDML mice. These observations suggest that PTD treatment causes strong inflammation, thereby leading to hippocampal degeneration and impairing CREB signaling pathway followed by impairments in hippocampus-dependent memory formation.

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Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.11/C50

Topic: C.01. Brain Wellness and Aging

Support: NIH AR-067667

Title: The role of gut microbiome in age associated cognitive impairment

Authors: J. BEHERA, J. ISON, K. KELLY, *N. TYAGI;
Physiol., Univ. of Louisville, Louisville, KY

Abstract: Alzheimer's disease (AD) remains a major clinical problem in older individuals. However, the pathophysiology of aging-associated AD remains unresolved. Interestingly, recent findings suggest that the gut microbiota may also play an important role in restraining immune responses in the brain via the brain-gut axis during AD. Through, it is unknown, whether age-related disruption of the gut-barrier integrity or dysbiosis, causes impaired cognitive function. Allyl sulfide (AS), an organosulfur compound from garlic. It has many health benefits and is known for its H_2S production. Therefore, we proposed, that administration of allyl sulfide (AS) can mitigate the gut dysbiosis and cognitive function by regulating the non-coding RNA, in part, by histone acetylation regulatory mechanism during aging.To test the hypothesis, we used (1): 5-

months age-matched mice as a control group (Young),(2): 24-months old age-matched mice as an aging group (AG),(3): 21-months (AG)+ allyl sulfide (AS) at the dosage of 2mg/kg/b.wt/per day through oral gavage (AG+AS). We found that, compared to young and AG+AS mice, AG mice had significantly increased levels of inflammation and AD biomarkers. The results also showed impaired blood-brain barrier (BBB) function and reduced cerebral blood flow as well as compromised learning and memory in aging mice. The gut microbiota is significantly altered as assessed by the denaturing gradient gel electrophoresis (DGGE) analysis in AG group as compared to Young and AG+AS group. Mechanistic study shows that AG mice display an increased plasma level of circulating non-coding RNA-Hotair (lncRNA-Hotair), as assessed by RT2 lncRNA qPCR Assay, This lncRNA-Hotair can cause a specific deregulation of histone H3 lysine 27 (H3K27) acetylation (ac) at the neuronal derived natriuretic factor (NDNF) promoter, and fail to initiate hippocampal activity associated with memory consolidation. However, administration of AS in the AG group was shown to ameliorate gut dysbiosis and memory functions. Furthermore, restoration of NDNF using recombinant NDNF-therapy promotes the recovery of cognitive abilities. In conclusion, our study suggests that the aging process involves deleterious changes in brain metabolic, vascular and cognitive functions, and gut microbiome structure and diversity, all of which may lead to inflammation and thus increase the risk for AD. Also, AS could serve as potential anti-aging therapeutics in treating aging individuals. Funding Information: This work is financially supported by the National Institute of Health grant AR-067667-NT, is greatly appreciated.

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Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.12/C51

Topic: C.01. Brain Wellness and Aging

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Finance Code 001.

Title: Adipocyte-derived exosomal microRNAs from cerebrospinal fluid are associated with downregulation of IL-8 and IL-6 pathways in aged animals

Authors: *L. R. CECHINEL[1], M. GOLDBERG[2], B. HARMON[2], R. FREISHTAT[2], I. SIQUEIRA[1];
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Abstract: It is well established that aging is associated with adipose tissue dysfunction, which is characterized by redistribution of fat depots and changes in the profile of cytokines released. Moreover, this age-related dysfunction is related to shortened lifespan and increased age- related diseases, including dementia. In this context, studies have proposed that adipocyte- derived exosomes might play a central role in the mechanisms by which adipose tissue dysfunction impact other tissues during aging. Exosomes can transfer several molecules such as microRNAs and they are able to cross the blood-brain barrier, releasing their cargo in the central nervous system (CNS). However, the effect of adipocyte-derived exosomal cargo, specifically microRNA, on the CNS needs to be better studied. Our aim was to investigate the microRNA profile of adipocyte-derived exosomes during aging process and their impact on central nervous system. The Local Ethics Committee (CEUA - Comissão de Ética no Uso de Animais - UFRGS; #29818) approved all animal procedures and experimental conditions. Male Wistar rats of 3- and 21-month-old were used and cerebrospinal fluid (CSF) was obtained from the cisterna magna. Exosomes were isolated and then the adipocyte-derived were selected (Fatty Acid Binding Protein 4 positive). microRNA was isolated from exosomes and profiled on Affymetrix GeneChip miRNA 4.0. Ingenuity Pathway Analysis (IPA) was used to identify pathways regulated by significantly altered miRNAs. The analysis of global microRNA expression revealed 47 differentially expressed ($p<0.05$; fold change of 1.1) between aged and young-adult animals of which 20 miRNAs were significantly up-regulated and 27 were down-regulated in aged animals compared to young-adult. A conservative filter was applied on IPA and only experimentally validated and highly conserved predicted mRNA targets for each microRNA was used. IPA analysis showed that IL-8 and IL-6 pathways are ranked as highly predicted targets for these differentially expressed microRNAs ($p<0.0001$). Moreover, IPA demonstrated that those canonical pathways are downregulated in aged animals when compared to young-adult. Interestingly lower levels of IL-8 and IL-6 were reported in subjects with dementia. Considering that, our results indicate that CSF adipocyte-derived exosomal miRNAs are predicted to influence neuroinflammatory pathways involved in neurodegenerative disorders.

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Poster

374. Aging: Molecular Mechanisms II

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Program #/Poster #: 374.13/C52

Topic: C.01. Brain Wellness and Aging

Support: HL 13331
HL 124576
AG054104

Title: Chronic short sleep initiates gender-dependent limbic system neurodegeneration

Authors: *J. E. OWEN, Y. ZHU, P. FENIK, K. SHULMAN, S. VEASEY;
Dept Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Introduction: Chronic short sleep (CSS) is prevalent in society and evidence is accumulating for the role of sleep loss in neurodegenerative processes, including Alzheimer's disease (AD). Sleep loss can accelerate AD pathology in transgenic animals that overexpress either Abeta42 or tau. However, it is not known whether sleep loss can initiate AD like pathology in non-transgenic mice. The present study tested the hypothesis that CSS in early adulthood would lead to neurodegenerative changes in old age that resemble the beginning stages of AD.
Methods: The present study subjected male and female C57B6 mice to 12 weeks of CSS (8hrs lights-on sleep loss for 3d/wk) as young adults. Spatial memory tests were performed at 18 months of age on CSS mice and rested control littermates. Subsequently, brain tissue was collected and analyzed for neurodegeneration including hippocampal (HC) and entorhinal cortex (EC) atrophy (stereological Cavalieri's volumes) and AD-suggestive immunopathology.
Results: Control mice had intact spatial memory ($p<0.05$), while CSS mice did not ($p>0.05$). CSS mice showed increased volume of the lateral ventricles (51%, $p<0.05$) and decreased total HC volume (-5%, $p<0.05$) as well as specific loss in the CA1 (-5%, $p<0.05$), CA2 (-19%, $p<0.05$) and EC (-5%, $p<0.05$). When analyzed separately, CSS exposed males had increased lateral ventricle volume (83%, $p<0.05$) and decreased total HC volume (-6%, $p<0.05$) as well as specific loss in CA2 (-26%, $p<0.05$). Female CSS versus control mice did not show any significant volume changes. Overall, CSS mice had increased AT8, a marker of phosphorylated tau, in the HC ($p<0.01$) and EC ($p<0.01$); increased punctate Ab42 ($p<0.05$) and LAMP2 ($p<0.01$), an autolysosomal marker in the HC and increased CD68, a marker of microglial activation, in the HC ($p<0.05$) and EC ($p<0.01$). When analyzed separately, female CSS mice had increased AT8 ($p<0.05$) and CD68 ($p<0.05$) in the EC only, not in HC, whereas male CSS mice had increased AT8 in the EC ($p<0.05$) and HC ($p<0.05$) and increased LAMP2 in the HC ($p<0.05$).
Conclusion: These results suggest chronic short sleep early in life in wild type mice can initiate a neurodegenerative process, which appears more significant in males than in females.

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Poster

374. Aging: Molecular Mechanisms II

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.14/C53

Topic: C.01. Brain Wellness and Aging

Support: Hunter Medical Research Institute Grant
Greaves Family Philanthropic Scholarship Hunter Medical Research Institute

Title: Aging of the blood-CNS barriers

Authors: *M. J. CUMMINS[1,2], E. T. CRESSWELL[1,2], D. W. SMITH[1,2];
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Abstract: Background: The blood-brain and blood-spinal cord barriers maintain the neural environment and protect it from ionic variations and potential toxins in the systemic circulation. Aging is thought to alter barrier permeability, resulting in neuronal dysfunction, but the literature is conflicted. We used molecular and functional assays to assess the effects of aging on blood-CNS barriers. The research contains both confirmatory and exploratory components.
Methods: Animals: Young (2-4 mo) and old (≥24 mo) C57Bl6 male mice were used for all studies.
RNA seq: RNA-seq was carried out on the frontal cortex (CTX) and spinal cord (SC), and differential gene expression (DEG) between young (n=4) and old (n=4) groups determined using cuffdiff, deseq2, and edgeR. DEGs were compared to cell-specific gene lists for endothelial (EC), pericyte, and astrocyte cells, as well as other barrier related genes.
CNS Regions: A subset of 29 barrier genes were investigated by qPCR across 5 regions (frontal cortex (CTX), corpus callosum (CC), hippocampus (HIP), spinal cord grey matter (SCGM), and spinal cord white matter (SCWM)) in young (n=8) and old (n=8) mice.
Time course of barrier changes: 12 genes were investigated by qPCR across 5 ages (2.5, 4, 8, 14, 26 months, n=6/group) in SC.
Microvessel-specific effects: Microvessels containing ECs and pericytes were microdissected from young (n=6) and old (n=6) CTX and SCGM and expression of 12 genes was determined by qPCR.
Barrier permeability: The effects of aging on permeability were assessed by CNS water content (n=6/group), and sodium-fluorescein (NaFl) permeability (n=10-12/group).
Statistics: For 2 group comparisons, the Mann-Whitney U Test was used. For the time course, the Kruskal-Wallis Test with the Games Howell post-hoc was used. p-value <0.05 was set for significance.
Results: RNA seq: A number of EC (CTX 19%, SC 19%), pericyte (20%, 40%), astrocyte (23%, 27%), and barrier (18%, 32%) specific genes were changed in old CNS. In general the SC was more affected than CTX.
CNS Regions: Of 29 genes investigated, 4 CTX, 7 HIP, 9 CC, 12 SCGM, and 14 SCWM were significantly changed in old CNS.
Time course of barrier changes: 4 barrier genes were significantly altered with aging and changes generally occurred at older ages.
Microvessel-specific effects: Only 1 gene was changed, in the SCGM.
Barrier permeability: Water weight was unchanged in whole brain and increased in whole SC.

NaFl permeability was significantly reduced in HIP, and SC, but not in CTX and CC (combined).
Conclusion: Overall, our data indicate barrier function in the SC may be affected by aging, but other regions remain intact. Barrier changes appear to occur later in the lifespan.

Disclosures: M.J. Cummins: None. **E.T. Cresswell:** None. **D.W. Smith:** None.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.15/C54

Topic: C.01. Brain Wellness and Aging

Title: Endothelial senescence mediated by $p16^{INK4a}$ leads to cerebrovascular dysfunction in mouse brains

Authors: *T. AIKAWA[1], Y. CHEN[2], I.-A. VETELENAU[1], H. OUE[1], M.-L. HOLM[1], B. Y. S. KIM[2], T. KANEKIYO[1];
[1]Neurosci., Mayo Clin., Jacksonville, FL; [2]MD Anderson Cancer Ctr., Univ. of Texas, Houston, TX

Abstract: As the cerebrovascular system plays a critical role in maintaining brain homeostasis, disturbances of this pathway can lead to cognitive impairment and dementia during aging. Interestingly, population-based epidemiologic studies have revealed that cerebrovascular damage due to hypertension, diabetes or smoking is also associated with the increased risk for Alzheimer's disease (AD). While AD and vascular cognitive impairment and dementia (VCID) are major causes of dementia, the prevalence and the incidence of both diseases remarkably increase in an age-dependent manner. Although the process of aging is complex, current evidence indicates that aging is caused by the accumulation of senescent cells, where the increase of $p16^{INK4a}$ is the major hallmark. Thus, we investigated how the forced expression of $p16^{INK4a}$ in vascular endothelial cells impacts cerebrovascular function, using an in vivo gene delivery system through a unique recombinant adeno-associated virus 2 (rAAV2) with a modified capsid. We confirmed that intraperitoneal injection of the rAAV2, harboring an enhanced green fluorescent protein (EGFP) reporter gene under the control of the CAG promoter, successfully induced the specific expression of EGFP in cerebrovascular endothelial cells in mice. When $p16^{INK4a}$ or EGFP was expressed in cerebrovascular endothelial cells in wild-type mice at 3 months of age, results showed an increase in IgG leakage into the brain parenchyma in the $p16^{INK4a}$-expressed mice in comparison with the control EGFP-expressed mice, 4 weeks after the rAAV2 injection. Whereas we also examined the coverage of endothelial cells with pericytes and major tight junction proteins, there were no significant differences between two mouse groups. When cerebral blood flow was measured by in vivo 2-photon

imaging 10 weeks after the rAAV2 injection in the mice, we found that cerebrovascular endothelial $p16^{INK4a}$ overexpression substantially reduced blood flow in arterioles and capillaries in the cortex. Together, our results indicate that $p16^{INK4a}$-induced endothelial senescence disturbs cerebral blood supply as well as blood-brain barrier integrity, which may contribute to the pathogenesis of AD and VCID.

Disclosures: T. Aikawa: None. **Y. Chen:** None. **I. Vetelenau:** None. **H. Oue:** None. **M. Holm:** None. **B.Y.S. Kim:** None. **T. Kanekiyo:** None.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.16/C55

Topic: C.01. Brain Wellness and Aging

Title: Single cell analysis of aged brain vasculature reveals capillary endothelium as locus of neurodegeneration implicated gene changes and therapeutic target

Authors: *Z. LI[1], L. ZHAO[1], J. S. VONG[2], L. YAN[3], H. LAI[1], X. CHEN[1], K. SY[1], X. TIAN[4], Y. HUANG[4], E. CHAN[5], W. CHIU[2], Y. LO[2], H. SO[4], C. MOK[1], H. KO[1];
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Abstract: Ageing is the strongest risk factor for neurodegenerative diseases. With advances in single-cell transcriptomics, molecular signatures of cells constituting the neurovascular units (NVUs) are revealed in unprecedented details. We performed high-throughput single-cell RNA sequencing (scRNA-seq) and RNA fluorescence *in situ* hybridization (RNA-FISH) of endothelial and mural cells in young adult and aged mice brains to characterize ageing-associated gene expression changes that concur with compromised blood brain-barrier (BBB) integrity. We identified shared and divergent transcriptomic changes in endothelial and mural cell subtypes along the arterio-venous axis, implicating altered energy metabolism pathways in multiple endothelial cell (EC) subtypes. Among the distinct NVU-constituting cell subtypes, capillary ECs exhibit the most gene expression changes with strong neurodegenerative disease-implicated gene enrichment. Furthermore, we demonstrated that exendin-4 (Ex4) treatment normalizes many gene expression level changes capillary ECs and highly reduces BBB leakage in aged brain, pointing to a novel neurovascular mechanism of action underlying the therapeutic efficacies of glucagon-like peptide11 receptor (GLP-1R) agonists in neurodegenerative diseases widely reported in pre-clinical and clinical studies.

Disclosures: **Z. Li:** None. **L. Zhao:** None. **J.S. Vong:** None. **L. Yan:** None. **H. Lai:** None. **X. Chen:** None. **K. Sy:** None. **X. Tian:** None. **Y. Huang:** None. **E. Chan:** None. **W. Chiu:** None. **Y. Lo:** None. **H. So:** None. **C. Mok:** None. **H. Ko:** None.

Poster

374. Aging: Molecular Mechanisms II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 374.17/C56

Topic: F.03. Neuroendocrine Processes

Support: RSF Grant 19-15-00039

Title: Neurochemical properties of the mediobasal hypothalamic neurons during ageing

Authors: *P. M. MASLIUKOV[1,2], K. MOISEEV[1], V. PORSEVA[1], A. EMANUILOV[1], M. KORZINA[1];
[1]Yaroslavl State Med. Univ., Yaroslavl, Russian Federation; [2]Petrozavodsk State Univ., Petrozavodsk, Russian Federation

Abstract: The hypothalamus is the most important integrator of the autonomic and endocrine regulation and is responsible for growth, development, reproductive function and metabolism. In recent years, evidence has been obtained that the hypothalamus also controls aging. The ventromedial (VMH) and dorsomedial (DMH) hypothalamic nuclei are important hypothalamic nuclei critical for regulating feeding and maintaining whole body energy homeostasis.
The aim of the study was to analyze the expression of neuronal nitric oxide synthase (nNOS), calbindin (CB), sirtuin 1 (Sir1), neuropeptide Y and steroid factor 1 (SF1) in the VMH and DMH hypothalamic nuclei using immunohistochemistry and western blotting in young (3-month-old) and aged (2-years-old) rats during aging.
The results have shown that in DMH, the percentage of CB-immunoreactive (IR) neurons was significantly reduced, and the percentage of CR-IR neurons increased in DMH and VMH during aging ($p<0.05$). The expression of SF1 significantly declined in the VMH during ageing ($p<0.05$). In old rats, there was an increase in the density of NPY-IR fibers in the VMH and a decrease in the DMH ($p<0.05$). The number of NPY-IR neurons in DMH of young and aged rats did not change significantly ($p>0.05$). The number of Sir1-immunoreactive (IR) neurons decreased in both VMH and DMH in old rats when compared to young animals ($p<0.05$). In young rats, nNOS-IR neurons had low intensity of fluorescence in the VMH and DMH. In old animals, the number of nNOS-IR neurons and the degree of immunofluorescence to nNOS significantly increased in all studied nuclei ($p<0.001$).
Thus, neurochemical properties of the hypothalamic neurons change during ageing. There are differences of age-dependent changes between VMH and DMH.

Disclosures: **P.M. Masliukov:** None. **K. Moiseev:** None. **V. Porseva:** None. **A. Emanuilov:** None. **M. Korzina:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.01/C57

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA T32 AG000222
NIA/NIH R00AG047336-05

Title: Elucidating the cell specific roles of apolipoprotein E in Alzheimer's disease

Authors: *R. J. JACKSON[1], J. C. MELTZER[1], H. N. NGUYEN[1], B. T. HYMAN[2], E. HUDRY[3];
[1]Massachusetts Gen. Hosp. Inst. for Neurodegenerative Dis., Charlestown, MA; [2]Massachusetts Gen. Hosp., Charlestown, MA; [3]Dept. of Neurol. Alzheimer Res. Unit, MGH, Charlestown, MA

Abstract: **Background:** Late-onset sporadic Alzheimer's disease (LOAD) represents about 95% of all Alzheimer's disease cases and has become a global health crisis with more than 3 million cases of AD in the US alone. More than two decades ago, apolipoprotein E (apoE) was identified as the strongest genetic modulator of LOAD, with the apoE4 isoform increasing the risk of developing LOAD by a factor of 3 (heterozygote) to 12 (homozygote) and apoE2 decreasing this risk by half as compared with the most common apoE3 isoform. Despite those compelling genetic findings and numerous experimental studies that have established apoE as a multifaceted player in AD, the field remains largely conflicted about the best way to target apoE for clinical improvement. In our recent work, we have demonstrated that the complete absence of apoE in a model of amyloidosis (APP/PS-APOEnull) reduces the overall amount of neuritic plaques in the brain, the density of reactive astrocytes and microglia, and more importantly preserves synaptic integrity and rescues Aß-dependent neuronal dysfunction (assessed by array tomography and in vivo multiphoton calcium imaging in awake animals, respectively). The present follow-up study aims at unraveling the cell-autonomous role of APOE in the brain, with an emphasis on the distinct effects of astrocyte- or microglia-derived APOE. **Approach:** Using healthy and diseased human brain samples, we will establish the topology of APOE expression in astrocytes and microglia by RNAscope in situ hybridation and determine whether APOE genotype or the disease course modulate APOE mRNA profile in each glial cell types. In addition, we have established novel humanized mouse models in which the expression of APOE3 or APOE4 can be conditionally switched off in astrocytes and microglia upon recombination to determine the contribution of astrocyte- and microglia-derived apoE in wild-type mice or in the context of amyloidosis. **Results:** We have successfully established the detection of apoE messengers in

fresh frozen brain tissue using RNAscope, and a large stereological analysis is ongoing. In addition, we have generated cx3cr1-Cre^{ERT2}xAPOE (microglial knockdown) and Aldh1l1-Cre^{ERT2}xAPOE mouse lines (on a wild-type or AD mouse model backgrounds) and observed that, at baseline, the microglial contribution for apoE messengers is minimal as compared to astrocytes. Whether or not the ratio between microglia- and astrocyte-derived apoE will change upon amyloid deposition and whether the genetic knockdown of apoE in either cell type will impact their phenotype as neuroinflammation progresses is under evaluation.

Disclosures: R.J. Jackson: None. **J.C. Meltzer:** None. **H.N. Nguyen:** None. **B.T. Hyman:** None. **E. Hudry:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.02/C58

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CureAlzheimer's Fund

Title: Neuroinflammatory effects of apolipoprotein E4 in the brain

Authors: *J. M. IANNUCCI[1,2], A. SEN[1], M. MAJCHRZAK[1], W. RENEHAN[1], P. GRAMMAS[1];
[1]George & Anne Ryan Inst. for Neurosci., [2]Interdisciplinary Neurosci. Program, Univ. of Rhode Island, Kingston, RI

Abstract: Mutations to the cholesterol transport protein apolipoprotein E (ApoE) have been identified as a major genetic risk factor for the development of sporadic or late onset Alzheimer’s disease (AD), with the E4 allele representing an increased risk and the rare E2 allele having a reduced risk compared to the primary E3 form. The reasons behind the differences in risk are not entirely understood, though ApoE4 has been connected to inflammation-related changes in both the brain and the periphery. It is our hypothesis that markers of both inflammation and oxidative stress are increased in response to apolipoprotein E4. We tested this hypothesis using both *in vivo* and *in vitro* models. Brains of mice with the human E4 gene were collected at 10 months of age along with age-matched wild-type controls. Western blot was used to assess whole-brain protein expression. Human astrocytes, human brain microvessel endothelial cells (HBMVECs), and a human immortalized microglia cell line (HMC3), were grown *in vitro*. Cells were treated for 24 hours with cholesterol (100 µM) with or without ApoE2/3/4 (20 nM). Following treatment, supernatant and lysate were collected, and western blot was used to assess protein expression. Our results indicate changes in expression of inflammation- and oxidative stress-related proteins both *in vivo* and *in vitro. In vivo,* mice

expressing the E4 allele exhibited changes in expression of inflammation-related proteins (GFAP, cd11b, Iba1, TNFalpha, IL-6, IL-1beta), and oxidative stress-related proteins (iNOS, eNOS, and NOX4). Similar results were seen *in vitro*, with astrocytes and microglia showing increased expression of pro-inflammatory cytokines including IL1beta, TNFalpha, and IL-6 in response to ApoE4. Similarly, HBMVECs *in vitro* showed altered expression of oxidative stress-related proteins, particularly NOX4 in the presence of ApoE4. Overall, results indicate that ApoE isoform affects the expression of inflammation-related proteins both *in vivo* and *in vitro*, with ApoE4 favoring a pro-inflammatory phenotype. Further studies are needed to better understand the mechanism by which different ApoE isoforms may drive inflammatory processes in the brain.

Disclosures: J.M. Iannucci: None. **A. Sen:** None. **M. Majchrzak:** None. **W. Renehan:** None. **P. Grammas:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.03/C59

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: T32 GM118292-02
NIH Grant RF1-AG057754

Title: Apolipoprotein E isoform impact on the neuroinflammatory state of the human Alzheimer’s disease brain

Authors: *C. M. KLOSKE[1,2], A. DUGAN[1], A. WOOLUMS[1], T. L. SUDDUTH[1,2], S. ANDERSON[1,2], E. PATEL[1,2], E. L. ABNER[1,2], P. T. NELSON[1,2], D. FARDO[1,2], D. M. WILCOCK[1,2];
[1]Univ. of Kentucky, Lexington, KY; [2]Sanders-Brown Ctr. on Aging, Lexington, KY

Abstract: The combination of two neuropathological hallmarks distinguish Alzheimer’s Disease (AD) from other dementias: amyloid plaques, composed of amyloid-beta peptide, and neurofibrillary tangles. Additionally, the brain experiences a robust inflammatory response to these amyloid plaques, which induces the activation and proliferation of microglia. Researchers have identified multiple genetic risk factors for developing AD. One of the most significant genetic risk factors is Apolipoprotein E (ApoE), a cholesterol transport lipoprotein in the brain. Of the three identified ApoE alleles, ApoE3 is believed to be the “control” phenotype, ApoE4 confers an increased risk of AD, and the ApoE2 allele may be protective for AD. Interestingly, ApoE binds to a surface receptor on microglia, triggering receptor expressed on myeloid cells 2 (TREM2). Evidence suggests that, amyloid plaques interact with ApoE, which then bind to

microglial TREM2. This activates a neuroinflammatory cascade and leads to microglial phagocytosis of the amyloid plaque, suggesting a role for ApoE in the inflammatory response seen in AD. While differences are evident, the field knows very little about how each ApoE allele affects the activation of the neuroinflammatory cascade. To test the impact of each ApoE allele, we used the Human Neuroinflammation NanoString panel to assess the inflammatory profile in the superior medial temporal gyrus (SMTG) and cerebellar regions of age and sex matched cases with the following pathology: ApoE3/3 Braak V/VI (ApoE3/3-AD; N=9), ApoE4/4 Braak V/VI (ApoE4/4-AD; N=10), and ApoE3/3 Braak I/II (ApoE3/3 without AD; N=5). Total RNA was extracted from frozen SMTG and frozen cerebellum. NanoString results were analyzed with NanoStringDiff. Furthermore, qPCR was performed to verify RNA trends from NanoString. We found significant differences in expression of a number of inflammatory genes expressed between the ApoE3/3-AD and ApoE4/4-AD brains. Additionally, the data suggests the ApoE3/3-AD cases exhibited an altered neuroinflammatory response from those ApoE3/3 without AD patients suggesting that ApoE3 may also react to the amyloid plaques. ApoE4/4-AD patients tend, instead, to be in a state of neuroinflammation similar to that found in ApoE3/3 patients without AD, suggesting they cannot respond to the amyloid plaques. These results suggest differences in neuroinflammation between ApoE3/3 and E4/4 patients and may be implicated in the progression of the disease.

Disclosures: C.M. Kloske: None. **A. Dugan:** None. **A. Woolums:** None. **T.L. Sudduth:** None. **S. Anderson:** None. **E. Patel:** None. **E.L. Abner:** None. **P.T. Nelson:** None. **D. Fardo:** None. **D.M. Wilcock:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.04/C60

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: APOE4 induces region-specific neuronal dysfunction in the entorhinal cortex of APOE target replacement mice

Authors: *H. A. ARAIN[1], H. Y. FIGUEROA[1], E. AREA-GOMEZ[2], K. E. DUFF[1], T. NURIEL[1];
[1]Taub Inst. for Alzheimer's and the Aging Brain, [2]Dept. of Neurol., Columbia Univ. Med. Ctr., New York, NY

Abstract: To date, the greatest genetic risk factor for sporadic Alzheimer’s disease (AD) is the possession of the apolipoprotein E4 (*APOE4*) variant. Yet the mechanism by which *APOE4* increases AD susceptibility is unclear. Our lab has revealed that *APOE4* may have unique effects on the regulation of important biological processes in the entorhinal cortex (EC), which is one of

the first regions of the brain to be affected by AD pathology. Using aged *APOE4* targeted replacement mice, we investigated the effects of *APOE4* expression in the EC using a combination of omics analysis, immunohistochemistry, and biochemical assays. Our data revealed *APOE4*-associated alteration in genes, lipids, and metabolites in the EC related to neuronal activity and bioenergetics. Follow up studies have elucidated the ways in which *APOE4* expression causes dysregulation of these vital biological pathways in the EC, and further studies are currently underway to understand how these pathways dysregulations are related to one another and whether they are instrumental in the AD pathology that accumulates early on in the EC. These findings provide novel insights into the ways in which *APOE4* expression may increase AD disease risk by revealing region-specific alterations in vital biological pathways such as neuronal activity and bioenergetics in AD-specific brain regions. We believe that these results will point to novel ways in which AD can be slowed or prevented, especially among *APOE4* carriers.

Disclosures: H.A. Arain: None. **H.Y. Figueroa:** None. **E. Area-Gomez:** None. **K.E. Duff:** None. **T. Nuriel:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.05/C61

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 NS 100704-02
NIH R01 NS 100704-S1

Title: The effects of high fat diet on metabolism and cognition in APOE mice

Authors: *N. JONES[1], G. W. REBECK[2];
[2]Neurosci., [1]Georgetown Univ., Washington, DC

Abstract: Obesity is a strong physiological risk factor for AD and *APOE4* is the strongest genetic risk factor for AD. Since these factors are present in high percentages of the population, it is imperative that the combined effects of a high fat (western) diet (HFD) and the *APOE4* allele be defined. Male and female human *APOE3* and *APOE4* knock-in mice were fed either a HFD (45% kcal fat) or a low fat diet (LFD) (10% kcal fat) for 3 months (from 6 to 9 months of age). At 9 months, metabolic measurements were determined: weight, baseline glucose levels, glucose tolerance, and visceral adipose tissue (VAT) accumulation. For behavioral or cognitive effects, we tested the mice on the Open Field, Elevated Zero, and Barnes Maze (BM). HFD had similar effects on *APOE4* and *APOE3* mice in terms of weight gain (22% vs 14%) and baseline glucose levels (15% vs 14%). HFD had greater effects in *APOE4* mice compared to *APOE3* mice

in a measure of glucose intolerance (34% higher), and in VAT accumulation (33% higher). Weight gain was positively correlated with glucose intolerance in *APOE3* mice, but not *APOE4* mice indicating *APOE4* mice have deficits in glucose metabolism independent of weight gain. On the BM, *APOE4* mice performed significantly worse than *APOE3* mice, but HFD did not impair spatial learning in either genotype. There were no significant differences in the other behavioral tasks. In summary HFD caused increased weight and metabolic disturbances in both *APOE3* and *APOE4* mice, with *APOE4* mice more affected. Given the highly inflammatory nature of VAT, the higher VAT accumulation in *APOE4* mice could cause higher levels of peripheral and CNS inflammation. Current research is addressing measures inflammation, as well as neuronal complexity, in order to test the effects of HFD on central mechanisms.

Disclosures: N. Jones: None. **G.W. Rebeck:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.06/C62

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RF1 AG057517

Title: Western diet affects the level of brain exosomes in an APOE-genotype dependent manner in mice

Authors: *L. RACHMANY[1,5], S. F. NEWBURY[2], S. BHAMDEO[2], B. S. EAST[3], G. M. FLEMING[3], K. Y. PENG[2,6], D. A. WILSON[3,4,7], E. LEVY[8], P. M. MATHEWS[2,5]; [2]Ctr. for Dementia Res., [3]Emotional Brain Inst., [4]Dept. of Child & Adolescent Psychiatry, [1]Nathan S. Kline Inst., Orangeburg, NY; [5]Dept. of Psychiatry, [6]Dept. of Neurol., [7]Dept. of Neurosci. & Physiol., New York Univ. Langone Hlth., New York, NY; [8]Nathan S Kline Inst., Orangeburg, NY

Abstract: Apolipoprotein E (APOE) transports cholesterol from astrocytes to neurons, contributing to the lipid composition of neuronal membranes. The three human alleles APOE2 (E2), APOE3 (E3), and APOE4 (E4) are linked epidemiologically to Alzheimer’s disease (AD). In a dose-dependent manner, expression of the APOE4 allele is the greatest genetic risk factor for Alzheimer’s disease. The common APOE3 allele is considered to be a neutral-risk factor for AD, while the less common APOE2 allele is protective. Prior findings have shown that expression of the APOE4 compared to APOE3 allele affects the neuronal endosomal-lysosomal system, including brain exosomes levels. Exosomes are phospholipid bilayer enclosed vesicles that are secreted into the tissue extracellular space following their formation by the invagination of the membrane of late endosome/multivesicular body (MVB) around cytoplasmic materials. In

humanized APOE2, APOE3, and APOE4 mice, we compared olfactory behavior and exosome biology in mice fed a standard diet to those fed a “Western diet” that contains increased cholesterol and fat. Initial cohorts of mice were fed the Western diet for one month, beginning at 12 months of age, and assessed behaviorally using an olfactory odor habituation test, previously shown to be affected by APOE genotype in mice of this age. Brain exosomes were then isolated by centrifugation through a differential-density gradient. One month of Western diet altered odor habituation only in APOE3 mice, while this behavior was unchanged in APOE2 and APOE4 mice following one month of the diet. Additionally, the Western diet resulted in changes in brain exosomes levels in an APOE-genotype dependent manner, with APOE3 mice appearing to be the most sensitive to diet-induced changes. Additional cohorts of mice assessed at the same age but fed the Western diet for 6 months are being examined. Thus, the APOE-genotype may modify the response to a diet high in cholesterol and fat, with our current findings suggesting APOE genotype-dependent responses to the diet in both behavior and brain exosomes.

Disclosures: L. Rachmany: None. **S.F. Newbury:** None. **S. Bhamdeo:** None. **B.S. East:** None. **G.M. Fleming:** None. **K.Y. Peng:** None. **D.A. Wilson:** None. **E. Levy:** None. **P.M. Mathews:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.07/C63

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 15K15712, (to M.M.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Title: Cholesterol regulates exosome release in cultured astrocytes

Authors: *M. ABDULLAH, T. FERDOUS, M. MICHIKAWA*;
Dept. of Biochemistry-I, Nagoya City Univ., Nagoya, Japan

Abstract: Extracellular vesicles (EVs), particularly exosomes, have emerged in the last 10 years as a new player in the progression of Alzheimer’s disease (AD) with high potential for being useful as a diagnostic and treatment tool. Apolipoprotein E (ApoE), secreted from astrocytes plays an essential role in high-density lipoprotein (HDL) generation by removing cholesterol from the cells. Aβ is known to binds ApoE-HDL, taken up by the cells, and degraded. Recent studies have demonstrated that exosome release is mediated by SM and ceramide in the cells. Flotillin, a marker for exosome, exists in lipids raft, which is rich in cholesterol and SM, suggesting that cholesterol may regulate exosome release from astrocytes. In this study, we performed experiments to determine whether cholesterol regulates exosome release in cultured

astrocytes.
Initially we found that exosome release demonstrated by flotillin and HSP90 levels in the conditioned media is significantly reduced in cultured astrocytes prepared from ApoE-KO mouse, compared with that in astrocytes prepared from WT, ApoE3- knock-in (KI), and ApoE4-KI mice. The reduced level of flotillin and HSP90 were accompanied by the elevation of cholesterol levels. PI3K/Akt phosphorylation was enhanced in ApoE-deficient astrocytes. Addition of cholesterol to the astrocytes significantly reduced exosome release demonstrated by flotillin and HSP90 levels. Whereas, depletion of cholesterol by the treatment with β-cyclodextrin recovered the secretion levels of exosome in ApoE-deficient astrocytes to a similar level of WT astrocytes. In addition, reduced levels of exosome release by the addition of cholesterol were recovered by the treatment of PI3K inhibitor (LY294002). These results suggests that, exosome release is regulated by cholesterol via stimulation of PI3K/Akt signal pathway.

Disclosures: M. Abdullah: None. **T. Ferdous:** None. **M. Michikawa*:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.08/C64

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DGIST MIREBraiN program
NRF of Korea 2018R1D1A1B07046356

Title: Investigating the contribution of astrocytic cholesterol transport in neuronal amyloid beta generation using human-induced pluripotent stem cells

Authors: *W. JEONG, H. LEE, J. SEO;
Brain and Cognitive Sci., DGIST, Daegu, Korea, Republic of

Abstract: Alzheimer's disease (AD) is the most common form of irreversible dementia characterized by progressive neurodegeneration and accumulation of Aβ plaques. In brain, neurons are the major source of Aβ, and cholesterol-rich membrane domains, lipid rafts are thought to offer a structural platform for Aβ generation and aggregation. Previous study showed increased cholesterol levels in human induced pluripotent stem cell (iPSC)-derived astrocytes carrying *APOE4* variant compared to its APOE3 astrocytes. We aimed to address the role of hiPSC-derived astrocytes with *APOE4* alleles on the formation of lipid rafts and Aβ production in neurons. By performing lipidomic analysis, we first examined differentially secreted lipid metabolites by APOE isoforms. We found that cholesterol is the one of the up-regulated metabolites by *APOE4* variant in astrocytes. To investigate the effect of astrocytic cholesterol

transport to neurons, we applied cholesterol or inhibitor of cholesterol synthesis (*MβCD*) to rat primary neurons and measured the area and intensity of lipid rafts on plasma membrane. Cholesterol-treated neurons displayed increased area and intensity compared to non-treated neurons, whereas inhibition of cholesterol synthesis showed opposite phenotypes. We further observed that lipid rafts in rat primary neurons were indeed regulated by APOE isoform when they were co-culture with hiPSC-derived astrocytes carrying either *APOE3* or *APOE4* alleles. We are currently investigating whether cholesterol dysregulation in APOE4 astrocytes is sufficient to alter neuronal Aβ production, which will be presented at the meeting.

Disclosures: W. Jeong: None. **H. Lee:** None. **J. Seo:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.09/C65

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cure Alzheimer's Fund
JPB Foundation (DMH)

Title: Differential effects of astrocyte and microglia apolipoprotein E production on the development of amyloid-beta pathology

Authors: *T. E. MAHAN[1], J. D. ULRICH[1], T.-P. V. HUYNH[1], N. SCOTT[1], J. REMOLINA SERRANO[1], R. E. TANZI[2], D. M. HOLTZMAN[1];
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Abstract: The ε4 allele of apolipoprotein E (apoE) is the strongest genetic risk factor for developing late-onset Alzheimer disease (AD). One of the key characteristic pathologies of AD is the formation of extracellular amyloid-β (Aβ) plaques. Previous studies have shown that both the level of apoE and the extent of lipidation of apoE-containing lipoprotein particles dramatically influence the development of Aβ pathology. While astrocytes are the primary producer of apoE in the CNS, studies have shown that microglia also produce apoE and upregulate their apoE mRNA levels as Aβ pathology develops. To address whether apoE produced by astrocytes vs. microglia might be playing specific roles in the development of Aβ plaque pathology, we first compared apoE-containing lipoprotein particles from microglia to those produced by astrocytes. We found that microglia produce smaller, likely much less lipidated apoE particles than astrocytes. Additionally, we found that microglia expressing the human apoE4 isoform produce greater amounts of smaller apoE particles relative to apoE2 and apoE3 expressing microglia. Next, we looked *in vivo* to investigate how removal of apoE from

astrocytes would affect the development of Aβ plaque pathology in APPPS1-21 mice expressing human apoE3 or human apoE4 and compared the results to previous work with global apoE KO in APPPS1-21 mice. We found that the loss of astrocytic apoE resulted in changes to Aβ plaque size and morphology, similar to what was seen in the global apoE KO. Plaques were larger and less compact in the absence of astrocyte-produced apoE. However, the loss of astrocytic apoE did not dramatically reduce the levels of fibrillar Aβ plaques, which is contrary to what was seen with global apoE KO's. Global apoE KO's had previously been shown to have a significant decrease in microgliosis around fibrillar amyloid plaques. The astrocyte-specific-apoE knockouts, however, did not display a similar decrease in microgliosis. Additionally, we found isoform dependent differences on Aβ plaque development between the apoE3 and apoE4 expressing mice. These results suggest that astrocyte-apoE plays an important role in regulating amyloid plaque morphology. The results also suggest that apoE produced by cells other than astrocytes, including microglia, or apoE produced by astrocytes, microglia, and other cells in the brain may be critical in facilitating fibrillization of Aβ and its local consequences in brain parenchyma. These results also suggest that the presence of microglial apoE and/or apoE produced by other CNS cell types is sufficient for microgliosis to occur around Aβ plaques.

Disclosures: **T.E. Mahan:** None. **J.D. Ulrich:** None. **T.V. Huynh:** None. **N. Scott:** None. **J. Remolina Serrano:** None. **R.E. Tanzi:** None. **D.M. Holtzman:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.10/C66

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UH2 NS100127
UH3 NS100127
UIC Chancellor's Proof-of-Concept-Award
NIH 1T32AG057468-01 Grant
UIC Provost Deiss
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Title: The plasma lipoprotein profile in a novel transgenic mouse model is modulated by Alzheimer's disease (AD) risk factors: Potential as an AD biomarker

Authors: B. XIANG[1], K. PANCHAPAKESAN[1], Y. SALEH[1], S. CORONEL[1], N. FAULK[1], J. E. MALDONADO WENG[1], J. M. YORK[1], A. C. VALENCIA[1], *M. LADU[1], A. J. KARSTENS[2,1];
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Abstract: Alzheimer's disease (AD) is the 6th leading cause of death in the United States with no cure, few palliative treatments and no prognostic biomarker. While age is the greatest risk factor for AD, the greatest genetic risk factor is *APOE4*, increasing risk 4- to 12-fold compared to the common *APOE3*, while the rare *APOE2* reduces risk. Female (♀) *APOE4* carriers have greater lifetime risk and rate of cognitive decline compared male (♂) *APOE4* carriers. *APOE* encodes apolipoprotein E (apoE), a protein component of plasma lipoproteins and the primary apolipoprotein in the brain, expressed by glial cells. In the brain, apoE plays a major role in lipid transport and clearance of soluble, oligomeric amyloid-β (oAβ), likely the proximal AD neurotoxin. Separate from the brain, plasma lipoproteins in the blood are heterogenous, including very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Blood lipid panels typically report total lipid levels and lipid levels associated with isolated lipoproteins, with an emphasis on HDL/LDL cholesterol. In contrast, plasma lipoprotein profiles (PLP) generated via fast liquid protein chromatography (FPLC) are the age- and *APOE*-dependent signature of peripheral lipid transport. FPLC elutes lipoproteins from largest to smallest (chylomicrons/VLDL, LDL, HDL-2, HDL-3, and free proteins) and resulting fractions can be further analyzed for lipid/protein content (e.g., cholesterol, oAβ). Thus, the PLP could be a physiologically relevant biomarker for AD, and the current study determines the effects of age, *APOE* and sex on the lipoprotein profiles of the EFAD transgenic mouse model. The EFAD mice overexpress human Aβ42 and express human *APOE3* (E3FAD) or *APOE4* (E4FAD), allowing for analysis of the synergistic effects of age, *APOE* and sex on the PLP. We hypothesize a leftward dyslipidemic shift (>VLDL/LDL; <HDL) in the PLP with age, *APOE4*, and ♀ sex. Further, oAβ will preferentially distribute to the larger particles. PLP fractions from EFAD mice ages 8- and 18-months (M) were analyzed for lipids and oAβ. With age, lipid transport appeared compromised, diminishing the *APOE* and sex effects on PLPs. Across age and sex, dyslipidemic PLPs were E4FADs > E3FADs, an effect driven by ♀E4FADs. Lipidation of VLDL was ♀E4FADs > ♀E3FADs = ♂EFADs, while HDL was ♀EFADs < ♂EFADs. The distribution of oAβ showed similar age, *APOE,* and sex effects. In conclusion, EFAD dyslipidemic PLPs correspond with AD pathology in EFAD mice and AD risk in humans. Thus, future studies will use human samples to validate the PLP as a surrogate biomarker for cognition in AD.

Disclosures: B. Xiang: None. **K. Panchapakesan:** None. **Y. Saleh:** None. **S. Coronel:** None. **N. Faulk:** None. **J.E. Maldonado Weng:** None. **J.M. York:** None. **A.C. Valencia:** None. **M. LaDu:** None. **A.J. Karstens:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.11/DP06/C67

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R44ES026268 Assay of chemicals for Parkinson's toxicity in human iPSC-derived neurons
NIH Grant R44ES026268-02S1 Assay of environmental toxicants for toxicity related to Alzheimer's Disease utilizing human iPSC-derived- neurons and microglia
NIH Grant R43AG062012-01 The Alzheimer's Therapeutics Screening Assay: a high-throughput drug-discovery platform utilizing neurons and microglia derived from human induced pluripotent stem cells and Kinetic Image Cytometry

Title: Phosphorylation and cytoplasmic translocation of TDP-43 in response to a neurotoxic fragment of apolipoprotein E4 (ApoE4): A mechanism that may underlie loss of cognition in Alzheimer's disease and Alzheimer's disease related dementias (ADRD)

Authors: *P. MCDONOUGH[1], K. L. GORDON[1], R. C. B. BASA[1], S. RAMESH[1], C. HANDLEY[1], T. T. ROHN[2], A. WARD[3], J. WANG[3,4], J. H. PRICE[1,4];
[1]Vala Sci. Inc, SAN DIEGO, CA; [2]Boise State Univ., Boise, ID; [3]Allele Biotech., San Diego, CA; [4]Scintillon Inst., San Diego, CA

Abstract: Elevated phosphorylation and cytoplasmic localization of TDP-43 (transactive response DNA-binding protein of 43kDA), is found in brain sections from patients that develop dementia due to Alzheimer's Disease (AD), Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB), Frontotemporal Dementia (FTD), Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Dementia (FTD), stroke (Vascular contributions to cognitive impairment and dementia [VCID]) and traumatic brain injury (TBI) (collectively referred to as Alzheimer's Disease Related Dementias, ADRD). The presence of the *APOE-ε4* gene variant, which encodes apolipoprotein E4 (ApoE4), increases the tendency for dementia to develop for patients with these afflictions, but the mechanisms responsible for this effect remain to be elucidated. In experiments utilizing glutamatergic neurons representing excitatory cortical neurons derived from human induced pluripotent stem cells (hiPSC-Gluts) we have found that a fragment of ApoE4 (aa1-151) is neurotoxic and induces phosphorylation and translocation of TDP-43 from the nucleus to cytoplasmic puncta (likely corresponding to stress granules) consistent with TDP-43 proteinopathy observed in AD and ADRD. In ongoing research, the assay system will be modified to include cocultured hiPSC-microglia, and hiPSC-astrocytes, cell types that may also be affected by ApoE4 fragments. HiPSC-neurons representing different CNS types (e.g., inhibitory or dopaminergic) will also be tested, as will neurons, microglia, and astrocytes derived from hiPSCs featuring mutations that predispose subjects to AD and ADRD. The assay system features cells cultured in 384-well dishes, in which TDP-43 phosphorylation and translocation are quantified via automated digital microscopy and image analysis, enabling high throughput testing of chemicals, biologics, and genomic constructs to elucidate the mechanisms of ApoE4 and TDP-43 neurotoxicity and to identify agents that may provide therapeutic benefit against dementia.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.12/C68

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIGMS GM105561

Title: Lipid peroxidation product 4-hydroxynonenal alters tertiary conformation and fold of apolipoprotein E in an isoform dependent manner

Authors: *M. ABEER, S. CRUZ, V. TRAN, V. NARAYANASWAMI;
Chem. and Biochem., California State Univ. Long Beach, Long Beach, CA

Abstract: Problem statement: Post mortem tissues from brains of Alzheimer's disease (AD) patients show higher levels of 4-hydroxynonenal (HNE)-modified proteins, with HNE arising as a result of oxidative stress and lipid peroxidation. The overall goal of our study is to understand the effect of HNE modification on the structure and function of apolipoprotein E3 (apoE3) and apoE4, which play a critical role in brain cholesterol homeostasis. Individuals carrying the *APOE* ε4 allele are at a higher risk of developing AD. In the present study, we report the biophysical analysis of HNE modification of apoE3 and apoE4 in terms of protein fold and conformation. **Methods:** Recombinant apoE3 and apoE4 were modified by HNE followed by Western blot with HNE specific antibody to confirm modification. The modified samples were subjected to circular dichroism (CD), fluorescence (intrinsic and 1-anilinonaphthalene-8-sulfonic acid (ANS) fluorescence) spectroscopic and guanidine hydrochloride (GdnHCl)-induced unfolding analyses. Cellular uptake studies of lipid-associated HNE modified apoE isoforms were performed with brain endothelial cells and monitored by confocal microscopy. **Results:** Western blot analysis of apoE3 and apoE4 that were treated with 20-200 μM HNE confirmed modification, with a major band appearing at ~36 kDa. HNE-modified apoE3 and apoE4 were highly helical (60.5 ± 3.1 and 61.0 ± 2.7 %, respectively, n=3) comparable to that of unmodified proteins (56.4 ± 1.5 and 57.9 ± 3.3 %, respectively, n=3) as revealed by far UV CD spectroscopy. A significant decrease in the intrinsic fluorescence emission was noted for both HNE-apoE3 and HNE-apoE4, compared to the corresponding unmodified proteins. GdnHCl-induced denaturation monitored by changes in intrinsic fluorescence revealed a notable difference in terms of increased susceptibility to unfolding for HNE-apoE4, but not HNE-apoE3. Further, ANS fluorescence emission spectra revealed a 10 nm red shift in the wavelength of maximal fluorescence emission for HNE-apoE4 (but not for HNE-apoE3) compared to unmodified protein. Incubation of HNE-apoE3 or -apoE4

with endothelial cells revealed punctate, perinuclear vesicles suggesting cellular uptake via the lipoprotein receptor family of proteins and/or scavenger receptors. **Conclusions:** Taken together, our data indicate that there are isoform-specific differences in protein conformation and tertiary fold as a consequence of modification of apoE by HNE. Further studies are needed to understand the mechanism of cellular clearance of HNE modified apoE at the neurovascular junction and the role of oxidatively modified apoE4 as a risk factor for AD and amyloid pathology.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.13/C69

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS100459
NIH Grant AG039452

Title: The role of pericytes and blood-brain barrier in regulation of soluble tau clearance from brain

Authors: *A. P. SAGARE, A. ARMENDARIZ, E. J. LAWSON, A. NELSON, B. V. ZLOKOVIC;
USC, Los Angeles, CA

Abstract: Recent studies suggest that pericyte loss and cerebrovascular dysfunction contributes to pathology and cognitive decline in Alzheimer’s disease (AD). In addition to Aβ deposition, accumulation of tau may also play a significant role in neurodegeneration. We have shown that brain vascular pericytes internalize and clear Aβ by low density lipoprotein receptor-related protein 1 (LRP1) in an apoE isoform-specific mechanism. However, the role of pericytes and blood-brain barrier (BBB) in the clearance of tau is still not completely elucidated. Here, we studied the ability of primary mouse brain microvascular pericytes to internalize and clear Cy3-labeled human recombinant tau (Cy3-tau), and the role of LRP1 in iodinated labeled human recombinant monomeric tau (^{125}I-tau) clearance across the BBB. Our results show that pericytes from *Apoe*$^{+/+}$ mice cultured on coverslips internalize Cy3-tau and that silencing *Lrp1* or *Apoe* by siRNA inhibits Cy3-tau uptake, whereas silencing of other lipoprotein receptors such as *Lrp2*, *Ldlr*, *Vldlr*, and *Apoer2* or scrambled siRNA (si*Ctr*), did not affect Cy3-tau clearance. Pericytes from *Apoe*$^{-/-}$ mice did not clear Cy3-tau; however, the addition of human lipidated apoE3, but not apoE4, reversed Cy3-tau clearance by *Apoe*$^{-/-}$ pericytes. We used an *in vivo* brain clearance technique to study tau clearance across the BBB in *Apoe*$^{+/+}$ and *Lrp1*$^{lox/lox}$; *Tie2-Cre* transgenic mice. After intracerebral microinjection of ^{125}I-tau (20 nM) and ^{14}C-inulin (an inert reference

molecule), we analyzed the brain at 30 minutes. Our data show that ^{125}I-tau is cleared rapidly compared to ^{14}C-inulin in *Apoe*$^{+/+}$ mice via transport across the BBB, but poorly cleared from *Lrp1*$^{lox/lox}$*; Tie2-Cre* mice with endothelial-specific *Lrp1* deletion. These results indicate that tau is cleared by pericytes and across murine BBB *in vivo* in an LRP1-apoE-isoform-dependent manner. Collectively these results suggest that improving vascular clearance may reduce brain accumulation and spread of tau pathology thereby minimizing neuronal injury in AD.

Disclosures: A.P. Sagare: None. **A. Armendariz:** None. **E.J. Lawson:** None. **A. Nelson:** None. **B.V. Zlokovic:** None.

Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.14/C70

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant

Title: Vascular specific expression of apoE4 enhances CAA pathology and promotes cerebrovascular damages

Authors: *C.-C. LIU, Y. YAMAZAKI, Y. A. MARTENS, A. YAMAZAKI, Y. CHEN, L. JIA, C. M. LINARES, J. KNIGHT, B. KIM, G. BU;
Mayo Clin., Jacksonville, FL

Abstract: Alzheimer’s disease (AD) is the leading cause of dementia in the elderly. Accumulating evidence has shown that risk factors for vascular diseases such as hypertension, diabetes, and hypercholesterolemia are associated with increased risk for AD. The ε4 allele of the apolipoprotein E (*APOE*) gene is the strongest genetic risk factor for late-onset AD and cerebral amyloid angiopathy (CAA) compared to the common ε3 allele. ApoE4 promotes the aggregation and deposition of amyloid-β (Aβ), principal component of both parenchymal amyloid plaques and CAA. Thus, understanding the effects of apoE isoforms on CAA formation represents a great opportunity to both uncover pathogenic mechanisms underlying AD and also explore new strategies for AD therapy.
ApoE is expressed in the brain mostly by astrocytes. Vascular mural cells (VMCs), the major cell types in the cerebrovasculature including smooth muscle cells and pericytes, also express abundant apoE. However, how vascular apoE isoforms modulate parenchymal plaques and CAA is unknown due to the lack of appropriate model systems to distinguish cell autonomous versus non-autonomous effects. Towards this, we have developed novel mouse models expressing human apoE3 or apoE4 in an inducible, cell type-specific manner. After breeding to *SM22α*-Cre, we generated VMC-specific human apoE3 and apoE4 mouse models in the murine *Apoe*-

knockout background. Upon breeding to amyloid model mice, we found that expression of apoE exclusively in VMCs exacerbated CAA pathology with apoE4 exhibited stronger effect compared to apoE3. Differential effects on blood-brain barrier integrity, amyloid deposition in the brain parenchyma, neuroinflammation and cerebrovascular transcriptomic signatures are also observed between vascular apoE4 and apoE3 mice. Together, our findings provide novel mechanistic insights into the specific role of vascular apoE in AD pathology and cerebrovascular integrity, and have implications in designing new therapeutic strategies targeting apoE.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.15/C71

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Preventing cerebrovascular decline in $APOE^{E4}$ mice

Authors: *K. FOLEY[1,2], L. GRAHAM[1], S. YANG[1], G. HOWELL[1,2];
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Abstract: The primary burden of dementia cases worldwide is attributed to Alzheimer's disease (AD) and Vascular dementia (VaD). Recent evidence suggests there is significant pathological overlap between these two diagnoses, implicating some form of cerebrovascular damage as a contributor to more than 90% of dementia cases. Therefore, preserving cerebrovascular integrity throughout aging is an underexplored therapeutic option for dementia. Importantly, mice carrying the humanized *APOE*$^{\varepsilon 4}$ gene, the greatest genetic risk factor for dementia, show cerebrovascular leakage by 2wks of age. We hypothesize that these early cerebrovascular deficits increase risk for dementia in later life. We propose that exercise can overcome cerebrovascular dysfunction, reducing risk for dementia. However, the interactions between genetic risk and environmental factors (such as exercise) are not well understood.
To evaluate exercise as an intervention to improve cerebrovascular health, young (1-2mos) and midlife (12mos) B6 mice were provided a running wheel for 12wks. Midlife runners showed a natural variation in running distance, which allowed us to determine the impact of various amounts of running on brain health during aging. Transcriptional profiling of the hippocampus and cortex revealed significantly enriched vascular remodeling terms such as extracellular matrix organization and angiogenesis in the young running mice but not in the midlife running mice. This suggests a reduction in cerebrovascular plasticity with age, and that interventions should be considered during youth.

We next examined whether the benefits of running at a young age will have similar effects on mice carrying human *APOE* $^{\varepsilon 4}$. Male and female B6.*APOE*$^{\varepsilon 3/\varepsilon 3}$, B6.*APOE*$^{\varepsilon 3/\varepsilon 4}$, and B6.*APOE*$^{\varepsilon 4/\varepsilon 4}$ were provided access to a running wheel at 1-2 months of age for 12 weeks. Lipid profiling revealed a sex by activity interaction for HDL levels, indicating running may alter serum lipids differently between the sexes. Although there was no difference in running distances between genotypes, there was similar variation in distance ran and running intensity between genotypes. This allows for interrogation into running intensity for each sex and genotype and ultimately, its effects on angiogenesis and vascular integrity. Transcriptional profiling by RNA-sequencing of the cortex and hippocampus from mice from all groups, as well as assessment of vascular density and blood brain barrier integrity by immunofluorescence is underway. Ultimately, our work aims to assess running as a prevention/intervention to strengthen cerebrovascular integrity for those with a genetic predisposition for dementia.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.16/C72

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 NS100704
NIH R35 CA197289
NIH R01 CA129769
NIH P30 CA51008
NIH TL1 TR001431

Title: APOE4 predisposes to cancer chemotherapy-induced cognitive impairment in a mouse model

Authors: *G. W. REBECK[1], T. C. DEMBY[2], O. C. RODRIGUEZ[3], Y. LEE[3], C. ALBANESE[3], J. S. MANDELBLATT[4];
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Abstract: The E4 allele of Apolipoprotein E (APOE) is the strongest genetic risk factor for cognitive impairment in late-onset Alzheimer’s disease (AD). Human genetic studies of breast cancer survivors have shown that *APOE4* carriers also have a significantly higher risk for cognitive impairments after cancer chemotherapy. Cognitive impairments to attention, executive function, and learning and memory are prevalent and debilitating side effects of many cancer chemotherapies. To test the association of chemotherapy-induced cognitive impairment with *APOE4*, we used human *APOE* targeted replacement mice, a model lacking the most overt

neuropathological signs of AD. We treated one year old female *APOE3* and *APOE4* mice by intraperitoneal injection with the common cancer chemotherapeutic agent doxorubicin or with saline (n=14-15 per group). We treated mice with 10 mg/kg doxorubicin, in two doses separated by one week, which caused a small (less than 5%), and temporary, reduction in body weight but no overt signs of toxicity. Five weeks following treatment, mice were tested for spatial learning and memory by the Barnes Maze. As expected, under control conditions *APOE4* mice were mildly impaired in their ability to identify and escape through the target hole compared to *APOE3* mice. Doxorubicin treatment had no effect on this behavior in *APOE3* mice. In contrast, doxorubicin caused a strong and significant impairment in *APOE4* mice over several training days ($p<0.01$). Other behaviors, including movement in an Open Field maze, exploration in an Elevated Plus maze, demonstration of Pre-Pulse Inhibition, and Fear Conditioning, were not significantly affected by doxorubicin in either the *APOE3* or *APOE4* mice. Mice were euthanized at 21 months of age, and their brains are being analyzed by voxel based morphometry, immunohistochemistry, and biochemical assays to explore possible long-term morphological and molecular mechanisms for this *APOE*-related impairment by doxorubicin. These findings extend our earlier published work in younger (six month old) *APOE* mice, which also demonstrated an effect of doxorubicin on spatial learning specifically in the *APOE4* mice. Together, these studies support the human studies that *APOE4* positive individuals undergoing cancer chemotherapy are at higher risk of adverse cognitive side effects, knowledge of which may affect their treatment decisions. Furthermore, the work supports the important hypothesis that *APOE4* predisposes to cognitive impairment via mechanisms beyond those specific to AD pathogenesis.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.17/C73

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Oxidative stress responsive kinase signaling and its accompanied insulin resistance precede overt AD pathology

Authors: *W.-B. SHEN, S. CAO, W. LUO, P. YANG;
Univ. Maryland Sch. Med., Baltimore, MD

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia in the elderly. AD manifests the deposit of extracellular amyloid plaques and intra-cellular neurofibrillary tangles containing hyper-phosphorylated tau. However,

the exact mechanism underlying AD pathogenesis remains obscure. Studies in the past few years have revealed metabolic dysregulation and insulin resistance in the AD brain. We hypothesize that oxidative stress responsive kinase-induced insulin resistance is involved in AD pathogenesis. To test this hypothesis, we analyzed kinases and insulin receptor signaling in the cortical tissues of non-AD controls (*APOEε3* carriers) and non-AD experimental group (*APOEε4* carriers) at age of around 50 years. *APOEε4* is associated with significantly increased AD frequency. We found no β-amyloid plaques and neurofibrillary tangles in the brain tissues of both groups. However, thioredoxin 1, an endogenous inhibitor to the oxidative stress responsive kinase ASK1 (Apoptosis signal-regulating kinase 1), was significantly downregulated in the *APOEε4* carriers with concurrently increased levels of phospho-ASK1, and phospho-JNK1/2 (c-Jun N-terminal kinases 1 and 2) and phospho-p38 MAPK (mitogen-activated protein kinase), two kinases downstream of ASK1. Furthermore, we found that in *APOEε4* carriers expression of the insulin receptor (IR) were downregulated, whereas phospho-IRS1 (insulin receptor substrate 1) at Serine 636/639, an inhibitory index of IR signaling, was upregulated and phospho-AKT1, a collective output of the IR signaling, was downregulated. We further verified these findings in an AD mouse model, the APP-PSEN1 Transgenic mouse. β-amyloid deposits were manifested in the cortex at 5.5 months in this AD model. At the age of 3.5 months or earlier, there was no β-amyloid deposit formed but levels of phospho-ASK1, phospho-p38MAPK and phospho-IRS1 at Serine 636/639 were significantly upregulated. However, insulin receptor expression was not affected at this age. Because studies in other systems have demonstrated that activation of the ASK1-JNK1/2/p38MAPK signaling contributes to insulin resistance, our studies implicate that oxidative stress responsive kinase signaling and its accompanied insulin resistance precede overt AD pathology.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIGMS P20GM103423

Title: Understanding Alzheimer’s disease risk: Early behavioral changes and physiological biomarkers associated with the human APOE4 allele in a knock-in rat model

Authors: L. BLACKMER-RAYNOLDS, L. AUGUSTIN, J.-Y. PARK, A. DOAK, *M. J. GLENN;
Psychology, Colby Col., Waterville, ME

Abstract: The human apolipoprotein E e4 (hAPOE4) allele is the greatest known genetic risk factor for Alzheimer's disease (AD) and is associated with numerous aspects of its symptomatology. Despite the importance of the hAPOE4 allele in one's risk of developing AD, the early life changes associated with it are not well understood. There is some evidence, derived from mouse models with a hAPOE4 knock-in, of memory impairments and elevated anxiety behavior, but only as early as 6 months of age; little is known about whether the hAPOE4 allele may affect features of cognitive and emotional functioning earlier in the lifespan. Furthermore, there exists no published research thus far using a hAPOE4 knock-in rat model. Significant insights are possible with rats given their long-standing use as behavioral models over the last century. Thus, the goals of the present study were to 1) carefully study behavior using a hAPOE4 knock-in rat model, and 2) begin these assessments early in life. To address these goals, 20 hAPOE4 knock-in and 20 wildtype Sprague Dawley rats (10 females and 10 males of each genotype) were acquired at 4 weeks of age. After a short period of acclimation to the facility, a series of behavioral tasks were conducted over a 3-month period, followed by an examination of rats' corticosterone reactivity to an acute stressor. Cognitive tests included assays of object, place, and context memory along with object-in-place scene memory. Emotion-based tests included the open field and elevated plus maze to assess rats' hesitation to explore and extent of exploration of novel, anxiety-provoking spaces. Overall, the results revealed subtle, sex-dependent effects of the hAPOE4 knock-in: hAPOE4 females displayed deficits in remembering the location of objects and decreased inhibition in both the open field and the elevated plus maze tests. Consistent with decreases in anxiety-like behavior, hAPOE4 females, compared to wildtype females, also showed significantly faster declines in serum corticosterone after 20 min of acute restraint stress. A similar, smaller, result was found in male rats' corticosterone reactivity, though few differences in their behavior as a function of genotype were evident. Taken together, the results of the present study demonstrate that the hAPOE4 allele has measureable impacts on behavior and physiology, particularly in female rats, during late adolescence and early adulthood. Continued monitoring of these rats across their lifespan is planned and may offer important indices of how early patterns of cognitive and emotional functioning map onto deficiencies that emerge later in life because of the hAPOE4 risk allele for AD.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

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Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant AA023797

Title: Lentiviral vectors for expressing human amyloid precursor protein or apolipoprotein E gene variants associated with increased risk of Alzheimer's disease

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Abstract: Variants of two human genes are associated with the greatest risk of Alzheimer's disease (AD)—the E4 allele of apolipoprotein E (APOE) and the Swedish mutation of amyloid precursor protein (APP). Studies suggest that risk is related to dosage, in that APOE E4/E4 has higher risk than E4/E3 or other APOE genotypes, and overexpression of APP, either Swedish or wild-type, leads to enhanced AD markers. Therefore, we constructed a library of lentiviruses designed to express selected variants of each gene. One group of plasmids is based on the pTet-O backbone so that expression is induced by doxycycline (dox), when co-transduced with rtTA, the required regulatory factor. Another version provides constitutive expression under control of the UBC promoter and is based on FUGW. The plasmids co-express either the expression marker mCherry or a drug-selectable resistance gene, each separated by a "self-cleaving" T2A peptide sequence. The 17-aa C-terminal T2A peptide can also be used as an epitope tag for specific detection of the exogenous protein. The APP plasmids include the wild-type sequence (NM_201414), the Swedish mutation (KM670/671NL), or the protective Icelandic mutation (A673T). The APOE (NM_000014) plasmids express E2, E3, or E4 variants. To demonstrate expression, we transduced cells and detected expression by mCherry fluorescence in live cells, by Western blotting, or using immunocytochemistry. Results indicate that expression from the dox-inducible promoter is responsive to dose of dox and that the UbC promoter expresses protein levels similar to normal expression. Imaging with mCherry is consistent with immunocytochemical detection of co-expressed protein. Transduced glial cells provide a source of extracellular APOE suitable for co-culture with neurons or preparation of conditioned media. Transduction of neurons with APP alters AD pathways. Transduction of APOE-null induced pluripotent stem (iPS) cells with the dox-inducible APOE provides a convenient starting material to study human neurons with controlled expression of individual APOE alleles. These lentiviruses provide effective tools for studying AD mechanisms in cultured cells or *in vivo.*

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.20/C76

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AA020103-S1
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NIH Grant AA025328

Title: The role of apoE isoforms in Alzheimer's disease-related neuropathology in isogenic human neuronal cells

Authors: S. O. ADEOSUN[1], X. CHEN[3], R. ALKAWAS[3], L. SHI[3], E. WICKS[3], B. ZHENG[3], T. MOSLEY[3], Y.-Y. MO[3], ***J. WANG**[2];
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Abstract: Studies indicate that the prevalence of Alzheimer's disease (AD) differs among subjects with different APOE isoforms (E3, E4, or E2), with E4 being higher and E2 lower versus E3. The pathogenic mechanism of APOE isoforms in AD progression is not completely clear. One of the hurdles is that the analyses are often performed in cells derived from resources with different genetic backgrounds. In this work, we sought to generate isogenic apoE neuronal cell lines in a human immortalized cell line with adrenergic and dopaminergic phenotype -the SH-SY5Y cell. gRNAs were constructed in EF1α-hspCas9 all-in one Cas9 SmartNuclease plasmid; the homologous right and left arm (with ApoE2, E3, E4, or ApoE null exon4) were constructed in pHR2 plasmids with a GFP-T2A-Puro-polyA reporter on the 3-prime end of Exon4. We delivered the respective donor constructs and dual gRNA plasmids to replace, or remove the whole exon 4 in the genome of SH-SY5Y cells, respectively, using electroporation. After selection with puromycin, the phenotype of GFP positive cells were confirmed by sequencing the PCR products of the genomic DNA and RT-PCR products of the transcripts. In addition to evaluate the effects of ApoE isoforms on the expression of neuro-pathological markers and cell behaviors. We have successfully generated isogenic APOE4/3/2 and APOE knockout SH-SY5Y neuronal cell lines. These cells are used to compare the specific roles of individual apoE isoforms in the expression of known and new AD neuro-pathologic markers, including coding and non-coding genes, in response to neurotoxin (e.g. alcohol, rotenone, etc.) exposure. We are currently using a similar protocol to generate isogenic AD patient-derived fibroblasts and monocytes in our laboratory.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grants-in-Aid for Scientific Research (C) 17K08272
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Title: Epigenetic regulation of apolipoprotein E by sphingosine-1-phosphate signaling

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Abstract: Background: Sphingosine-1-phosphate (S1P) is a pluripotent lipophilic mediator working as a ligand for G-protein coupled S1P receptors (S1PR). Previously, we reported that sphingosine kinase (SphK2), which phosphorylate sphingosine to produce S1P, is upregulated in Alzheimer disease (AD) brains. And we also found that S1P signaling regulates the production of Amyloid-β peptide (Aβ) which is a pathogenic molecule for AD. However, the effect of S1P signaling on glial function remains unclear. Because S1P is reported as an epigenetic modulator, we examined the effect of this signaling on the one of the AD therapeutic target, nuclear receptors RXR/LXR agonists, which upregulate the Apolipoprotein E (ApoE) and ABCA1 expression. Method: Using specific inhibitor or RNA interference methodology, we analyzed the effect of SphK2/S1P signaling on a human astrocytoma cell line or primary astrocyte. Results: We identified that SphK2/S1P signaling specifically inhibited the induction of ApoE transcription, meanwhile, genetical and chemical inhibition of SphK2 activity enhanced ApoE induction by RXR/LXR agonists. Interestingly, the upregulation of ABCA1 by RXR/LXR agonists is not altered. By screening with RNA interference, we identified the candidate S1P receptor which may epigenetically regulate ApoE expression. Conclusion: Our results indicate that SphK2/S1P signaling is the key epigenetic regulator for ApoE, and the sensitivity of RXR/LXR agonists are changed by this lipid mediator. We will report the significance of this mechanism on AD pathogenesis.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

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Topic: C.02. Alzheimer's Disease and Other Dementias

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American Health Assistance Foundation
Consortium for Frontotemporal Dementia Research

Title: Endosomal pH drop ameliorates ApoE4-mediated synaptic impairments and reduces amyloid plaque load *in vivo*

Authors: *T. POHLKAMP, X. XIAN, M. DURAKOGLUGIL, C. H. WONG, F. PLATTNER, J. HERZ;
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Abstract: The pathological hallmarks of Alzheimer's disease (AD) are extracellular aggregates of the Amyloid β (Aβ) peptide, called plaques, and neurofibrillary tangles of hyperphosphorylated Tau. Processing of the Aβ precursor protein (APP) at the β- and γ-sites leads to Aβ production. Aβ forms neurotoxic oligomers and accumulates in plaques. Apolipoprotein E (ApoE) isoform ε4 is the highest genetic risk factor for AD and is thought to be involved in plaque deposition. ApoE is a cholesterol transporter in the brain and delivers its cargo by receptor-mediated endocytosis. The three main ApoE isoforms seen in humans are ApoE2 (ε2), ApoE3 (ε3), and ApoE4 (ε4). Due to two single nucleotide polymorphisms in the ApoE gene the amount of positively charged residues differs between the isoforms. The least common isoform ApoE2 has one positive charge less than the most common isoform ApoE3. ApoE4 has one positive charge more than ApoE3. Each allele of ApoE4 reduces the age of AD onset by approximately three years, whereas ApoE2 has protective effects, when compared to ApoE3. The difference in the quantity of positively charged residues alters the net charge and the isoelectric point (IEP) of ApoE. Importantly, the IEP of ApoE4 matches the pH 6.4 present in early endosomes. **We hypothesize that the match of the ApoE4 IEP with the early endosomal pH prevents ligand-receptor dissociation resulting in a trafficking defect.** In neurons ApoE, its receptor Apoer2, and glutamate receptors traffic through the same endosomal compartments. The neuronal stimulator Reelin is an alternative ligand of Apoer2 and can stimulate the increase of glutamate receptor expression on the neuronal surface resulting in enhanced long-term potentiation. Importantly, the AD risk factor ApoE4 impairs the surface expression of glutamate receptors upon Reelin stimulation. **Our recent data show that endosomal acidification, achieved by chemical or genetic depletion of the endosomal proton leakage channel Na/H Exchanger 6 (NHE6), prevents this ApoE4-mediated recycling deficit. Conditional NHE6 knockout alleviates ApoE4-mediated deficits in synaptic plasticity *in vivo*.** Moreover **NHE6 knockout in an Aβ producing AD mouse model led to a reduction in plaque load by approximately 80%. Fewer plaques were accompanied by increased activation of microglia and astrocytes in the NHE6 knockout brains.** There was no change in β-site cleavage by NHE6-ablation, which indicates that phagocytosis of Aβ deposits might be the underlying mechanism. Together these data suggest endosomal acidification as a promising drug target to prevent AD.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.23/C79

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Title: Fragment-based discovery of an apolipoprotein E4 (apoE4) stabilizer

Authors: *E. G. MOHLER[1], A. V. KOREPANOVA[1], C. G. JAKOB[1], W. QIU[1], S. C. PANCHAL[1], J. WANG[1], J. D. DIETRICH[1], F. POHLKI[2], A. KLING[2], K. C. WILCOX[1], V. LAKICS[2], L. BAHNASSAWY[2], P. REINHARDT[2], S. KARUNAN PARTHA[1], P. BODELLE[1], M. R. LAKE[1], E. I. CHARYCH[3], V. S. STOLL[1], C. C. SUN[1], A. M. PETROS[1];
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Abstract: Apolipoprotein E is a 299-residue lipid carrier protein produced in both the liver and the brain. The protein has three major isoforms denoted apoE2, apoE3, and apoE4 which differ at positions 112 and 158 and which occur at different frequencies in the human population. Genome-wide association studies (GWAS) indicate that the possession of two E4 alleles is a strong genetic risk factor for late-onset Alzheimer’s disease (LOAD). We have found that apoe4 is less stable to thermal denaturation than either aope2 or apoe3 and our hypothesis is that this decreased stability of apoe4 may lead either to some toxic loss of function, or, to some toxic gain of function for apoE4 compared to apoE2 and apoE3 and thereby be a contributing factor to the greater risk of developing Alzheimer’s disease for apoe4 carriers. In an attempt to identify a small molecule stabilizer of apoE4 that may have utility as a therapy for Alzheimer’s disease, we initially carried out an NMR-based fragment screen on the N-terminal domain of apoE4 and identified a benzyl amidine core that exhibited robust binding to the protein. Binding of the core was also characterized by thermal shift analysis, surface plasmon resonance, and its effect in a liposome breakdown assay. A crystal structure of the fragment bound to the apoE4 N-terminal domain was used to guide synthetic chemistry which yielded a ligand exhibiting single-digit micromolar affinity for apoE4. The resulting compound was also evaluated for mouse pharmacokinetics and in a cell-based functional assay and found to be efficacious in ameliorating readout of the inflammatory cytokines IL-6 and IL-8.

Disclosures: **E.G. Mohler:** A. Employment/Salary (full or part-time):; AbbVie. **A.V. Korepanova:** A. Employment/Salary (full or part-time):; AbbVie. **C.G. Jakob:** A. Employment/Salary (full or part-time):; AbbVie. **W. Qiu:** A. Employment/Salary (full or part-

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.24/C80

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cedars Sinai Institutional Support
Alzheimer's Association (SAGA-17-419408)
National Institutes on Aging (RFI AG058068)

Title: APOE4 and sex mediate microglial transcription profiles in an Alzheimer’s disease mouse model and in patient induced pluripotent stem cells

Authors: *V. A. MOSER[1], M. J. WORKMAN[2], S. SANCES[3], T. E. MORGAN[5], C. E. FINCH[5], M. LADU[7], C. J. PIKE[6], C. N. SVENDSEN[4];
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Abstract: A number of risk factors for late-onset sporadic Alzheimer’s disease (AD) have been identified, with apolipoprotein E4 (APOE4) being the largest genetic risk factor and driving greater disease risk in females. Though there are a number of mechanisms through which APOE4 may be acting to drive increased risk of AD, there is substantial evidence that neuroinflammation and glial function are key pathways in its effects. APOE4 has been shown to alter microglia, which are one of the major immune cells of the brain and are known to have a role in AD. Specifically, microglia are more pro-inflammatory both at baseline and after being

stimulated, when in the presence of APOE4. Recent reports have demonstrated that microglia have unique transcriptional profiles in the context of AD and other neurodegenerative conditions. Whether these signatures are exaggerated in the context of APOE4 or if APOE4 microglia have their own unique transcriptional phenotype is unknown. Thus, we used RNAseq to profile APOE4 microglia, both from a mouse model of AD as well as from patient induced pluripotent stem cell (iPSC)-derived microglia. CD11b+ microglia were isolated from male and female EFAD mice, which carry either human APOE3 or APOE4 and the 5xFAD transgenes. Additionally, iPSCs from healthy, non-demented APOE3 and APOE4 carriers were differentiated into microglia and treated with various inflammatory stimuli. RNAseq performed on both mouse and human iPSC-derived microglia uncovered that APOE4 and sex are associated with altered transcriptional phenotypes in microglia. Determining how APOE4 affects microglial transcription will be critical in understanding how this AD risk factor alters the function of microglia and contributes to disease. We have identified microglial networks that are altered in the context of APOE4 and sex, enabling us to now examine how these pathways may be impacting AD risk and progression.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.25/C81

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: Cure Alzheimer's Fund
Alzheimer’s Association SAGA-17-419408
NIA RF1 AG058068

Title: APOE genotype and sex affect TREM2-dependent microglial interactions with amyloid plaques in EFAD mice

Authors: T. STEPHEN[1], M. CACCIOTTOLO[1], D. BALU[2], T. E. MORGAN[1], M. LADU[2], C. E. FINCH[1], ***C. J. PIKE**[1];
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Abstract: Microglia affect the pathogenesis of Alzheimer’s disease (AD), by protecting against amyloid accumulation in early phases of the disease and promoting neuropathology in advanced stages. Recent research has identified that specific microglial interactions with amyloid plaques exert important protective functions including attenuation of early pathology. It is unknown how these microglial interactions with plaques are affected by apolipoprotein E (*APOE*) genotype and

sex, two well-established AD risk factors that modulate microglial function. We investigated this question using high-resolution confocal microscopy to compare microglial interactions with amyloid plaques in EFAD mice (AD model that is hemizygous for the 5xFAD transgenes and homozygous for human *APOE3* or *APOE4*). In male and female E3FAD (*APOE3)* and E4FAD (*APOE4)* mice at 6 months of age, we observed that microglial coverage of plaques is highest in male E3FAD mice with significant reductions in coverage observed in female E3FAD and E4FAD mice. Plaque compaction, a beneficial consequence of microglial interactions with plaques, showed a similar pattern in which female E3FAD and E4FAD mice were associated with significantly lower values. Within the plaque environment, microglial expression of triggering receptor expressed on myeloid cells 2 (TREM2), a known regulator of microglial plaque coverage, was highest in male E3FAD mice and reduced in female E3FAD and E4FAD. These differences in plaque interactions were unrelated to the number of microglial processes in the plaque environment across groups. Interestingly, the pattern of amyloid burden across groups was opposite to that of microglial plaque coverage, with female E3FAD and E4FAD mice showing the highest amyloid levels. Alongside this, a similar pattern in the levels of dystrophic neurites, within the plaque environment, was observed. Together, these findings suggest a possible mechanism by which microglia may contribute to the increased AD risk associated with *APOE4* genotype and female sex.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.26/C82

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: Cure Alzheimer's fund
National Institutes on Aging RF1 AG058068
Alzheimer’s Association SAGA-17-419408

Title: Interaction among sex, APOE genotypes and TREM2 on primary mouse microglial functions

Authors: *T. IIDA[1], C. J. PIKE[2];
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Abstract: Microglia are significant modulators of Alzheimer’s disease (AD) risk and pathogenesis. An important but understudied issue is how AD-related microglia actions are

affected by individual and interactive effects of AD risk factors. Three important factors are sex, apolipoprotein E (APOE) genotype, and triggering receptor expressed on myeloid cells 2 (TREM2). First, AD is characterized by numerous sex differences. One important sex difference is that the predominant genetic risk factor for late-onset of AD, the ε4 allele of APOE (APOE4), enhances AD risk more in women than men. Sex also affects microglial phenotypes and functions, including those implicated in AD. Interestingly, APOE4 carriers show enhanced microglial activation, which is thought to contribute to pronounced neuroinflammation. Third, a TREM2 mutation that results in partial loss of function strongly increases AD risk. In brain, TREM2 is mainly expressed in microglia and signals with APOE protein to regulate several microglial functions. The extent to which microglial actions are modulated by interactions among sex, APOE genotype, TREM2 expression is not known. To investigate this issue, we utilized primary mixed glial cultures generated from young adult (age 3-3.5 months) male and female mice homozygous for human APOE3 or APOE4. To model TREM2 partial loss of function, we used TREM2 knockdown. Transfection of TREM2 siRNA decreased protein and mRNA levels of TREM2 compared to scrambled siRNA. Inflammatory cytokines secreted from microglia in response to various stimuli contribute to progression of AD. Thus, we examined mRNA expression of inflammatory cytokines (e.g., IL-1β, IL-6 and TNFα) under basal conditions and following stimulation with lipopolysaccharide or oligomeric amyloid-β (Aβ). Furthermore, microglia clear Aβ from brain, decreasing accumulation of neurotoxic Aβ. To assess microglial clearance, we evaluated (1) microglial phagocytosis of fluorescent beads, and (2) phagocytosis and digestion of fluorescence-labeled Aβ. A range of individual and interactive outcomes were observed among TREM2 expression, APOE genotypes and sex. These findings provide new insight into the role of AD risk factors in modulating microglial actions relevant to development of late-onset AD.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 375.27/C83

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG032361

Title: Sex- and APOE genotype-associated differences in cortical thickness in healthy, middle-aged adults

Authors: *K. N. LECLAIRE, J. K. BLUJUS, I. DRISCOLL;
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Abstract: The Apolipoprotein E (APOE) ε4 allele is the best-established genetic risk for late-onset Alzheimer's disease (AD). Moreover, APOE ε4 allele confers even greater risk for AD in females than males. Extant literature has largely focused on characterizing sex-APOE interactions on structural brain integrity in older adults (aged 65+). It is important to better characterize these relationships in middle-aged adults, well prior to the onset of overt clinical symptoms and already substantial structural damage that accompanies this stage, in order to aid in early prevention or intervention efforts. We investigated the relationships between sex, APOE status, and cortical thickness in healthy, non-demented, middle-aged adults (ages 40-60, *M*=49.97, *SD*=6.04, N=128; 77 females). All participants underwent structural MR imaging, were genotyped and classified as ε4 carriers (i.e., ε4/ε4, ε3/ε4, ε2/ε4; n=38) or noncarriers (i.e., ε3/ε3, ε2/ε3, ε2/ε2; n=90). Main effects of both sex and APOE status were significant, even after controlling for multiple comparisons. Females had greater cortical thickness bilaterally in the superior frontal gyrus ($p<0.01$), the left pars triangularis ($p<0.03$) and middle temporal gyrus ($p<0.03$) compared to males. Thinner cortices were observed in the left rostral middle frontal gyrus ($p<0.02$) in ε4 allele carriers compared to noncarriers. Furthermore, there was a significant sex-APOE interaction. Female ε4 noncarriers had thicker cortices in the left caudal anterior cingulate cortex, banks of the superior temporal sulcus, superior frontal gyrus, and superior parietal cortex, as well as the right precentral gyrus ($p's<0.01$), compared to male ε4 noncarriers. Female ε4 allele carriers had thicker cortices bilaterally in the superior frontal gyrus ($p<0.01$) compared to male ε4 allele carriers. Female ε4 carriers also had thinner cortices in the left rostral anterior cingulate cortex compared to female noncarriers ($p<0.03$). No differences in cortical thickness were observed in males regardless of APOE status. Overall, our findings suggest that the associations between APOE risk and cortical thickness may be more pronounced in females and can be observed in middle age, well before the onset of overt symptoms associated with pathological aging.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association (SAGA-17-419408)
National Institutes on Aging (RF1 AG058068)
National Institutes on Aging (P01 AG26572)

Title: Effects of APOE genotype and estrogen status on systemic and neural effects of acute immune challenge in female mice

Authors: *J. LIU, C. SAMPLE, C. J. PIKE;
USC, Los Angeles, CA

Abstract: Alzheimer's disease (AD) is an age-related neurodegenerative disease with multiple risk factors. An accumulating literature provides evidence that the innate immune system is an important regulator of both the initiation and progression of the disease. The predominant genetic risk factor for late-onset AD is the ε4 allele of apolipoprotein E (APOE4), which is implicated in contributing to AD risk in part by increasing susceptibility to inflammation. Consistent with a regulatory role in innate immune responses, acute challenge with the endotoxin lipopolysaccharide (LPS) is reported to yield higher inflammation in both human carriers of APOE4 and mice with knock-in of human APOE4. Another AD risk factor is age-related depletion of the estrogens in women. Estradiol, the primary bioactive estrogen, is also a regulator of inflammation. Low estradiol level is associated with changes in the peripheral immune response that can be partially reversed by hormone therapy. Uncertain are the extent to which APOE genotype may modulate innate immune responses in females and whether this relationship is affected by estradiol levels. To study these questions, we studied the peripheral, neural, and behavioral effects of acute LPS challenge on adult female mice homozygous for human APOE3 or APOE4 in the presence or absence of estradiol. Peripheral LPS administration was associated with hypothermia and sickness behaviors - likely to be important defense responses to severe systemic inflammation - that were more pronounced in APOE4 mice, particularly under low estradiol. LPS induced robust increases in cytokine expression, which was assessed in liver, brain, and plasma. Overall, patterns of cytokine levels were similar across APOE genotype and estrogen status though there was an unexpected general trend for APOE3 genotype to exhibit higher cytokine expression under basal and challenge conditions. Whereas prior evidence suggests APOE4 can increase vulnerability to immune challenges, the current findings indicate that APOE4 also can exert relatively protective outcomes in acute inflammatory response.

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Poster

375. Alzheimer's Disease: APOE and Associated Pathways

Location: Hall A

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Program #/Poster #: 375.29/C85

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG026572
NIA T32 AG052374

Title: Metabolic and cognitive effects of obesity: Regulation by estradiol in female APOE3, APOE3/4 and APOE4 mice at early middle age

Authors: *A. CHRISTENSEN, C. J. PIKE;
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Abstract: The predominant genetic risk factor for late onset Alzheimer's disease (AD) is the apolipoprotein E ε4 allele (*APOE4*). AD risk associated with *APOE4* disproportionately affects women. Further, human and rodent studies indicate that the cognitive deficits associated with *APOE4* are greater in females. Several modifiable lifestyle factors also affect AD risk. One such factor is obesity during middle-age. Given that ~65% of US adults are overweight, it is important to understand how obesity affects AD risk, how it interacts with *APOE4*, and the extent to which its detrimental effects can be mitigated with therapeutics. One intervention often considered for women is estrogen-based hormone therapy, which can exert wide-ranging health benefits when administered in early middle-age but may be harmful in late middle-age. No experimental studies have explored the interactions among *APOE4*, obesity, and hormone therapy in aging females. To look at these issues, we considered how obesity outcomes are affected by treatment with estradiol at the onset of middle-age in female mice with human *APOE3* and *APOE4*. Transgenic mice with targeted replacement of mouse *APOE* with human *APOE3* or *APOE4* (called EFAD non-carriers, EFAD-NC) were generated from breeding of EFAD-Tg mice, which are homozygous for human *APOE* against a hemizygous background of 5xFAD-Tg mice. In addition to studying *APOE3* and *APOE4* mice, we also explored how gene dosage would be affected by obesity and estradiol treatment, so we also generated mice hemizygous for *APOE4* (*APOE3/4)*. Female EFAD-NC mice were examined over a four-month period that spans the transition into reproductive senescence, which models many aspects of human perimenopause. Beginning at 5 months of age, mice were maintained on a control diet (10% fat) or high-fat diet (HFD; 60% fat). After 8 weeks, by which time obesity was present in all HFD groups, mice were implanted with an estradiol or vehicle capsule that was maintained for the final 8 weeks of the experiment. Animals were assessed on a wide range of metabolic and neural measures. Three general patterns emerged: (1) Under normal diet, *APOE4* was associated with poorer metabolic function and cognitive performance; (2) With HFD, *APOE3* showed greater obesity-induced impairments in metabolic function and cognitive performance; (3) Estradiol treatment improved metabolic and cognitive outcomes across all groups, with *APOE4* generally exhibiting the greatest benefit. Together, these findings highlight the importance of *APOE* genotype as a modulator of not only consequences induced by the AD risk factor obesity, but also beneficial outcomes of estradiol, a putative AD preventive intervention.

Disclosures: A. Christensen: None. **C.J. Pike:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.01/C86

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SAF2015-63935R
RTI2018-095793-B-I00
S2017/BMD-3827

Title: Restoration of the autophagic flux by melatonin treatment in the early stages of tauopathy prevents cognitive decline

Authors: *M. G. LOPEZ[1], E. LUENGO[1], C. FERNÁNDEZ-MENDÍVIL[1], P. TRIGO-ALONSO[1], P. NEGREDO[2], B. HERNÁNDEZ-GARCÍA[3], S. LANTIGUA[1], E. DEL SASTRE-LÓPEZ[1], J. A. BERNAL[4], T. IKEZU[5], R. LEON[6], I. BUENDIA[6];
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Abstract: Alterations in autophagy are increasingly being recognized in the pathogenesis of different neurodegenerative diseases such as tauopathies. Therefore, regulation of this process in the early stages could be a promising disease modifying strategy. Melatonin is a neurohormone that has shown a good neuroprotective profile in Alzheimer related models. The aim of this study was to evaluate if melatonin treatment, before cognitive decline, could restore the autophagic flux and prevent dementia. We have used as tauopathy model the injection of AAV-hTau^{P301L}/GFP viral vectors (under the promotor of synapsin) and treatment/injection with okadaic acid in *ex vivo*, human brain slices and *in vivo* models. In the *in vivo* studies, intracerebroventricular injection of AAV-hTau^{P301L} increased oxidative stress, neuroinflammation and tau hyperphosphorylation in the hippocampus 7 days after the injection, without inducing cognitive impairment; however, 28 days later, animals presented cognitive decline in the NOR test. Administration of melatonin (10 mg/Kg) in the drinking water, from day 7 to day 28, reduced oxidative stress, neuroinflammation, tau hyperphosphorylation and caspase-3 activation; these observations correlated with restoration of the autophagy flux and memory improvement. This study highlights the importance of autophagic dysregulation in tauopathy and how melatonin treatment in the early phases of the disease can restore the autophagy flux, and thereby, prevent cognitive decline. Therefore, the development of drugs that improve the autophagy flux such as melatonin or melatonin derivatives could be useful for the treatment of proteinopathies like AD.

Disclosures: **M.G. Lopez:** None. **E. Luengo:** None. **C. Fernández-Mendívil:** None. **P. Trigo-Alonso:** None. **P. Negredo:** None. **B. Hernández-García:** None. **S. Lantigua:** None. **E. Del Sastre-López:** None. **J.A. Bernal:** None. **T. Ikezu:** None. **R. Leon:** None. **I. Buendia:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.02/C87

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (CBB-APQ-02044-15)
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Conselho Nacional de Desenvolvimento Científico e Tecnológico 424588/2016-1
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)
IBRO-SfN Travel Grant awardee

Title: CDPPB prevents neurotoxic effects induced by amyloid-beta 1-42 and improve neuropathological changes in app transgenic mice

Authors: *P. M. Q. BELLOZI[1], M. C. M. DA SILVA[1], G. F. GOMES[1], I. V. A. LIMA[1], C. R. A. BATISTA[1], W. O. C. JUNIOR[1], J. G. DORIA[1], T. WYSS-CORAY[2], F. M. RIBEIRO[1], A. C. P. DE OLIVEIRA[1];
[1]Univ. Federal de Minas Gerais, Belo Horizonte, Brazil; [2]Stanford Univ., Palo Alto, CA

Abstract: Alzheimer’s disease (AD) is the most incident neurodegenerative disorder. Despite the advances in the understanding of its pathophysiology, none of the available therapies prevents its progress. Glutamate-induced excitotoxicity plays an important role AD. However, the role of metabotropic glutamate receptors (mGluR) in neuronal cell death is not completely understood. Recent data indicates that CDPPB, a positive allosteric modulator of mGluR5, has neuroprotective effects. Thus, we investigated the effects of CDPPB in Aβ-induced pathological alterations and in a mouse overexpressing APP. Hippocampal neuronal cultures prepared from newborn C57Bl/6 mice were treated with CDPPB (1 μM), followed by Aβ, to assess the neuronal death. Male C57Bl/6 mice submitted to Aβ 1-42 intra-hippocampal injection were treated for 8 days with CDPPB (1 or 5 mg/Kg, i.p.) or its vehicle and then submitted to object recognition task or contextual conditioned fear test. Subsequently, animals were euthanized and their hippocampal brain slices were stained with FluoroJade C (FJC), NeuN, Iba-1, CD68 and GFAP. APP41 (T41) transgenic mice, 14 months old, received CDPPB (5 mg/Kg, i.p.) or its vehicle for 28 days and were submitted to the same behavioral tasks. After euthanized, their hippocampal slices were stained with NeuN, Iba-1, CD68 and GFAP; the hippocampal Aβ content was measured; and serum levels of hepatic enzymes were assessed. CDPPB prevented *in vitro* Aβ-induced neuronal death. Despite not preventing memory deficits in T41 mice, the drug recoveried Aβ-induced memory impairment. CDPPB ameriorated neuronal viability both after

Aβ injection and in T41 mice and partially reversed gliosis in CA1 region of T41 mice. The mGluR5 modulator, however, did not change Aβ levels and, importantly, seric hepatic enzymes content, in T41 mice. Therefore, CDPPB has a potential neuroprotective effect in AD, especially associated with the prevention of neuronal loss. Thus, there should be considered additional evaluations of the drug, either with earlier interventions in AD models or with longer treatments.

Disclosures: P.M.Q. Bellozi: None. **M.C.M. da Silva:** None. **G.F. Gomes:** None. **I.V.A. Lima:** None. **C.R.A. Batista:** None. **W.O.C. Junior:** None. **J.G. Doria:** None. **T. Wyss-Coray:** None. **F.M. Ribeiro:** None. **A.C.P. de Oliveira:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.03/C88

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Discovery of novel small molecule therapeutics for Alzhiemer's disease by targeting steric zippers involved in tau aggregation

Authors: *M. APOSTOL[1], M. GRAF[1], A. LAY[1], M. SCHULTZE[1], J. SCHERRER[1], G. NAUMANN[1], A. P. WRIGHT[1], I. HERNANDEZ[2], G. LUNA[2], K. S. KOSIK[2], J. J. TREANOR[1];
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Abstract: Frontotemporal dementia and Alzheimer's disease (AD) are progressive neurodegenerative diseases characterized in part by aggregation of the tau protein. At the molecular and cellular levels the aggregation of tau leads to the formation of neurofibrillary tangles (NFTs) that cause a loss of neuronal connectivity, initiates neurodegeneration, and result in the cognitive decline that is the hallmark of the disease. Tau aggregation is dependent on two highly aggregation-prone hexapeptide segments or "steric zippers"--VQIVYK and VQIINK--that are located in the repeat domains in the tau protein. ADRx has used a structure-based approach to design and optimize novel peptidic Tau Aggregation Inhibitors (pTAI) that bind to these segments in their aggregation-competent conformation. Here we show that pTAIs are potent inhibitors of Tau aggregation both *in vitro*biochemical and cellular assays and *in vivo*into transgenic rTg4510 mice using a novel viral delivery approach. Based on the ability of pTAIs to specifically bind to Tau in its aggregated state, we have designed a competition assay to enable high throughput screening to find small molecules that elicit the same effects in the developed suite of assays. We show here that by screening a library of over 200,000 compounds we were able to identify a series of small molecule TAIs that block aggregation of Tau *in vitro*biochemical and cellular assays. Because of the nature of the unique competition assay used

for screening, the discovered molecules promise a level of specificity and potency superior to previously offered aggregation inhibitors. Results from these studies enhance our understanding of aggregation-prone segments of tau as a therapeutic target and of targeted small molecule TAIs as a potential therapy for AD and other tauopathies.

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.04/C89

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: T32-NS007222
AG052934
R01-HL55374

Title: Role of plasminogen activator inhibitor-1 (PAI-1) in cerebrovascular morphometry and cognition in a mouse model of Alzheimer's disease

Authors: ***T. K. STEVENSON**[1], R. C. STEVENSON[2], E. J. SU[3], D. A. LAWRENCE[5], G. G. MURPHY[4];
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Abstract: BACKGROUND: There is accumulating evidence which suggest that cerebrovascular pathologies are associated with cognitive decline and neurodegeneration in Alzheimer’s disease (AD) patients. Indeed, risk factors known to promote vascular dysfunction in cardiovascular disease (CVD) are also risk factors for the development of AD. Of particular interest is the serine protease inhibitor, PAI-1, plasminogen activator inhibitor-1, which is a well-established, independent CVD risk factor that increases with age. Recently, PAI-1, which is known to regulate angiogenesis, was found to be significantly upregulated in the tauopathy AD mouse model Tg4510; importantly, Tg4510 mice were shown to have severe cerebrovascular-patterning abnormalities. OBJECTIVE: It is unclear what role PAI-1 is playing in vascular remodeling and if similar changes in vascular remodeling are present in an amyloidogenic AD mouse model (5xFAD). In addition, it is unclear what effect these changes on the vasculature have on cognition, cerebrovascular physiology, and AD pathology. To address these questions, 5xFAD mice and littermate controls were given a novel PAI-1 inhibitor, and cognition, vascular

morphometry, and amyloid-beta (Aβ) plaque load were assessed. METHODS: The Morris water maze was used to assess cognition in 8-month old 5xFAD (n = 9) and littermate controls (n = 5) treated with the PAI-1 inhibitor. Following completion of the Morris water maze, the cerebrovasculature was double-labeled with a tail-vein injection of DyLight 594 tomato-lectin and a TRITC-Dextran gelatin solution. Brains were sectioned (500µm) and treated with the Pittsburgh compound B (6-OH-BTA-1) to visualize Aβ plaques. SeeDeepBrain technology was then used to optically clear the sectioned tissue and a ~0.1mm^3 volume of tissue was imaged using confocal microscopy. Hippocampal vascular morphometry statistics, including vessel diameter and length and vascular density, were gathered using custom Matlab and Mathematica software programs. RESULTS: Cognitive assessment of learning and memory from the Morris water maze suggests that inhibition of PAI-1 improves cognition in 8 month old 5xFAD mice. Moreover, preliminary analysis of hippocampal vascular morphometry indicates that the 5xFAD mouse model has differences in vessel branch density, and vessel diameter and length. Treatment with the PAI-1 inhibitor appears to ameliorate these changes in the 5xFAD mouse model. CONCLUSIONS: This data will provide important insights into AD pathology; specifically, how changes in the vasculature architecture can affect cerebrovascular physiology and AD progression.

Disclosures: T.K. Stevenson: None. **R.C. Stevenson:** None. **E.J. Su:** A. Employment/Salary (full or part-time):; MDI Therapeutics (Novi, MI). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MDI Therapeutics (Novi, MI). **D.A. Lawrence:** A. Employment/Salary (full or part-time):; MDI Therapeutics (Novi, MI). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MDI Therapeutics (Novi, MI). **G.G. Murphy:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.05/C90

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus A2018169S

Title: Targeting the Alzheimer’s disease-associated risk gene, TREM2, with antisense oligonucleotides

Authors: *K. M. SCHOCH[1], R. N. BANNON[1], L. EZERSKIY[1], P. JAFAR-NEJAD[2], F. RIGO[2], T. M. MILLER[1];
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Abstract: Genetic variants within the *TREM2* gene show strong association with increased Alzheimer's disease (AD) risk. To enhance understanding of TREM2 loss, mice haploinsufficient or null for *TREM2* have been crossed with amyloid beta-depositing mouse models, yielding both positive and negative effects of TREM2 reduction on amyloid beta deposition. While these ongoing studies have identified important relationships between TREM2, microglia, and AD pathology, they are challenging to interpret in the context of varying microglial phenotypes and disease progression. We hypothesized that short-term *TREM2* reduction in the adult mouse may alter amyloid pathology and microglial pathology, thereby helping to guide *TREM2*-targeted therapeutic strategies for patients. We developed antisense oligonucleotides (ASOs) that potently lower *TREM2* mRNA throughout the brain. ASOs were administered to the lateral ventricle via a single bolus injection in male APP/PS1 mice at 10 months of age. One month later, mice were assessed for plaque deposition and microglial responses. *TREM2*-targeted ASO treatment in APP/PS1 mice substantially decreased *TREM2* mRNA compared to control ASO treatment across multiple brain regions. When evaluated for amyloid plaques, brains from ASO-mediated *TREM2* knockdown treated mice exhibited significantly reduced plaque deposition and less microglial association around plaque deposits. Results obtained from cultured microglia suggest phagocytosis activity may be increased with *TREM2* reduction, consistent with the decreased plaque load identified *in vivo*. These results confirm a role for TREM2 in mediating increased plaque deposition and suggest that *TREM2* lowering may reduce pathological markers of neuroinflammation. While these data are seemingly contradictory to the presumption that *TREM2* genetic variants confer a loss of function, an ASO approach allows for greater understanding of TREM2 interventions throughout disease progression, which may depend on the timing and location of TREM2 signalling. Overall, ASOs that target *TREM2* will be highly informative on *TREM2*-mediated AD pathogenesis and may be effective at modulating disease.

Disclosures: K.M. Schoch: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ionis Pharmaceuticals. **R.N. Bannon:** None. **P. Jafar-Nejad:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. **F. Rigo:** A. Employment/Salary (full or part-time):; Ionis Pharmaceuticals. **T.M. Miller:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ionis Pharmaceuticals. **L. Ezerskiy:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ionis Pharmaceuticals.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.06/C91

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant UGTO-PTC-623, Secretariat of Public Education and the Program for the Professional Development for the Superior Type (PRODEP)
Grant CIIC-222-2018, Research and Postgraduate Support Direction (DAIP) of the University of Guanajuato

Title: Design and assessment of antiepileptic drug nanocarriers as a therapeutic strategy for pharmacoresistant seizures control

Authors: *A. ROSILLO-DE LA TORRE, F. ROMERO-ARGOTE, I. A. QUINTERO-ORTEGA, L. E. CASTELLANO-TORRES, J. DELGADO-GARCÍA;
Chemical,Electronic and Biomed. Engin. Dept., Univ. of Guanajuato, León, Mexico

Abstract: Nanocarriers represent a novel strategy for the transport and delivery of drugs in several pathologic conditions, which are characterized by the overexpression of efflux proteins, such as, P-glycoprotein (P-gp). The aim of the present study was to synthesize and physicochemically characterize phenytoin (PHT) loaded silica nanocarriers (SnC) and, evaluate their effect in an animal model of refractory seizures with P-gp overexpression. SnC were synthetized by the Stober method and PHT was loaded by absorption. The PHT *in vitro* release evaluation was performed simulating physiological conditions. P-gp overexpression was induced in male Wistar rats by repeated administration of 3-mercaptopropionic acid (3MP, 37.5 mg/kg i.p.), every 12 hours for 5 days. Microscopy analysis verified the sphere-shaped morphology with a mean diameter of 69.51 ± 7.63 nm and 3.62 mg of PHT were loaded in each 100 mg SnC. The infrared analysis revealed characteristic bands (3276 and 3212 cm^{-1}) attributed to the functional groups of the AE drug. The drug release kinetics from the SnC presented a two-phase behavior: first an initial burst discharge (308.31 ± 19.86 μg/mL) followed by a sustained release (618.44 ± 86.81 μg/mL) throughout 48 h. For in vivo evaluation, Pgp-PHT-SnC group (n=5) were administered with PHT loaded SnC (210 mg/kg, i.p.) one hour before a new (11^{th}) 3MP injection. Other experimental groups also were evaluated: a) Pgp-SnC group (n=5), rats were manipulated as Pgp-PHT-SnC group, but injected with SnC not loaded with the AE drug; b) Pgp-PHT group (n=5), animals were handled as Pgp-PHT-SnC group, but administered with PHT not loaded in SnC; c) 3MP group (n=5) that only received repetitive 3MP administration and d) PHT group (n=5), rats were manipulated as the Pgp-PHT group, except that the animals do not received repetitive 3MP injections. Incidence to tonic-clonic seizures were assessed during 30 min immediately after the last 3MP injection. All the rats (100%) from the 3MP presented tonic-clonic seizures while none of the subjects of the Pgp-PHT group presented seizures. Pgp-SnC and Pgp-PHT groups presented similar incidence for tonic-clonic seizures (80 and 70%, respectively), when compared with 3MP group. In contrast, PHT loaded in SnC protected all the animals of the Pgp-PHT-SnC group (0%, $p<0.05$), to present tonic-clonic seizures. These findings show that the SnC could be a therapeutic tool for the transport of AE drugs in refractory disorders with overexpression of efflux proteins, as P-gp. Nevertheless, more studies should be done for evaluating the positive or negative bearings of SnC in experimental models of refractory epilepsy.

Disclosures: A. Rosillo-De La Torre: None. **F. Romero-Argote:** None. **I.A. Quintero-Ortega:** None. **L.E. Castellano-Torres:** None. **J. Delgado-García:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.07/C92

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Bluefield Foundation

Title: Characterization of an HDAC corepressor complex inhibitor for the treatment of FTD-GRN

Authors: *M. IVARSSON, B. A. LYNCH, A. PIRONE, N. O. FULLER;
Rodin Therapeut., Cambridge, MA

Abstract: The familial form of progranulin-associated progressive frontotemporal dementia (FTD-GRN) is caused by haploinsufficiency at the progranulin gene (*GRN*); FTD-GRN has no significant treatments at this time. Upregulation of progranulin expression from the remaining normal *GRN* allele in patients is a potential therapeutic strategy that is being explored. The HDAC inhibitor (HDACi) Vorinostat, or SAHA, an approved cancer drug, was first reported as upregulating *GRN* expression in *in vitro* cell systems. Vorinostat is a broad spectrum HDACi, which inhibits multiple members of the multi-class HDAC family. Broad spectrum HDACi such as Vorinostat generally have significant toxicity, especially hematological toxicity, and combined with low brain exposure creates an impediment to using HDACis as a therapy for FTD-GRN treatment. We are developing more selective HDACis to treat FTD-GRN. Evidence supports that some, but not all, Class I selective HDACi can upregulate progranulin expression; furthermore, most known Class I selective HDACis still exhibit significant toxicity. We've previously screened a collection of proprietary, complex-selective HDACi, with much-improved safety profiles relative to Vorinostat and other known Class I HDACi, in an *in vitro* neuroblastoma cell line assay (SH-SY5Y), and we have identified compounds, including representative compound R8, that upregulate progranulin *in vitro*. We have also tested R8 effects on gene expression in an *in vitro*, differentiated SH-SY5Y cells, which were treated with the compound under various conditions. Extracted RNA was analyzed by the Nanostring neuropathology probe set, and changes in levels of mRNA were compared across treatments. We report the effects of treatment with R8 on gene expression, noting specific effects on neuronal, and learning and memory-related genes and pathways. Additionally, sub-chronic oral administration of R8 in WT mice resulted in increased expression of progranulin in brain. Interestingly, this result correlates with a significantly increased spine density in the CA1 region of the dorsal hippocampus of WT mice after sub-chronic *in vivo* treatment with R8. Together, our results describe the properties of a new, selective and safe HDACi (R8) which upregulates

progranulin levels as well as synaptic formation, representing a new potential therapeutic intervention in FTD-GRN.

Disclosures: **M. Ivarsson:** A. Employment/Salary (full or part-time):; Rodin Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rodin Therapeutics. **B.A. Lynch:** A. Employment/Salary (full or part-time):; Rodin Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rodin Therapeutics. **A. Pirone:** A. Employment/Salary (full or part-time):; Rodin Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rodin Therapeutics. **N.O. Fuller:** A. Employment/Salary (full or part-time):; Rodin Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rodin Therapeutics.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.08/D1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Department of Biology, Georgia Southern University
Office of Faculty Development, Georgia Southern University

Title: Carbachol-induced phosphorylation of glycogen synthase kinase 3 beta (GSK3beta) and extracellular signal-regulated kinase (ERK 1/2) in the telencephalon of adult zebrafish is mediated by muscarinic acetylcholine receptors

Authors: *R. A. MANS, G. POWERS;
Georgia Southern University-Armstrong Campus, Savannah, GA

Abstract: The zebrafish (*Danio rerio*) represents a widely used model organism for the study of vertebrate development, and it has recently gained in prominence for the study of brain function and disease. While it is known that zebrafish share approximately 70% genetic similarity with humans, the physiology of the zebrafish brain remains largely uncharacterized. In mammals, the proteins glycogen synthase kinase 3 beta (GSK3beta) and extracellular-signal regulated kinase (ERK1/2) are integral for normal and pathogenic brain processes including cell survival, synaptic plasticity, and inflammation, and the activity of mammalian GSK3beta and ERK1/2 are regulated by muscarinic acetylcholine receptors (mAChRs). Recently, we demonstrated that cholinergic stimulation of the adult zebrafish brain using the non-specific cholinergic agonist carbachol (CCh) induces phosphorylation of GSK3beta and ERK1/2 in the zebrafish

telencephalon, a structure with functional homology to the human hippocampus and striatum. The current study was conducted to investigate the AChR subtypes mediating CCh-induced phosphorylation of GSK3beta and ERK1/2. To this end, male and female adult zebrafish brains were isolated and maintained *ex vivo* in oxygenated artificial cerebrospinal fluid (aCSF) before being subjected to pharmacology and Western blot analysis. It was demonstrated that blockade of mAChRs using scopolamine attenuated CCh-induced increases in GSK3beta (N=16) and ERK1/2 (N=9) relative to vehicle-treated controls, indicating mAChRs contributed to the increased phosphorylation observed in CCh treatments. Additionally, selective stimulation of mAChRs with oxotremorine-M increased the phosphorylation of GSK3beta (N=11) and ERK1/2 (N=9) relative to vehicle-treated controls. These results provide evidence for the coupling of GSK3beta and ERK phosphorylation to mAChRs in the adult zebrafish telencephalon, which indicates functional conservation between the cholinergic systems of zebrafish and mammals (no sex differences were detected). Future experiments investigating the roles of GSK3beta and ERK1/2 in AChR dependent processes will further elucidate the degree by which zebrafish may be used to model mammalian brain function and disease.

Disclosures: R.A. Mans: None. **G. Powers:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.09/D2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DGAPA-UNAM-PAPIIT-IA208118
DGAPA-UNAM-PAPIIT-IN216918

Title: Novel synthetic BDNF derived peptides protects dental pulp mesenchymal stem cells (DPSCs) against oxidative stress through the TrkB receptor

Authors: *M. SILVA-LUCERO[1], G. LOPEZ-TOLEDO[2], T. PADILLA[1], V. TORRES-ROJAS[3], L. ZHANG[1], M. CARDENAS-AGUAYO[1];
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Abstract: Dental Pulp Stem Cells (DPSCs) are adult mesenchymal stem cells, with a great capacity for differentiation towards different lineages. The *in vitro* differentiation of DPSCs depends mainly on the presence of growth factors and cytokines. Currently the goal of cell therapy is to promote the regeneration of lost or damaged tissue by means of the implantation of cells, undifferentiated or differentiated into the damage tissue, for this purpose it is necessary to establish the appropriate conditions to culture the DPSCs and differentiate them into the required

cell type. There is great interest in using DPSCs in the treatment of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's, since they share similarities with the stem cells of the neural crest and can be differentiated into neuronal and glial cells *in vitro*, in the presence of growth factors. Neurotrophic factors are of great importance in the development, differentiation, maintenance and regeneration of neurons. Although it has been demonstrated that BDNF favors the survival and differentiation of neural precursors, it does not have appropriate pharmacological properties, due to its high molecular weight, and its low permeability in the blood-brain barrier, thus our gold is to evaluate the effect of small synthetic peptides derived from the active site of BDNF that mimic the functions of BDNF, but are more permeable through the blood-brain barrier and stable in the plasma. Therefore, we evaluated the trophic role of two hexa- (B3E and B5E) derived from the active site of BDNF, on the proliferation, survival and differentiation of normal female (46 years old) DPSCs, in order to find peptides with possible therapeutic applications for neurodegenerative diseases, we challenge the cells culturing them in stress conditions, such as oxidative stress in the presence or absence of the peptides. We evaluate the effect of the peptides on the viability (by WST-1), proliferation and differentiation of DPSCS, through the detection of stem cell and neuronal markers by Western blot and immunofluorescence techniques, as well as by RT-PCR. We conclude that BDNF synthetic peptides activate TrkB receptor and promote survival of DPSCs, therefore these small molecules have therapeutic potential.

Disclosures: **M. Silva-Lucero:** None. **G. Lopez-Toledo:** None. **T. Padilla:** None. **V. Torres-Rojas:** None. **L. Zhang:** None. **M. Cardenas-Aguayo:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.10/D3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CSIR-SRF by Council of Scientific and Industrial Research, New Delhi, India
IBRO-SfN Trave Award (2019) by International Brain Research Organization

Title: Alendronate, an antiresorptive drug, confers neuroprotective effect against Alzheimer's disease like pathological changes in mice induced by high fat diet and aluminium trichloride plus D-galactose

Authors: *S. ZAMEER[1], D. VOHORA[2], J. ALI[2], M. AKHTAR[2];
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Abstract: In the present scenario, millions of people throughout the world are affected by various age-linked neurological disorders such as neurodegenerative diseases. Alzheimer’s

disease (AD) is a most prevalent and complex neurodegenerative disorder which is manifested by progressive cognitive depletion, senile plaques, neurofibrillary tangles, neuronal and synaptic death. The elusive etiopathology and constant clinical trial failure directed the researchers to explore therapeutic target for AD. Neurodegenerative disorders and osteoporosis were observed to have some similar risk factors such as increasing age, altered isoprenoids and their downstream components. Supporting this notion, bisphosphonates (BPs), recommended for treatment of bone disorders were demonstrated to be beneficial in attenuation of dementia in osteoporotic patients.
Alendronate, a nitrogen-containing BPs, acts by blocking farnesyl pyrophosphate synthase (FPPS) enzyme in Mevalonate pathway and modulate isoprenoids and also cited to inhibit acetylcholinesterase enzyme and cholesterol in brain. Thus the present hypothesis was designed to explore the effect of alendronate against high fat diet (HFD) induced pathologies resembling AD in mice. It was observed that oral administration of alendronate for 15 days in mice exposed to HFD for 14 weeks and AlCl3+D-galactose for 6 weeks showed improvement in neurobehavior, reduction in hippocampal neuroinflammatory cytokine (IL-1β, TNF-α), cholinesterases, Aβ, APP processing, and oxidative stress markers and isoprenoids serum cholesterol (in HFD model). These neuroprotective findings of alendronate were supported by histopathological analysis done by Congo red staining. Our current outcomes suggest the prominent role of alendronate against AD like pathologies by targeting Mevalonate pathway, supporting this NBPs a potential target for AD treatment.
Key words: Alzheimer’s disease, alendronate, isoprenoids, amyloid beta, APP processing, GSK-3β

Disclosures: **S. Zameer:** None. **D. Vohora:** None. **J. Ali:** None. **M. Akhtar:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

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Program #/Poster #: 376.11/D4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS Grant No. 16H03288

Title: An automated microliter-scale high-throughput screening system (MSHTS) using quantum-dot nanoprobes for amyloid aggregation inhibitors

Authors: *R. SASAKI[1], R. TAINAKA[1], Y. ANDO[1], M. KURAGANO[1], K. OTA[2], K. MONDE[3], K. UWAI[1], K. TOKURAKU[1];
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Abstract: Alzheimer's disease (AD) is the most popular disease in dementia. Recent studies have shown that aggregation and accumulation of amyloid β (Aβ) and tau are involved in the pathogenesis of AD. Therefore, substances inhibiting the aggregation and accumulation of Aβ and tau have great potential as a lead compounds of prophylactic and therapeutic drugs for AD. In this study, we tried to develop an automated real-time microliter-scale high throughput screening (MSHTS) system for Aβ and tau aggregation inhibitors using quantum-dot (QD) nanoprobes. We also analyzed 504 crude extracts from plants and 134 low molecular weight aromatic compounds using this automated system. To automate the MSHTS system, first, we tried to optimize the sample dilution and the mixing steps by JANUS G3 automated workstation (Perkin Elmer). The results demonstrated that the mixing process of $A\beta_{42}$and inhibitor significantly affected the evaluation of inhibitory activity. Therefore, we examined optimal pipetting conditions (number of times, speed, volume) in the mixing process of Aβ and inhibitor. Next, we examined the type of QDs to visualized aggregates. In previous reports, we used QD655 which emits fluorescence at 655 nm. Since chlorophyll-derived absorption of plant extracts at 650-700 nm may affect to the fluorescence intensity of QD655, we used QD605, which does not overlap with the absorption of chlorophylls. In addition, for rapid analysis, we also examined automatic imaging conditions using a fluorescence microscope system (ECLIPSE Ti-E, Nikon).With these trials, we were able to capture all 1536 wells within 1 hour, and it became possible to correctly analyze the amount of aggregates without depending on the brightness and darkness of the samples. Screening 504 plant crude extracts revealed the relationship of Aβ aggregation inhibitory activities of plant extracts using a plant-based classification. Within the eudicots, rosids, Geraniales and Myrtales showed higher activity. Screening of low molecular weight aromatic compounds demonstrated that the structure of tropolone endows it with potential Aβ aggregation inhibitory activity. The activity of the most active tropolone derivative was higher than that of rosmarinic acid that we previously identified from summer savory. MSHTS also identified three chaperone molecules as tau aggregation inhibitors. These results demonstrate that our automated MSHTS system is a novel and robust tool that can be adapted to a wide range of compounds and aggregation-prone polypeptides.

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.12/D5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Combined beneficial actions of gallic acid remediate Alzheimer pathology and restore cognition in mice

Authors: *T. MORI[1], N. KOYAMA[1], T. YOKOO[2], T. SEGAWA[3], M. MAEDA[3], D. SAWMILLER[4], J. TAN[4], T. TOWN[5];
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Abstract: Recent focus has been given to natural dietary compounds (so-called ‘nutraceuticals’) as Alzheimer’s disease (AD) modifying therapies. Plant-derived phenolics make ideal nutraceuticals, because they are well-tolerated and have drug-like properties including anti-inflammatory and anti-oxidant activities. Here, we investigated the therapeutic potential of gallic acid (GA) using the pre-clinical mutant human amyloid β-protein precursor and presenilin 1 (APP/PS1) transgenic mouse model of AD. Beginning at 12 months of age, we orally administered GA (20 mg/kg) or vehicle once daily for 6 months to APP/PS1 mice that have accelerated cerebral amyloidosis. At 18 months of age, GA treatment reversed transgene-related learning and memory impairment by most outcome measures but did not alter behavior in nontransgenic littermates. GA-treated APP/PS1 mice had mitigated cerebral amyloidosis, including brain parenchymal and cerebral vascular β-amyloid deposits as well as decreased cerebral abundance of amyloid β-protein species *versus* vehicle-treated APP/PS1 mice. These beneficial effects co-occurred with a shift toward nonamyloidogenic APP processing. In support, α-secretase candidate and nonamyloidogenic soluble APP-α protein expression were increased, whereas β-secretase protein expression together with amyloidogenic soluble APP-β and amyloidogenic β-C-terminal APP fragments were decreased. Additional significant benefits included alleviation of neuroinflammation and oxidative stress. Collectively, these results demonstrate that six months of GA treatment exerts disease-modifying effects by tandemly impacting α- and β-cleavage of APP in parallel with stabilizing neuroinflammation and oxidative stress. We propose dietary supplementation with GA as a promising prophylaxis for AD.

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.13/D6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC 81671259

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NSFC 81801269

Title: Effects of fluoxetine on the neurons and synapses in the hippocampus of early APP/PS1 transgenic AD mice

Authors: *Y. TANG, C. N. ZHOU, Y. ZHANG, L. JIANG, F. L. CHAO, L. ZHANG, J. MA; Dept. of Histology and Embryology, Chongqing Med. Univ., Chongqing, China

Abstract: It has recently reported that fluoxetine (FLX) shows positive effects on the AD patients who have depression and anxiety. It is unclear, however, whether FLX can affect the pathogenesis of early AD. To address this issue, we designed the present study. The present study is the first study to investigate the effects of FLX on the neurons and synapses in the hippocampus of early APP/PS1 transgenic AD mice using the unbiased stereological techniques and other techniques. 8-month-old male APP/PS1 mice were randomly divided into an APP/PS1+NS group and an APP/PS1 + FLX group. 8-month-old male wild-type (WT) littermates were randomly divided into a WT+NS group and a WT + FLX group. The mice in the WT + FLX group and APP/PS1 + FLX group were intraperitoneally injected daily with FLX (10 mg/kg i.p. dissolved in 0.9% NS) regime for 10 weeks. The mice in the WT+NS group and APP/PS1+NS group were intraperitoneally injected daily with NS (equivalent 0.9% NS i.p.) for 10 weeks. At last two weeks injection, the spatial learning and memory ability of the mice was detected with Moriss water maze. After 10 weeks, 6 mice were randomly selected from each group. The total numbers of the neurons, immature neurons and synapses were estimated with the unbiased stereological methods. The newborn neurons and $5HT4R^{+}/NeuN^{+}$ cells in the hippocampal subregions were counted with immunofluorescence technique. Immunofluorescence was used to detect the changes of amyloid plaques, 5HT1A receptor and the density of PSD95 in the hippocampus of each group mice. The changes of phosphorylated Tau protein, the levels of GSK3β and p-ser9-GSK3β and the levels of SYP and BDNF in the hippocampus of each group mice were detected with ELISA technique. The present results indicated that FLX treatment could protect the neurons and synapses in the hippocampus of early AD through 5-HT system, which might be the important structural bases for the FLX-induced improvement of the spatial learning and memory ability of early AD. Moreover, our results suggested that the FLX may be a safe and effective drug for delaying the progress of AD, which might provide a starting point for further research into the new preventative measures and treatments of AD.

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.14/D7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Abbvie

Title: ACAT inhibition in the cns as a therapeutic target for APOE4-induced Alzheimer's disease risk

Authors: *A. C. VALENCIA, N. FAULK, D. BALU, E. LOUKENAS, J. M. YORK, M. LADU;
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Abstract: *APOE4*, the gene encoding apolipoprotein E4 (apoE4), is the greatest genetic risk factor for Alzheimer's disease (AD), compared to common *APOE3*, and *APOE2*, which is protective but rare. While the mechanism underlying *APOE*-modulated AD risk remains unclear, *APOE4* is associated with accelerated amyloid-beta (Aβ) accumulation, both as amyloid plaques and soluble oligomeric forms of Aβ (oAβ), the latter considered a proximal neurotoxin. In addition, apoE4 levels in the brains of humans and transgenic mice (Tg) expressing human *APOE* are lower compared to apoE3, suggesting that the poorly lipidated apoE4 particles are unstable. Thus, one possible therapeutic target for *APOE4* carriers is increasing the intracellular free cholesterol pool in neurons and glial cells to allow greater lipidation of apoE4 particles, with a parallel reduction in Aβ. This was tested using an Acyl-CoA: cholesterol-acyltransferase (ACAT) inhibitor avasimibe (AVAS). ACAT esterifies intracellular free cholesterol to produce cholesteryl ester droplets and reduce the free cholesterol pool. Therefore, our therapeutic target for *APOE4* carriers is increasing intracellular cholesterol via inhibition of ACAT, thus increasing extracellular transport of cholesterol to lipidate the apoE4 particles in the parenchyma. In this study, we treated male E4FAD-Tg mice, which specifically over-express human Aβ42and express human *APOE4,* with AVAS in a prevention paradigm (6-8M). AVAS treatment prevented memory loss and a reduction in synaptic proteins, reduced soluble and insoluble Aβ levels, Aβ deposition, amyloid deposition, and neuroinflammation. However, there was no evidence of indirect target engagement as measured by an increase in apoE4 lipidation. Thus, in the absence of indirect target engagement, AVAS demonstrates efficacy and produced significant changes in mechanistic pharmacodynamics readouts for both neuroinflammation and Aβ solubility/deposition, two critical components of AD-related pathology. Further investigation is ongoing to understand the mechanisms underlying ACAT inhibition and apoE4 lipidation as a therapeutic target for AD.

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.15/D8

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Neuroprotective role of Myo/Nog cells in a rat model of Alzheimer's disease

Authors: *A. PAYNE[1], S. JOSEPH-PAULINE[1], M. BRACCIA[1], G. GORSKI[1], R. BRAHMBHATT[1], S. YOUNG[1], J. GERHART[1], M. GEORGE-WEINSTEIN[1], J. STONE[2], S. PURUSHOTHUMAN[2], A. BRAVO-NUEVO[1];
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Abstract: Introduction: Alzheimer’s disease (AD) is a degenerative disorder of the central nervous system in which plaques of misfolded beta amyloid proteins accumulate in brain tissue over time, impeding cognitive function and causing memory loss.
Previous studies by our colleagues suggest the beta amyloid accumulation is due to spontaneous, clinically silent micro-hemorrhages (“tiny bleeds”) in the brain tissue that lead to plaque formation.
Recently, Myo/Nog cells, identified by their expression of the MyoD transcription factor and an inhibitor of bone morphogenetic proteins called noggin, have been discovered to have neuroprotective effects in nervous tissue in the retina. They migrate to sites of injury to mitigate subsequent damage.
The established needle-stick injury model was used to introduce micro-hemorrhages and plaque formation in the rat brain, and to explore the amount of Myo/Nog cells in the affected areas and their effects on neuronal cell death.
Methods: Sprague Dawley rats were injected with either Myo/Nog cells purified from the mouse brain by labeling them with the G8 monoclonal antibody and isolating labeled cells by magnetic cell sorting. Myo/Nog cells were depleted in the area of the needlestick by injecting the G8 mAb and complement. Additional experimental groups included rats that only received a needlestick, animals injected with complement alone following needlestick, and rats that were not subjected to needlestick injury. These treatment groups served to modify the number of Myo/Nog cells at the site of injury.

Tissue sections of the brain from all groups were then stained using double labeled with the G8 mAb and antibodies to NeuN to localize neurons, glial fibrillary acidic protein (GFAP) that is expressed in glial cells , and oligomeric amyloid-beta. Cell death was measured by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) technique.
Results: Our preliminary data show a negative correlation between Myo/Nog cells and stress induced by the needlestick in the brain. While depletion of endogenous Myo/Nog cells in the area of injury did not affect neuronal cell death, addition of brain-derived Myo/Nog cells did reduce cell death.
Conclusion: Myo/Nog cells appear in areas of injury. Addition of Myo/Nog cells is neuroprotective following brain injury. Further analysis is needed to confirm our results.

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.16/D9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1 AG056976
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NIH R21 AG056025
Kwanjeong Educational Foundation Overseas Scholarship

Title: Role of protein prenylation in the pathogenesis of Alzheimer’s disease

Authors: *A. JEONG[1], S. CHENG[1], W. QU[2], M. DISTEFANO[3], D. A. BENNETT[4,5], M. BERGÖ[6], L. LI[1];
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Abstract: Alzheimer’s disease (AD) is the leading cause of age-related dementia, but its pathogenesis is not fully understood. Protein prenylation, a posttranslational lipid modification of proteins, is dysregulated in aging and might contribute to AD pathogenesis. Many proteins, including the Ras superfamily of small GTPases, undergo prenylation by farnesyltransferase (FTase) and geranylgeranyltransferases (GGTase-I, GGTase-II). Small GTPases serve as molecular switches in signal transduction pathways that regulate diverse cellular processes and functions. We showed previously that systemic heterozygous deletion of FTase reduces amyloid

pathology and neuroinflammation, and rescues learning and memory in the APP/PS1 mouse model of AD. To further investigate the role of protein prenylation in AD pathogenesis, this study was undertaken to compare farnesylated or geranylgeranylated protein levels in human postmortem brain samples from individuals with a range of clinical diagnosis from no cognitive impairment (NCI), mild cognitive impairment (MCI), to AD. We found that the levels of farnesylated H-Ras and its downstream effector protein phosphorylated ERK were higher in MCI and AD brains compared to control. Moreover, farnesylated H-Ras levels correlated positively with tau tangle pathology, whereas levels of geranylgeranylated Rho GTPases did not correlated with either amyloid or tau pathology. To define the impact of inhibiting FTase in forebrain neurons on AD pathology, we generated neuronal-specific FTase knockout mice and bred them with APP/PS1 mice. Preliminary results revealed that FTase deletion ameliorates the neuropathology and memory deficits in APP/PS1 mice. Thus, these preliminary studies suggest that protein prenylation is dysregulated in AD pathogenesis and that protein farnesylation could be a potential therapeutic target for AD. Further analyses are underway to unravel the molecular mechanisms underlying the effects of FTase inhibition in forebrain neurons on multiple aspects of AD pathology.

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.17/D10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HEC Grant NRPU-4480

Title: Naringenin produces neuroameliorative effects and counteracts genotoxicity induced by $AlCl_3$ and D-gal in rat model of AD

Authors: ***S. HAIDER**[1], L. LIAQUAT[1], Z. BATOOL[2];
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Abstract: Currently available medication for the treatment of Alzheimer’s disease (AD) such as acetylcholinesterase inhibitors can only offer symptomatic relief. Such medications only work on intact neurons, but cannot stop the ongoing neurodegeneration. So, there is an intense need for the development of therapeutic strategy that can not only improve brain functions but can also prevent neurodegeneration. Since oxidative stress is one of the main causative factors of AD, various antioxidants are under investigations to prevent neurodegenerative mechanism of AD.

The present study was intended to investigate the neuroprotective effects of naringenin (NAR) against $AlCl_3$+D-gal induced AD-like animal model. For this purpose rats were orally pre-treated with NAR (50 mg/kg) for two weeks. Rats were then exposed to $AlCl_3$+D-gal (150 mg/kg+300 mg/kg) intraperitoneally for one week to develop AD-like animal model. On the basis of the cholinergic hypothesis of AD, scopolamine (SCO) and donepezil (DPZ) were used as standard drugs in present investigation. Results showed that NAR pre-treatment significantly protected behavioral disturbances in rats. Observed effects might be due to improved neurotransmission, prevention against histopathological alteration, DNA fragmentation and antioxidant defense system. In DPZ group, rats showed improved cognitive and cholinergic functions but neuropsychiatric functions were highly impaired and marked histopathological alterations were observed. NAR not only prevented $AlCl_3$+D-gal induced AD-like symptoms but also significantly prevented SCO induced cholinergic dysfunctions in rats. Results of present study strongly suggest neuroprotective and cognition enhancing functions of NAR. NAR may be considered as a neuroprotective compound in the future for therapeutic management of AD.

Disclosures: S. Haider: None. **L. Liaquat:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.18/D11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of soluble epoxide hydrolase in Alzheimer’s disease progression

Authors: *H.-Y. HU[1], T.-S. LEE[2], H.-T. LEE[1,3,4];
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Abstract: Amyloid-beta (Aβ) plaque is known for pathological evidence in Alzheimer’s disease (AD) which is a progressive neurodegenerative disorder in the brain over the age of 65. AD causes cognitive impair and behavioral dysfunction over time that affect daily activities. Neuroinflammation is characterized by reactive astrocytes and activated microglia in brain and also involved in AD. One of the role, astrocytes, are the most abundant cell type in the central nervous system which are essential for homeostasis and support to neuron. Here, we elucidate the potential mechanism in Aβ-induced inflammatory response in astrocytes. Aβ uptook by astrocytes which occurs after inflammatory response may not be entirely beneficial in AD. It would cause the accumulation and deposition of Aβ plaque leading to accelerate AD progression. Therefore, our strategy is to find a potential protein which is responsible for regulating

inflammatory response. Soluble Epoxide Hydrolase (sEH) is well-known for metabolism of epoxyeicosatrienoic acids (EETs). It exhibits two functional domains activities - C-terminus with epoxides hydrolase activity and N-terminus with phosphatase activity. Once sEH eliminates the effect of EETs, it results in inflammatory response. Thus, limited reports indicated that sEH inhibitor could be as the approach to against the response. In this study, we hypothesize that sEH may induce reactive astrocytes to increase Aβ uptake ability and neuroinflammation leading to exacerbate neuronal impairments in AD. We used primary mouse astrocytes and 4 months of AD mouse model (5xFAD) separately treated with different terminal-sEH inhibitors (AFC compound for inactive N-terminus and ADUA compound for inactive C-terminus). We examined phagocytosis ability, inflammatory level, brain tissue staining and animal behaviors test to verify our hypothesis. Our evidence showed that Aβ uptake and inflammatory level in astrocytes were decreased with inactivation of sEH treatment. Besides, immunohistochemistry data indicated Aβ/ GFAP were decreased in cortex and hippocampus. Furthermore, we found the learn/ memory, position balance, grip strength and motor coordination were also improved. It indicates that sEH has a potential role for neuroinflammation and Aβ aggregation which developing AD progression. In conclusion, sEH inhibition may be as a therapeutic potential approach for providing neuroprotective effect to against AD.

Disclosures: **H. Hu:** None. **T. Lee:** None. **H. Lee:** None.

Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.19/D12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Enhancing blood circulation to facilitate the efflux of amyloid β from the brain to peripheral blood circulation by xuefu zhuyu decoction

Authors: *L. CHAO[1], C.-W. YEH[1], Y. SKYE HSIN-HSIEN[2], H.-K. LIU[3], F.-S. SHIE[4], H.-J. TSAY[1], Y.-J. SHIAO[3];
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Abstract: The burden of amyloidosis and senile plaques in the brain of Alzheimer’s Diseases (AD) patients are determined by the production and the clearance of cerebral Aβ. The predominant mechanism is vascular-mediated efflux of Aβ from the brain into the circulation, and this mechanism is actively mediated by low-density lipoprotein receptor-related protein 1 at the brain blood barrier.

Xuefu Zhuyu Decoction is a traditional Chinese herbal formula and it is well-knownfor improving blood circulation and anti-inflammation. Also, XZD is used for the treatment of metabolic syndrome through removing blood stasis.Recently, we have reported that XZD ameliorating obesity, hepatic steatosis, neuroinflammation, amyloid deposition and cognition impairments in metabolically stressed APPswe/PS1dE9 mice. We have identified that the increase of serum Aβ42 induced by high fat diet (HFD) streptozotocin (STZ) is attenuated by XZD. However, the mechanism mediating the reduce of cerebral Aβ42 remains unclear. The therapeutic potential of XZD is based on multiple beneficial aspects including the enhancement of blood circulation and anti-inflammation.
The glycemic homeostasis and the plasma Aβ level of HFDSTZ AD mice with and without Chinese medicines be monitored weekly. The glucose usage acquired by positron emission tomography/computer tomography (PET/CT) was performed to evaluate the neuronal activity of HFD with and without Chinese medicines. Whole brain static PET images were acquired, reconstructed, and then corrected for attenuation based on CT imaging data. Regional radioactivity concentration (kBq/mL) of [^{18}F]flu-deoxyglucose ([^{18}F]FDG) was estimated from the maximum pixel. Furthermore, the effect of blood circulation improved by XZD in the brain of HFDSTZ AD is going to be done by using small animal PET/MRI. The efflux of Aβ of HFDSTZ AD mice brain by using XZD will be more complete.
For the measurement of Aβ, mice were sacrificed and the brain was removed. After processing the brain, we acquired SDS-soluble and SDS-insoluble Aβ samples. Both Aβ samples underwent enzyme-linked immunosorbent assay (ELISA) using Aβ40 and Aβ42 ELISA kits (Life Technologies, Carlsbad, CA, USA). The absorbance was measured at 450 nm using a TECAN plate reader (Sunrise, UK).

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 376.20/D13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS096275
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JPB Foundation GC223621

Title: Mechanism of gamma-secretase modulators

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Abstract: Amyloid-beta (Aβ) plaques are believed to be integral to Alzheimer's disease (AD) pathogenesis through their role in the "amyloid cascade hypothesis," in which the accumulation of Aβ peptides initiates a cascade of pathological events leading to neurodegeneration and AD. γ-Secretase is a transmembrane aspartyl protease which cleaves amyloid precursor protein in the final step of proteolysis to generate Aβ peptides, making γ-secretase an attractive drug target. However, γ-secretase inhibitors failed in clinical trials due to their unwanted side effects on other γ-secretase substrates such as Notch. ɣ-Secretase modulators (GSMs) selectively reduce levels of the pathogenic Aβ species without affecting Notch and overall APP processing. However, the precise mechanism of action of GSMs is still unclear. Using photoaffinity labeling in cross-competition studies, active site-directed inhibitor L-686, 458 (L458) enhances the labeling of GSM photoprobe E2012-BPyne to presenilin-1 (PS1), the catalytic subunit of γ-secretase, suggesting cooperative action between the active and allosteric GSM binding sites on γ-secretase. We aim to determine their exact peptide interactions and elucidate the mechanism of action for GSMs. We have demonstrated that L505, a probe developed to profile the active site, increases E2012-BPyne labeling of PS1-NTF. Furthermore, we have shown that an active site-directed probe containing a cleavable Dde linker also enhances PS1-NTF labeling of E2012-BPyne and are in the process of optimizing elution conditions. We have also characterized the labeling of other GSM photoprobes which can be enhanced by L458. Together these results will allow us to pull down the photolabeled targets and analyze them with LC-MS/MS to map the binding sites on γ-secretase. Identification of these sites will define the molecular mechanism of GSMs, leading to a better understanding of their modulation for AD drug development.

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

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Program #/Poster #: 376.21/D14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Longevity Science Committee of the Ministry of Health and Welfare of Japan
Scientific Research (C) (18K07385 MS) from the Ministry of Education, Science, and Culture of Japan
Study of prevention for neurodegenerative diseases by new antiaging methods in Hirosaki University Institutional Research Grant;

Development of Fundamental Technologies for the Production of High-value Materials Using Transgenic Plants by the Ministry of Economy, Trade, and Industry of Japan

Title: Oral immunization with soybean storage protein ontaining Aß4-10

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Abstract: Amyloid ß peptide (Aß) plays a central role in the pathogenesis of Alzheimer's disease (AD). Immunotherapies for Aß amyloidosis have therefore been developed as putative disease-modifying therapies (DMTs) of AD. Because AD pathologies begin two decades before the onset of dementia, prevention of Aß amyloidosis has been proposed as a means to block the pathological cascade leading to cognitive devastation. For this reason, safe and non-invasive interventions are necessary. Here, we generate a transgenic plant-based vaccine, a soybean storage protein containing Aß4-10, named Aß+, for oral Aß immunization. One mg of Aß+ or control protein (Aß-) was administered to TgCRND8 mice once a week from 9 weeks up to 58 weeks. Aß+ immunization raised both anti-Aß antibodies and cellular immune responses. Morris water maze test revealed significant prevention of spatial memory deterioration in escape latency and path-length in Aß+ immunized animals compared with littermates in the Aß- treatment group from 21 to 57 weeks. Quantitative ELISA for Aß40 and Aß42 in Tris phosphate buffered saline (TBS), sodium dodecyl sulfate (SDS), and formic acid (FA) serial extractions from brains showed that large amounts of Aß40 and Aß42 were recovered in SDS and FA fractions from both Aß+ and Aß- groups, with no significant differences between the groups, except for an increase of Aß40 in the FA fraction of the Aß+ group. In contrast, the levels of soluble Aß oligomers (AßOs) in the TBS soluble fraction were significantly decreased in the Aß+ treated group. Western blot analyses showed all sets of Aß species to have different solubility in 3 step extractions from Aß monomer, low to high molecular weight (LMW and HMW) AßOs, and Aß smears in TgCRND8 brains. These were increased with aging, and C-terminal ends of HMW AßOs were blocked. Decreased Aß oligomers in TBS fractions, a corresponding increase in HMW AßOs in SDS fractions, and Aß smears in FA fractions were observed in the Aß+ treated group. Processing of amyloid precursor protein, and tau levels were not different between Aß+ and Aß- groups. There was significant inhibition of histological Aß burden, especially in diffuse plaques and suppression of microglial inflammation. No evidence of amyloid-related inflammatory angiopathy was observed. These findings suggest that Aß+ oral immunization reduces toxic soluble AßOs and sequestrates them to form aggregated Aß amyloidosis. Thus, Aß+ oral immunization could be a promising, cheap, and safe DMT to prevent AD pathological process leading to cognitive impairment.

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Poster

376. Alzheimer's Disease and Therapeutic Strategies II

Location: Hall A

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Program #/Poster #: 376.22/D15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant (AG025493, NS074256)
Alzheimer's Association (NPSPAD-10-174543)

Title: BACE1 inhibitions affect on astrocytic amyloid beta clearance

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Abstract: Abnormal accumulation of amyloid beta peptide (Aβ) in the brain is regarded as the possible causative agent of Alzheimer's disease (AD). Increased production of Aβ or impaired clearance of Aβ results in the accumulation and aggregation of Aβ into insoluble, toxic plaques, which are surrounded by glia cells. BACE1 is the rate-limiting secretase required for production of Aβ through its initial cleavage of amyloid precursor protein (APP). Because of this, inhibiting BACE1 as a means to reduce Aβ production is being tested for treating Alzheimer's disease. A recent study using conditional deletion of BACE1 in an AD mouse during adulthood showed a reversal of previously formed plaques and rescues Aβ-associated behavioral deficits. One intriguing question is whether plaque reversal requires an action from reactive astrocytes surrounding plaques. In this study, we aimed to test our hypothesis that BACE1 inhibition likely facilitates astrocytic Aβ clearance. Using *in vitro* primary astrocyte cultures, we found that BACE1 inhibition enhances both astrocytic Aβ uptake and degradation. Furthermore, using single cell RNA sequencing, we found that BACE1 knockout mice had an increased number of reactive astrocytes and these reactive astrocytes from BACE1 knockout mice have transcriptomes distinct from reactive astrocytes from wild-type mice. Some of the gene signatures upregulated in BACE1 knockout astrocytes likely play a role in clearing Aβ. Our preliminary results identified a unique signature that could enhance Aβ clearance based on our *in vitro* characterization. Together, our study suggests a novel function of BACE1 in astrocytes.

Disclosures: J. Zhou: None. **R. Yan:** None.

Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.01/D16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: REAP Grant 10254-V1223

Title: A novel mechanoceutics treatment strategy for Alzheimer's disease

Authors: H. TOBEY[1], T. LUCAS[1], D. BLEDSOE[1], M. J. MYKINS[2], S. PAUL[3], S. S. BERR[3], P. G. BROLINSON[1], *B. G. KLEIN[4], **B. M. COSTA**[1];
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Abstract: In the aging brain, reduction in the pulsation of cerebral vasculature and fluid circulation cause impairment in fluid exchange between different compartments that pave a foundation for neuroinflammation that results in Alzheimer’s disease (AD). The knowledge on central nervous system lymphatic vessels in clearance of brain derived metabolic waste products opens an unprecedented capability to increase the clearance of macromolecules such as amyloid beta (Aβ) proteins. However, currently there is no pharmacological mechanism available to increase fluid circulation in the aging brain. In the present study, we demonstrate the influence of cranial osteopathic manipulative medicine (COM) therapy on spatial memory and changes in substrates associated with CNS metabolic waste clearance mechanisms using the naturally aged and transgenic (TgF344-AD) rat model of AD. The results obtained from Morris water maze assay indicate an improvement in spatial memory after seven days of COM therapy. Live animal positron emission tomography (PET) imaging and immunoassays reveal that COM treatment reduces Aβ levels, activates astrocytes and increases expression of their water channels, and improves neurotransmission. These findings demonstrate molecular mechanisms of COM treatment currently used in clinical practice. Further investigation in this direction will help clinicians practice COM as an evidence based adjunct treatment strategy for AD.

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Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

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Program #/Poster #: 377.02/D17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Institutional funds from the College of Medicine at UIC
Anonymous philanthropic contributions
NSF Bridge to the Doctorate fellowship

Title: The effects of sex and APOE genotype on the gut microbiome in young EFAD transgenic mice

Authors: *J. E. MALDONADO-WENG[1], I. PARIKH[3], Z. ISLAM[1], S. CORONEL[1], A. NAQIB[2], J. M. YORK[1], S. J. GREEN[2], S. ESTUS[3], M. LADU[1];
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Abstract: The gut microbiome (GM), the collective genome of microbiota residing within the gut, is emerging as an important component in neurological disorders such as Alzheimer's disease (AD), a fatal neurodegenerative disease diagnosed post mortem by the presence of amyloid plaques and neurofibrillary tangles. The brain is in constant bidirectional communication with the gut, allowing the brain to modulate the bacterial composition and metabolic function of the GM. With dysbiosis, the GM becomes a source of endotoxins rather than essential metabolites. To understand if dysbiosis plays a significant role in the GM of AD patients, the effects of the known AD risk factors must first be determined. Although rare, familial AD (FAD) is caused by autosomal dominant mutations that increase amyloid-β peptide (Aβ). Age is the greatest risk factor for AD and *APOE4*, the ε4 allele of *APOE*, is the greatest genetic risk factor for AD, increasing risk up to 15-fold compared to the common *APOE3*. *APOE4* is associated with increased Aβ, resulting in increased levels of both amyloid plaques and soluble neurotoxic oligomeric Aβ (oAβ). Importantly, female (♀) *APOE4* carriers have a greater risk for developing AD, an increased rate of cognitive decline and an accelerated accumulation of Aβ compared to male (♂) *APOE4* carriers. To study the interactions among these AD risk factors, we use the EFAD mice, transgenic mice that overexpress human Aβ42 and express human *APOE3* (E3FAD) or *APOE4* (E4FAD). **Our specific hypothesis is that *APOE* genotype and sex interact to modulate the GM composition in young EFAD mice.** Microbial analysis of fecal samples from 4-month (M) EFAD mice demonstrate that *APOE* genotype has a significant effect on the GM at various taxonomic levels. Additionally, there are significant differences in the GM of ♂E4FAD vs ♀E4FAD, manifested at the operational taxonomic unit (OTU) level. The synergistic effect of *APOE4* and female sex on the GM was further validated

visually by a bootstrap resampling plot. In addition, ♀E4FAD and ♂E4FAD samples cluster together with heatmap analysis based on the abundance of 29 specific bacterial OTU identified by a machine learning algorithm, again suggesting that *APOE* genotype and sex interact to alter the EFAD GM. Future work will include defining the interactive effect of age with *APOE* genotype and sex, with the goal of producing a distinct compositional and functional GM profile that distinguishes ♂E3FAD, ♀E3FAD, ♂E4FAD and ♀E4FAD. These data will be used to inform GM remodeling, for example, from ♀E4FAD to ♂E3FAD, producing a potential AD therapeutic that corrects the GM, particularly in the at risk ♀*APOE4* carriers.

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Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.03/D18

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Reduction of amyloid pathology by probiotic supplementation in a mouse model of Alzheimer's disease

Authors: *E. LEE, J. LEE, J. JUNG, H. KIM, H. PARK, T. KIM;
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Abstract: Alzheimer’s disease (AD) is one of the most common neurodegenerative disorders, which associates with impaired cognition. In the past decade, the understandings of the commensal microbiota in human have been expanded rapidly and their roles in neuropsychiatric disorders such as AD have been investigated. Studies in germ-free animals and animals exposed to pathogenic microbial infections, antibiotics, probiotics, or fecal microbiota transplantation propose a role of the gut microbiota in AD pathogenesis. However, there is a lack of studies that confirmed the effects of specific strains of probiotics on the brain of Alzheimer's disease. Therefore, we examined the effects of selected probiotics on the neuropathologic progression in the brain of Alzheimer’s disease. We used the 5XFAD mouse model of Alzheimer’s disease from the age of five weeks (N=95 males). Six strains of probiotics were selected, and the mice were grouped based on the strains (n=13 for each group). Additionally, groups of wild type (n=9) and sham administration (n=8) were treated with phosphate-buffered saline (PBS). Probiotics or PBS were administered daily per os for four months. At the age of 5 months, we sacrificed the mice and harvested the brain followed by the dissection of two brain regions (cortex, hippocampus). Soluble/insoluble amyloid-β 42 (Aβ42), and tau/p-tau proteins were quantified with enzyme-linked immunosorbent assay. Among the six probiotic groups, three groups showed

a decreased level of soluble Aβ42 in the cortex compared with the sham control group ($P<0.05$ by the two-sample t-test). Furthermore, the levels of soluble Aβ42 of the three strain groups were similar to that of the wild type group. In contrast, the levels of soluble Aβ42 in the hippocampi did not differ among all six strain groups and sham group, whereas insoluble Aβ42 in the hippocampi even increased in four strain groups, including two groups that showed decreased soluble Aβ42. These data suggest that certain strains of probiotic supplementation may induce the changes of soluble and insoluble Aβ42 in different regions of the brain. Given that soluble Aβ42, but not insoluble one has a significant association with the severity and progression of AD, it is highly likely that the reduction of soluble Aβ42 through probiotic supplementation may have therapeutic effects in AD. Further investigation in this novel approach as a therapeutic and/or preventive intervention for AD is warranted.

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Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

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Program #/Poster #: 377.04/D19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Title: Enriching enrichment: A novel 'enrichment track' protocol produces controllable and persistent cognitive enhancement in mice

Authors: ***H. A. COLLETT**[1], S. GATTAS[3], E. A. HUFF[1], S. D. CREIGHTON[1], S. E. WEBER[1], S. BUCKHALTER[2], S. A. MANNING[1], B. L. MCNAUGHTON[4], B. D. WINTERS[1]; [1]Psychology, [2]Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada; [3]Electrical Engin. and Computer Sci., Univ. of California, Irvine, Irvine, CA; [4]Dept. of Neurosci., The Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Cognitive reserve (CR) is a conceptual construct reflecting rich life experience, such as higher education, intellectual activity, travel, and multilingualism. It is the best predictor of preservation of cognitive function in humans with trauma, neurodegenerative or age-related brain changes. CR can be modeled in rodents using environmental enrichment (EE). In standard EE protocols, however, it is difficult to measure or control individual animal enrichment, making it difficult to compare treatment effects (such as drugs, lesions, age, or sex) on EE induced brain or behavioral changes. We developed a novel EE protocol, using reward-motivated running on tracks with built-in obstacles of progressive complexity, to enable quantification and control of enrichment experience and effects. 28 day-old male C57/BL6 mice were divided into 4 different

groups (n = 10/group): home cage enrichment (EE) using conventional EE procedures; enrichment track (ET); exercise control track (CT); or standard ("impoverished") housing control (SH). All but the EE group were housed in standard cages containing only simple nesting supplies. After 2 mo (with 1 h/d x 6 d/wk for the ET and CT groups), a 'difficult' version of the object recognition task with reduced sample exploration time was used to assess memory performance. Cognitive enhancement was evident for both the EE and ET groups with a 20-min retention delay, but only ET mice discriminated between familiar and novel objects with a 24-h delay. We also assessed ET effects on other object recognition tasks including category, view-invariant and cross-modal object recognition. The ET group demonstrated superior performance on the more difficult versions of these tasks, with longer retention delays, indicating that ET mice can use object representations with greater flexibility. Enhanced memory performance on such tasks was observed up to 4 mo post discontinuation of ET. The ET is thus an advancement over typical enrichment protocols, enabling more direct and measurable individual enrichment, and conferring sustained cognitive enhancement. The ET protocol may enable further characterization of experience-dependent mechanisms that contribute to the development of CR in mouse models of Alzheimer's disease or other brain disorders.

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Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.05/D20

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Beneficial effects of resistance exercise in improving cognitive functions and reducing neuropathology in postoperative cognitive dysfunctions and Alzheimer's transgenic mice

Authors: *R. C. C. CHANG[1,2], Y. LIU[1,3], J. M. T. CHU[1,3], C. HUANG[1,3], G. T. C. WONG[3]; [1]Lab. of Neurodegenerative Diseases, Sch. of Biomed. Sciences, LKS Fac. of Medicine, The Univ. of Hong Kong, Hong Kong, China; [2]State Key Lab. Brain and Cognitive Sciences, The Univ. of Hong Kong, Hong Kong, China; [3]Dept. of Anaesthesiology, LKS Fac. of Medicine, The Univ. of Hong Kong, Hong Kong, China

Abstract: Exercise is well-known to have great beneficial effects to the whole body. While majority of research demonstrates neuroprotective effects of aerobic exercise, a certain large population of patients who are too old or suffer from motor problem cannot gain the benefits of aerobic exercise. Some Alzheimer's disease (AD) patients cannot even perform proper aerobic exercise. Therefore, investigating the effects of resistance exercise is important for patients lying

on the bed and for some AD patients. We hypothesize that resistance exercise can improve cognitive functions. We proved the correction of cognitive functions by two experimental models. One model was the risk factor for developing dementia, namely postoperative cognitive dysfunctions (POCD), using C57BL/6 mice. Another model was to use AD transgenic mice bearing three types of mutations (3xTg mice). Resistance training was accomplished by a ladder-climbing protocol for 5 weeks with 3-4 training sessions per week. For each session, mice were forced to climb up a 1-meter ladder with progressively larger weight attached to tail for 15 times with 2 minutes rest during each trail. Effects of resistance training on cognition and muscle strength were checked by Y-maze and weight lifting test, respectively. For 3xTg mice, we used 9-month-old mice. For POCD, laparotomy was performed using aseptic procedures under sevoflurane (3-4%) as anesthesia (~20 min). Cognitive functions were assessed by Y-maze test and novel object recognition (NOR) test 2 weeks afterwards. Levels of synaptic proteins in the hippocampus were detected by western blot. Cytokines levels were measured by their expression of mRNA and proteins. Our results showed that resistance exercise could revert the cognitive dysfunctions induced by laparotomy and attenuate progressive cognitive loss in 3xTg mice in Y-maze test. Furthermore, western-blot analysis showed that it could ameliorate loss of synaptic proteins, tau protein phosphorylation, and inflammatory cytokines. Our studies demonstrate that resistance exercise can be an alternative way of exercise that can provide to patients who have motor deficit or difficulty in working out for aerobic exercise.

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Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R56-AG057895-01
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Title: Exercise mitigates cognitive dysfunction resulting from a graduate loss of ovarian function in an Alzheimer’s disease mouse model

Authors: J. R. WILLIAMS[1], C. GRANT[1], S. S. VASHISTH[1], I. KOC[1], S. A. AHMAD[1], S. V. MAURER[1], C. A. COLTON[2], E. A. FINCH[3], ***C. L. WILLIAMS**[1];
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Abstract: Over two-thirds of individuals with Alzheimer's disease (AD) are female, with the gradual loss of ovarian hormones during the perimenopausal transition thought to be a major factor contributing to greater female risk. A sedentary lifestyle also increases the risk for AD, and accumulating evidence suggests that physical activity may provide a therapeutic intervention to slow or mitigate AD progression. Here we investigated whether a graduate loss of ovarian function exacerbates AD neuropathogenesis and exercise might mitigate these effects. We induced transitional menopause (TM) in AD and control mice by injecting the ovotoxin 4-vinylcyclohexene diepoxide for 16 days, beginning at 9 wks of age; and exercise-trained mice for 12 wks at various stages of AD-like disease and TM using both voluntary (wheel running 5 days/wk) and enforced (treadmill running 2X/wk) exercise. We used the APPSwDI/mNos2$^{-/-}$ AD (CVN-AD) mouse model, which mimics familial AD by expression of mutated APP and a human-like immune environment through lowered NOS2 expression; and both C57BL/6 and mNos2$^{-/-}$ mice as controls. CVN-AD mice exhibit many neuropathological features of AD, and exacerbated AD-like neuropathogenesis and resistance to therapeutic intervention in females. TM mice had lower uterine weights than oil-treated control mice in each mouse line, confirming lower cycling estrogen levels following TM. Increased time-to-exhaustion on a treadmill test and lower body weights confirmed significant physiological benefits of exercise training in CVN-AD mice. When trained on a Novel Object Recognition task with a short retention interval, sedentary and exercised cycling mice showed intact short-term recognition memory. In contrast, the short-term memory of sedentary TM mice was impaired. However, as hypothesized, exercise-trained TM mice had no difficulty recalling the familiar object, indicating that exercise can mitigate the detrimental effects of menopause on short-term memory. Preliminary analysis of microglia suggests that exercise also prevents enhanced neuroinflammation caused by AD and TM. Our findings support previous reports that CVN-AD mice show progressive, age-related cognitive impairment and provide strong evidence that a gradual, menopause-like loss of ovarian hormones exacerbates AD-like cognitive decline and neuropathogenesis which can be mitigated by exercise training around the menopause transition. Ongoing studies are investigating the consequences of TM on other aspects of AD-like disease progression, and the response of females to therapeutic interventions with exercise at various stages of the menopausal transition.

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Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.07/D22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant MH090067

Title: Altered dopamine neuron activity in the FAB rodent model of Alzheimer's disease

Authors: *S. M. PEREZ[1], A. M. BOLEY[1], D. J. LODGE[2];
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Abstract: Individuals affected by Alzheimer’s disease (AD) often experience comorbid psychosis, which severely diminishes the quality of life for the patient and their family. Because of the potential risk antipsychotic medications present to the elderly, there is an immediate need to establish novel alternative therapies. Psychosis (including hallucinations and delusions) has been demonstrated to be associated with a dysregulation of the dopamine system. We have previously demonstrated that psychosis observed in schizophrenia, may be attributed to aberrant regulation of dopamine neuron activity by the hippocampus. Because the hippocampus has been identified as a site of pathology in AD, we posit that it may also be a key region contributing to comorbid psychosis in AD. Thus, we used the ferrous amyloid buthionine (FAB) rodent model of AD to model a sporadic form of the disease and examine alterations in dopamine system function. FAB rats display structural and functional alterations in the hippocampus, which is accompanied by a decrease in spontaneous low frequency oscillatory activity. Additionally, FAB rats exhibit robust increases in dopamine neuron population activity, consistent with what is observed in other models of psychosis. These data suggest that aberrant hippocampal activity in AD may contribute to dopamine dependent psychosis. We believe that understanding the pathophysiology leading to comorbid psychosis in AD, will lead to novel targets for the treatment of this disease.

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Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

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Program #/Poster #: 377.08/D23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Synapsis Foundation - Alzheimer Research Switzerland

Title: Mild uncoupling of astrocyte mitochondria as a strategy to alleviate spatial memory deficits in Alzheimer's disease

Authors: N. ROSENBERG[1], A.-B. ROCHER[1], L. RESTIVO[1], M. BRIQUET[1], Y. BERNARDINELLI[2], *J.-Y. CHATTON[1];

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Abstract: Hypometabolism and oxidative stress, along with neuronal death are often associated with the cognitive symptoms of Alzheimer's disease (AD). It has been shown in neuron-astrocyte co-cultures that uncoupling proteins 4 (UCP4) expressed by mitochondria of astrocytes decrease peroxide production, increase their glycolysis and lactate release, and enhance the survival rate of neurons. In parallel, lactate is known to exert beneficial effects in cerebral ischemia and has a non-metabolic effects modulating neural activity. In neurons, lactate has been shown to promote plasticity gene expression and is a key player for memory formation. We pose the hypothesis that in vivo overexpression of astrocytic mitochondrial UCP4 will provide support to neurons facing AD-associated injuries. Adeno-associated viruses (AAV) containing mCherry, as a fluorescent reporter, or UCP4 in combination with mCherry under the GFAP promoter, were stereotaxically injected in the CA1-subiculum of the dorsal hippocampus of wild-type (WT) and 3xTg-AD triple-transgenic mice. We assessed the cognitive status in a first cohort of 3- and 7-month-old mice. We found that the viral construct had no effect on the exploratory behavior in both either groups. While 3- and 7-month-old WT mice reacted to the spatial change, 3xTg-AD mice failed to selectively explore displaced objects. Uncoupling astrocyte mitochondria appeared to rescue spatial memory deficits in 7-month-old mice. The electrophysiological properties of CA1-subiculum pyramidal cells of 7-month-old 3xTg-AD mice showed alterations that were reversed by UCP4 treatment. They are possibly linked to abnormal calcium responses. Overall, astrocytic UCP4 overexpression appears to positively influence AD symptoms both at behavioral and cellular levels.

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Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: A study of the neural circuit mechanisms underlying the emotional symptoms associated with Alzheimer's disease

Authors: V. BANOV[1], Y. KOBAYASHI[1], R. ANDO[1], T. SAITO[1], T. SAIDO[1], S. ITOHARA[1], S. OGAWA[2], L. J. YOUNG[3,2], ***Q. ZHANG**[1,2];
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Abstract: Patients suffering from Alzheimer's disease (AD) demonstrate both cognitive symptoms (including deficits of learning, memory, attention and executive function) and emotional symptoms (including irritability and low mood). Depressive symptoms and behaviors are very common comorbidities observed in AD patients and generally precede the onset of AD. However, the vast majority of AD research has focused on the neurobiological mechanisms underlying the cognitive symptoms, whilst the mechanisms underlying depression associated symptoms have been grossly neglected and are poorly understood. In current study, we investigated the emotional phenotypes in a typical Alzheimer Disease (AD) animal model and discovered that the depression-like symptoms occurred before the onset of brain pathological changes and cognitive impairments. Further we showed that an improvement in emotional state either by anti-depression drugs or enriched environment can delay the initial deposition of Aβ.This work has important implications for future strategy for AD detection and treatment.

Disclosures: V. Banov: None. **Y. Kobayashi:** None. **R. Ando:** None. **T. Saito:** None. **T. Saido:** None. **S. Itohara:** None. **S. Ogawa:** None. **L.J. Young:** None. **Q. Zhang:** None.

Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.10/D25

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: How to stimulate: Basal forebrain DBS parameters to restore the attentional performance of rats with cholinergic losses

Authors: *M. NAZMUDDIN[1], H. A. RAO[2], T. VAN LAAR[1], M. F. SARTER[2];
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Abstract: The degeneration of basal forebrain (BF) cholinergic neurons is an index of the severity of cognitive impairment in Alzheimer disease (AD) and Parkinson's disease (PD). Moreover, in PD patients, gait and balancing deficits, and an increased propensity for falls have been attributed to cholinergic losses. Thus, Deep Brain Stimulation (DBS) of the BF has been considered a potential therapeutic intervention to improve cognition and movement control in these patients. However, efficacy of BF DBS in clinical populations has yet to be conclusively demonstrated. Likewise, the demonstration of beneficial effects of BF DBS in rodent models has

been hampered by uncertainties about useful animal models and behavioral tasks and, importantly, a lack of consensus concerning DBS parameters (duration, frequency, current, intermittent versus continuous, prior and/or during task, etc.). Here we assessed various DBS parameters in rats with a partial loss of the cortical cholinergic input system. In rats, such cholinergic losses have been frequently demonstrated to impair the detection of cues during the performance of a Sustained Attention Task (SAT) and to attenuate performance recovery following a distractor challenge (dSAT). In PD patients with cholinergic losses, attentional impairments were also attributed to cortical and thalamic cholinergic losses (Kim et al., 2017). The attribution of SAT impairments to cholinergic losses is consistent with evidence showing that the detection of cues and associated attentional control parameters depend on cortical cholinergic signaling (e.g., Howe et al., 2017). Here, rats acquired the SAT, received infusions of the cholino-specific neurotoxin 192-IgG-saporin into the BF, and were implanted bilaterally with BF unipolar stimulation electrodes. Initial DBS parameters consisted of continuous high (130 Hz) versus low (20 Hz) frequency stimulation, intermittent (20-s ON at 80 Hz and 40-s OFF) stimulation, with pulse width and amplitude kept constant at 100 µs and 100 µA, respectively. We first assessed the effects of these DBS parameters on the behavior of rats in an open field space and then when administered during, or only prior to (for 1 hr), SAT and dSAT performance. Ongoing experiments indicate that these stimulation parameters are well tolerated as indicated by the absence of effects on locomotor and exploratory activity. We predict that BF DBS will be particularly effective in restoring attentional performance in the dSAT condition. If confirmed, this finding will suggest that demonstration of efficacy in patients will require measures indicating their attentional capacities in response to taxing performance challenges.

Disclosures: **M. Nazmuddin:** None. **H.A. Rao:** None. **T. van Laar:** None. **M.F. Sarter:** None.

Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.11/D26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG058081
NIH Grant AG056976
NIH Grant AG056025
Wallin Neurosci Discovery Program, Academic Health Center of the University of Minnesota

Title: Modulation of Alzheimer’s neuropathology through peripheral circulation

Authors: W. LIU[1], D. A. HOTTMAN[1], A. GRAM[1], V. D. KRISHNA[2], M. C.-J. CHEERAN[2], W. C. LOW[3], ***L. LI**[1];

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Abstract: To date there is no effective treatment for Alzheimer's disease (AD), the most common form of dementia. Pathological hallmarks of AD include the deposition of amyloid-β peptide (Aβ) in the brain parenchyma (senile plaques) and in the cerebral blood vessels (cerebral amyloid angiography or CAA) and associated neuroinflammation, as well as neurofibrillary tangles. Previous studies have shown that administration of young blood/plasma or circulatory exchange by parabiosis ameliorates age-related changes and facilitates clearance of Aβ in mouse models. To further understand the role of peripheral circulation in the pathogenic process of AD, we performed parabiotic surgeries between C57BL/6 (B6) congenic APP/PS1 transgenic (Tg) mice and sex/age matched B6 congenic green-fluorescent-protein (GFP) Tg mice at 3 to 6 months of age (before or at the onset of Aβ deposition in the brain). The mice remained parabiosed until 7 to 10 months of age (n=7 pairs). The age/sex-matched APP/PS1 mice (n=7) without parabiosis served as controls. Success of the parabiotic surgery/circulatory exchange was confirmed two weeks after the surgery by the presence of GFP-expressing cells in the blood of paired APP/PS1 mice by flow cytometry analyses. At the end of the experiments, blood and brain tissue samples were collected for further analyses. Compared with age/sex matched non-parabiotic APP/PS1 mice, Aβ40 and Aβ42 levels in the plasma of parabiosed APP/PS1 mice were significantly decreased. Immunohistochemical analyses showed that Aβ deposition and associated neuroinflammation, represented by immunoreactivity to activated microglial marker Iba-1 and astrocytic marker GFAP, in the cortex and hippocampus were reduced significantly in the parabiosed APP/PS1 mice. In addition, CAA, quantified by Congo red staining, was also attenuated in the brain of the parabiosed APP/PS1 mice. Intriguingly, while substantial numbers of GFP-expressing cells circulated in the blood of parabiosed APP/PS1 mice, no or negligible GFP-expressing cells were found in the brain of parabiosed APP/PS1 mice, indicating that there was no or minimal recruitment of circulating cells from the parabiosed donor into the Aβ-bearing brain, despite evidence of GFP-chimerism in lymphoid organs. Taken together, these findings suggest that modulation of peripheral circulation could be a potential therapeutic approach against AD.

Disclosures: W. Liu: None. **D.A. Hottman:** None. **A. Gram:** None. **V.D. Krishna:** None. **M.C. Cheeran:** None. **W.C. Low:** None. **L. Li:** None.

Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.12/D27

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Learning the game of chess reduces age related cortical thinning in patients with mild cognitive impairment

Authors: *J. STOUT[1], T. MALONE[2], J. HEFFERNAN[1], T. MALMSTROM[2], P. RUPPERT[2], S. BROWN[2], R. BUCHOLZ[3], R. TAIT[2], G. HEADY[2], L. SCHWARZ[2];
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Abstract: Introduction: Patients with mild cognitive impairment (MCI) have memory and mental processing changes that are greater than expected within the normal aging process. Approximately 10-20% of adults ages 60 or older have MCI. The rate of progression to Alzheimer's disease is 12% per year for patients diagnosed with MCI, significantly higher than the 1-2% for older adults. Previous studies have shown cognitive training improves immediate and delayed recall in MCI patients. This study examines the effectiveness of a complex activity, learning and playing chess, on memory and mental function among older adults with MCI. The subset of data reported here examine the effects of learning and playing chess on longitudinal cortical thickness among MCI patients. **Methods:** This study is a 2-year, prospective, randomized, single-blinded, controlled trial to investigate the effects of learning and playing chess among older adults with MCI. Participants with MCI are randomized to either a chess intervention group or a non-chess control group. The MCI chess group (CG) is taught how to play chess by chess grand masters using a computer tablet and standard chess board, and the non-chess MCI group (Ctrl) is be instructed to continue to engage in their typical cognitive activities. This study is on-going. This analysis comprises patients with follow-up at 12 months, CG(N=11) and Ctrl(N=11). Data was acquired on a 3T Siemens Skyra scanner at St. Louis University Medical Center. MRI acquisition was performed at 0, 6, 12, and 18 month time points. T1 and T2 MRIs, were acquired using Human Connectome Project (HCP) acquisition sequences. Images were processed through the HCP anatomical pipeline for surface segmentation, parcellation, and cortical thickness evaluation. Longitudinal volume and surface processing was performed using Freesurfer. Connectome workbench was used to extract parcellated cortical thickness assessments using the Glasser parcellation. A T-test between CG and Ctrl patients was calculated on all parcels for 12 month vs baseline. **Results**: The ROIs with the most significant changes between CG and Ctrl patients are the following areas: Left peri entorhinal cortex, area TF, orbitofrontal cortex with p-values of .007, .008, .008 respectively. **Conclusions:** In this preliminary analysis we found a positive effect of chess training and gameplay on cortical thickness over time. The regions identified as most significant also correspond to regions that have previously been identified in MCI research. Future analysis will include additional subjects and neuropsych findings.

Disclosures: J. Stout: A. Employment/Salary (full or part-time):; Medical College of Wisconsin. **T. Malone:** None. **J. Heffernan:** None. **T. Malmstrom:** None. **P. Ruppert:** None. **S. Brown:** None. **R. Tait:** None. **R. Bucholz:** None. **L. Schwarz:** None. **G. Heady:** None.

Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.13/D28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSF Grant 1258111

Title: The effects of deep brain stimulation of the cholinergic forebrain on markers of nerve growth factor and beta-amyloid

Authors: *J. S. KUMRO[1], J. SWORD[1], S. A. KIROV[1], A. PILLAI[2], D. T. BLAKE[1]; [1]Neurosci. and Regenerative Med., [2]Psychiatry and Hlth. Behavior, Med. Col. of Georgia at Augusta Univ., Augusta, GA

Abstract: The cholinergic forebrain or nucleus basalis of Meynert (NBM) degenerates earlier and more rapidly than other brain regions during Alzheimer's disease (AD). The current frontline therapy for AD is cholinesterase inhibitors which improve cognition and slow structural degeneration, particularly of the basal forebrain, over the first three to four months prior to decline restarting. Our previous research demonstrated that intermittent electrical stimulation of the NBM improves working memory in a young adult primate model. Anticholinergic pharmacology suggested ACh release to be the responsible mechanism. In states of beta-amyloid (Aβ) plaque accumulation like AD, Aβ binds and antagonizes α7-nAChR, a nicotinic ACh receptor densely populated in the cholinergic forebrain, resulting in cholinergic neuronal degeneration. We hypothesize that intermittent NBM stimulation can treat this AD pathogenesis by stimulating α7-nAChR and promoting its role in increased expression of nerve growth factor (NGF) and its receptor TrkA, which has been shown to modify amyloid precursor protein (APP) cleavage reducing Aβ and maintain cholinergic axons respectively. Here we show that stimulation in mouse NBM significantly improves cerebral blood flow, measured using laser speckle imaging, and ACh synaptic release, measured using a fluorescent protein coupled acetylcholine receptor sensor in confocal imaging. This stimulation pattern in middle-aged adult rats prevented performance decline observed in sham controls. Additionally, unilateral stimulation for multiple weeks, in either APP/PS1 mice or old rats, increased markers indicating improved vitality of the basal forebrain neurons and reduction in Aβ production in the stimulated hemisphere.

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Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.14/D29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Seed grant from University of Kentucky Department of Neuroscience

Title: Feasibility of dynamic sleep enhancement in a mouse model of Alzheimer's disease

Authors: D. HUFFMAN[1], A. A. AJWAD[2], J. WANG[1], M. P. MURPHY[3], B. F. O'HARA[1], M. J. DUNCAN[4], ***S. SUNDERAM**[1];
[2]Biomed. Engin., [1]Univ. of Kentucky, Lexington, KY; [3]Molecr & Cell. Biochemi, Univ. Kentucky, Lexington, KY; [4]Neurosci., Univ. of Kentucky Med. Sch., Lexington, KY

Abstract: Alzheimer’s disease (AD) is a degenerative condition that is marked by memory impairment, cognitive deficits, and changes in personality. Neurofibrillary tangles and amyloid plaque buildup in the brain are believed to play an integral role in AD pathology, and are therefore active topics of research. However, disruptions or alterations in sleep commonly seen in this condition are less understood. There is growing evidence that disordered sleep is not merely a consequence of AD, but that it may also accelerate pathology. Therapeutic strategies for improving sleep quality may slow disease progression and are therefore desirable. To this end, we have developed a system to accomplish sleep enhancement through simple closed-loop control of ambient temperature (*Ta*), which is known to influence sleep through thermoregulation. We first applied this technique to wildtype mice to evaluate feasibility. With institutional approval, five adult C57/BL6 mice (3 female, 2 male) were instrumented for electroencephalogram (EEG) and electromyogram (EMG) recording in individual cages. After complete recovery, each mouse was subjected to a closed-loop protocol in which *Ta* was adjusted at regular intervals to promote EEG slow wave activity in the delta band (0.5-4 Hz), a recognized marker of deep sleep, for five hours during the light period, every other day over four days. The recordings were supplemented by motion signals from a noninvasive floor-mounted piezoelectric sensor (Signal Solutions, LLC), to better discriminate sleep and behavior. A comparison of sleep metrics from these experiments showed that the mice experienced significantly longer bouts of NREM and REM sleep as well as greater amounts of deep NREM sleep under the experimental protocol compared to the sham reference period in which *Ta* remained constant. Having thus established the feasibility of sleep enhancement in controls, we applied a similar protocol to an AD model. Six 5xFAD mice (6-8 months old; 3 male, 3 female) were instrumented for EEG/EMG recording and subjected to two weeks of *Ta* manipulation in the light period directed at sleep depth enhancement. For each animal, an age- and sex-matched sham-treated control was monitored in an adjacent cage that remained at room temperature.

These experimental recordings have been completed and data analysis is ongoing. Future work will investigate the effects of sleep enhancement on molecular and cognitive disease markers in this and other experimental models of AD.

Disclosures: S. Sunderam: None. **D. Huffman:** None. **A.A. Ajwad:** None. **J. Wang:** None. **B.F. O'Hara:** None. **M.J. Duncan:** None. **M.P. Murphy:** None.

Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.15/D30

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Assessment of the mechanisms of action of ethanolic extracts of bacopa floribunda and angraecum eichlerianum on amyloid beta (1-42) model of Alzheimer's disease in male Wistar rats

Authors: *M. OMOTOLA OYELEKE[1,2], O. L. AROKOYO[1], H. T. ONI[1], B. V. OWOYELE[2]; [1]Physiol., Afe Babalola Univ., Ado-Ekiti, Nigeria; [2]Physiol. Dept., Univ. of Ilorin, Ilorin, Nigeria

Abstract: This study investigated the effects of crude extracts obtained from *Bacopa floribunda* and *Angraecum eicherianum.* A total number of 48 (n=6) male wistar rats were used for this study. AE and BF extracts were given at a dose of 200mg/kg/day and Alzheimer's disease was induced by a single bi-lateral intra-cerebroventricular (ICV) injection of Amyloid beta protein (4µg/µl/site) using a stereotaxic apparatus. Pre-treatment and post-treatment models with BF and AE were employed for 21 days. Rats were grouped as follows; Group 1 (Normal saline), Group 2 (Amyloid beta without treatment), Group 3 (BF alone), Group 4 (AE alone), Group 5 (pre-treated with BF), Group 6 (pre-treated with AE), Group 7 (post-treated with BF) and Group 8 (post-treated with AE). Novel Object Recognition (NOR) and Y maze task were carried out on different days during the course of the study. Twenty-four hours after the last administration, rats were sacrificed and brain tissues excised using appropriate forceps. The hippocampus was isolated on cold surface and assayed for the levels of glutamate, acetylcholinesterase (AChE), Na^+ - k^+ ATPase activities and Amyloid beta deposition using ELISA kits. Data were analyzed using One-way ANOVA followed by a post-hoc test and expressed as Mean ± SEM. A significant increase was observed in the activities of AChE in group 6 when compared with every other group, likewise, a significant decrease was observed between groups 5 and 7 when compared with groups 1, 2, 3, 4 and 8. Results also showed that group 3 alone had the highest level of significance of Na^+- k^+ ATPase activities when compared with every other group. Groups 6, 7 and 8 revealed some levels of significance when compared with groups 1, 2, 4 and 5. Neurobehavioural scores were observed to be better in BF and AE groups compared to Aβ and normal saline groups. Glutamate excitotoxicity was equally observed in hippocampal

homogenates of rats in group 2 and this was suppressed by our extracts. In conclusion, Amyloid beta (1-42) caused an increase in the level of AChE and a decrease in Na^+ - k^+ ATPase level, while BF elicited its protective action by increasing the levels of Na^+ - k^+ ATPase and the mechanism of action of AE was by reducing the activities of acetylcholinesterase in Amyloid beta induced Alzheimer's disease. Therefore, our study suggests that these extracts possess cognitive enhancement properties and as such can relieve dementia to a great extent.
KEYWORDS: Alzheimer's disease; Amyloid; Rats.

Disclosures: M. Omotola Oyeleke: None. **O.L. Arokoyo:** None. **H.T. Oni:** None. **B.V. Owoyele:** None.

Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.16/D31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Institutes of Health (AG045656-05)
Alzheimer's Association (ZEN-15-321972)
Charles H. Smith Endowment Fund to G.C.

Title: Development of NeuroD1-mediated *in vivo* neuroregeneration therapy in an Alzheimer's disease mouse model

Authors: *X. HOU, Y. WANG, Z. WU, F. ZHANG, Z. PEI, Y. BAI, G. CHEN; Pennsylvania State Univ., University Park, PA

Abstract: Alzheimer's disease (AD) is a chronic neurodegenerative disorder with a plethora of pathological changes such as amyloid-β accumulation, intracellular tau tangles, synaptic dysfunction, reactive gliosis, and ultimately neuronal loss. AD is the most prevalent dementia among elderly people and ranked as the sixth leading cause of death in the United States. Currently available drugs are targeting cholinergic and glutamatergic neurotransmission and can only alleviate certain disease symptoms. So far, there is no disease-modifying therapy to prevent or reverse AD progression, largely because of the lack of a technology to prevent neuronal loss or to replenish the lost neurons after degeneration. In this study, we employed our AAV NeuroD1-based gene therapy to achieve high efficiency astrocyte-to-neuron conversion in the 5xFAD mouse model for AD. We found that after AAV NeuroD1-mediated conversion, the number of cortical neurons increased while the number of reactive astrocytes decreased, leading to a rebalanced neuron to astrocyte ratio in the cortex. Moreover, we observed a reduction of intracellular amyloid-β level accompanied with a reduction of inflammatory microglia. The NeuroD1-converted neurons showed long-term survival of more than 8 months in old AD mice,

and the NeuroD1-converted areas showed higher synaptic density and improved blood vessel integrity. In summary, our NeuroD1-based gene therapy not only regenerates a large number of functional new neurons through high efficiency astrocyte-to-neuron conversion but also changes the previously degenerative landscape into a neural regenerative environment.
Key words: Alzheimer's disease, NeuroD1, gene therapy, astrocyte-to-neuron conversion, *in vivo* reprogramming, intracellular Aβ, reactive astrocytes, neuron, microglia, blood vessel

Disclosures: X. Hou: None. **Y. Wang:** None. **Z. Wu:** None. **F. Zhang:** None. **Z. Pei:** None. **Y. Bai:** None. **G. Chen:** None.

Poster

377. Alzheimer's Disease and Other Dementias: Therapeutic Strategies I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 377.17/D32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG055577
Cecil H. and Ida Green Distinguished Chair fund
Carl J. and Hortense M. Thomsen Chair in Alzheimer's Disease Research

Title: Altered heme metabolism in Alzheimer's disease pathogenesis

Authors: *L. ZHANG[1], C. VIDAL[1], K. DAESCU[1], K. E. FITZGERALD[1], A. STAROKADOMSKA[2], I. BEZPROZVANNY[3];
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Abstract: Heme is a central molecule for mitochondrial respiration and for all processes involved in oxygen utilization. Multiple subunits in the mitochondrial respiration complexes also require heme in order to function. Particularly, neurons have an increased demand for cellular energy and are known to enhance heme biosynthesis and uptake. Therefore, it is not surprising that heme has been implicated with the progression of Alzheimer's disease. Heme has been shown to colocalize with Aβ in AD tissue. This Aβ heme complex has been shown to generate ROS, which can generate an array of neurotoxic products. Despite this evidence of the involvement of heme in AD pathogenesis it is not clear what heme-related events play a causative role in AD pathogenesis. To elucidate the role of heme in AD we identified heme-related proteins whose expression is altered in AD patients and APPPS1 mice. We found that the rate-limiting heme synthetic enzyme ALAS1 and heme degradation enzyme HO-2 are selectively reduced in AD brain hippocampi. Further, to identify the potentially early events in Aβ action, we employed the SH-SY5Y cell line to study the AD pathogenesis. Using the SH-SY5Y cell line, we monitored heme synthesis, uptake, and degradation, as well as heme-related proteins and

enzymes as neuronal differentiation progresses. Using fully differentiated SH-SY5Y cells we found that Aβ selectively reduces the levels of HO-2 and heme degradation which are elevated to support neuronal function. Our data show that heme degradation should also lead to lowered levels of biliverdin and bilirubin in neuronal cells, which would likely lead to oxidative stress in early stages of AD pathogenesis. Further experiments are underway to illuminate the roles of altered heme metabolism in AD pathogenesis.

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Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.01/D33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Center of Geriatric and Gerontology Grant
JSPS KAKENHI Grant Number JP 16H06277.
AMED under Grant Number JP18dm0107103.

Title: Propagation of neurodegenerative protein in human aging

Authors: *S. MURAYAMA[1], Z. TANEI[1], T. MATSUBARA[1], R. SENGOKU[1], Y. SAITO[2]; [1]Neurol and Neuropathology (the Brain Bank for Aging Research), Tokyo Metropolitan Geriatric Hosp. and Inst. of Gerontology, Tokyo, Japan; [2]Natl. Ctr. Hosp. Neurol. & Psy., Tokyo, Japan

Abstract: Introduction: Age- related neurodegenerative pathology presents relatively high incidence and extent, with certain symptomatic threshold for each pathogenic protein. The aim of this study is to provide full profile of Alzheimer pathology and Lewy body- related alpha synucleinopathy (LBAS) in human aging population in addition to other neurodegenerative proteins including four repeat tau and TDP43.
Method: The Brain Bank for Aging Research (BBAR) consists of consecutive autopsy cases of a general geriatric hospital, which supports an aging cohort of Tokyo Metropolitan suburban area. The registration to BBAR was based on the first kin of relatives' informed consent with or without patients' living will. The tissues were processed following BBAR method which was uploaded to www.mci.gr.jp/BrainBank/. We have screened not only the brain and spinal cord, but also the peripheral nervous system (PNS) of all the registered cases to BBAR immunohistochemically. The antibodies employed include antibodies against Abeta, phosphorylated (p) tau, p- synuclein (psyn), p- TDP43 and ubiquitin.
Results: Among the 534 cases registered to BBAR from 1995 to 2018, 181 cases (34%) contained LBAS either in the PNS and/ or the central nervous system (CNS). 46 out of the 181

cases fulfilled diagnostic criteria of Parkinson disease (PD) with (PDD) or without dementia, dementia with Lewy bodies (DLB) or pure autonomic failure (PAF). The most predilection site of LBAS was sympathetic ganglia in PNS and olfactory bulbs in CNS. In the earliest stage of Lewy body disease, these two sites were independent as we previously reported. PNS had strong connection with brain stem, while olfactory bulbs with limbic system. We classified incidental LB disease (LBD) to preclinical and prodromal stage either with or without loss of pigmentation in substantia nigra. Preclinical and prodromal PD, DLB and PAF could be recognized of lesser frequency than preclinical, prodromal and clinical AD, which roughly represents 20%, 10% and !0% of all the registered cases.
Conclusion: This data provides basic information about the propagation of AD pathology, LBAS and their combination in human aging. Our brain bank could provide resource for mechanistic research of multiple proteinopathy in human aging.

Disclosures: S. Murayama: None. **Z. Tanei:** None. **T. Matsubara:** None. **R. Sengoku:** None. **Y. Saito:** None.

Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.02/D34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIMH (R01-MH111604, Robison PI)
NIDA (R01-DA040621, Rudenko PI)

Title: *In vivo* redox regulation of ΔFosB's structure-function relationship in Alzheimer's

Authors: *H. LYNCH[1], C. MCCORNACK[2], V. BALI[1], G. RUDENKO[4], E. J. NESTLER[5], A. ROBISON[3];
[1]Physiol., [3]Neurosci., [2]Michigan State Univ., East Lansing, MI; [4]Pharmacology/Toxicology and Sealy Ctr. for Structural Biol., Univ. of Texas Med. Br., Galveston, TX; [5]Icahn Sch. Med. At Mount Sinai, New York, NY

Abstract: Many neurodegenerative diseases, including Alzheimer’s disease (AD), are driven by altered reduction/oxidation (redox) balance in the brain. Moreover, cognitive decline in AD is caused by neuronal dysfunction that precedes cell death, and this dysfunction is in part produced by altered gene expression. However, the mechanisms by which redox state controls gene expression in neurons are not well understood. ΔFosB is a neuronally enriched transcription factor critical for orchestrating gene expression underlying memory, mood, and motivated behaviors and is dysregulated in Alzheimer’s Disease (AD). ΔFosB regulates gene expression by dimerizing with JunD to form activator protein 1 (AP-1) which binds the promoter regions of

target genes to control transcription. In controlled *in vitro* conditions, AP-1 complex formation and DNA binding are modulated by redox-sensitive disulfide bonds, and by redox-sensitive conformational changes in ΔFosB. Here, we show that the redox-dependence of the structure-function relationship of fos-family proteins found *in vitro* is also conserved in ΔFosB *in vivo*; a characteristic that we find across the brain. Under non-reducing (oxidizing), fully denatured conditions, immunoprecipitation followed by Western blot reveals a shift in the molecular weight of ΔFosB from 37kDa to ~75kDa and ~150 kDa; potentially representing the binding of ΔFosB to other proteins through disulfide bridge formation between cysteine residues that has been demonstrated *in vitro*. We also demonstrate that JunD is part of this oxidation-dependent complex. In contrast, under reducing conditions, denatured ΔFosB remains at 37kDa, indicative of no oxidation-dependent covalent complex formation. Additionally, we delve into the ability of ΔFosB to form both heterodimers and homodimers under various redox conditions and the role of specific cysteine residues in these complexes in cultured Neuro2a cells. Taken together, these data suggest that ΔFosB complex formation in the brain is directly regulated by redox state through disulfide bonds. Better understanding of how the structure-function relationship of ΔFosB is regulated by cysteine oxidation may ultimately allow us to use ΔFosB as a therapeutic target for diseases associated with an altered redox state, like AD.

Disclosures: H. Lynch: None. **C. McCornack:** None. **V. Bali:** None. **G. Rudenko:** None. **E.J. Nestler:** None. **A. Robison:** None.

Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.03/D35

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Differential expression of PICALM in the cortex and hippocampus of Alzheimer’s disease (AD)

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Abstract: Phosphatidylinositol-binding clathrin assembly protein or PICALM is a genetic risk factor for AD based on recent genome wide association studies (GWAS). The preponderance of PICALM in the brain capillary endothelium makes it an ideal candidate for regulation of blood brain barrier function in vivo due to its ability to clear Aβ from the brain into circulation. Additional evidence also suggests that PICALM’s involvement in receptor mediated clearance

such as low-density lipoprotein receptor related protein (LRP1), which eventually binds to Aβ. By using a highly specific antibody, we studied the localization of PICALM in the temporal cortex (BA21), prefrontal cortex (BA10) and hippocampus across different stages of AD. Although recent studies suggest the downregulation of PICALM in AD disease progression, localization of PICALM in the present study does not support the earlier observations. At Braak stage I, most PICALM positive endothelial cells are present in the surface layer of the cortex and terminals were seen in the meningeal membrane; however, most of the axonal varicosities as well terminals were seen in the polymorphic layer of the hippocampus. At Braak stage III, a moderate number of PICALM positive terminals were still present in the cortex and, at Braak stage VI, very few PICALM positive terminals were seen in the cortex and hippocampus. PICALM positive endothelial cells are present in early Braak stages (I-III); however, this level increased across higher Braak stages. PICALM positive pre-tangles started to appear in Braak stages III-IV, but they are limited in the BA21 and BA10. However, most of the ghost tangles and flamed tangles that are PICALM can only be seen in the hippocampus at Braak stages V-VI. PICALM colocalized with phospho-Tau that were specific for either paired helical factor of phosphorylated Tau. Western-blot data also revealed that there is significant upregulation of PICALM expression in the hippocampus of AD patients compared to control. Additional studies correlating the expression of PICALM in the endothelium and brains and genotype of patients from control and AD patients would be useful to determine how genotypes such as APOE and other vascular risk factors can influence the progression of AD.

Disclosures: S. Sarkar: None. **Q. Gu:** None. **J. Raymick:** None. **E. Cuevas:** None. **H. Rosas-Hernandez:** None.

Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.04/D36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG15379
NIH Grant AG44486

Title: Linking gamma-secretase and glutamate homeostasis: Presenilin-1 and glutamate transporter GLT-1 interaction

Authors: *L. C. ANDERSON[1], M. MAESAKO[1], N. SEKULA[1], C. ZHANG[1], S. L. WAGNER[2,3], R. E. TANZI[1], O. BEREZOVSKA[1];
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Abstract: We recently discovered an unknown interaction between Presenilin 1 (PS1), a catalytic subunit of γ-secretase responsible for the generation of amyloid-β (Aβ) peptides, and GLT-1, a major glutamate transporter in the brain (a.k.a. EAAT2). This PS1/GLT-1 interaction may be the critical link between two major pathological aspects of Alzheimer's disease (AD): abnormal Aβ occurrence and neuronal network hyperactivity. We previously verified that PS1 and GLT-1 interact both *in vitro* and *in vivo* and that the interaction is not impacted by treatment with γ-secretase inhibitors. Here, we investigate the effect of PS1 conformation and familial AD mutations on the PS1/GLT-1 interaction. First, we employ a FRET-based assay, fluorescence lifetime imaging microscopy (FLIM), to characterize the PS1/GLT-1 interaction in the brain tissue of sporadic AD patients and control individuals (Experiment 1). Next, to identify possible modulators of the PS1/GLT-1 interaction, we use FLIM to explore the effect of gamma-secretase modulators (GSMs; Experiment 2) and of familial AD PS1 mutations (Experiment 3) in transiently transfected CHO and HEK PS1 and PS12 double knockout cells. Our findings indicate that there is significantly less interaction between PS1 and GLT-1 in sporadic AD brains, as measured by a decrease in FRET efficiency, compared to tissue of non-demented controls and Frontotemporal dementia (FTD) controls. Interestingly, treatment with PS1 conformation modulators (sGSM15606 and sGSM36), increase the PS1/GLT-1 interaction, as measured by an increase in FRET efficiency. Furthermore, pathological familial AD mutations in the PS1 protein, specifically Δ9, L166P and G384A, reduce PS1's binding to GLT-1. Of note, non-pathogenic mutation E318G had similar FRET efficiency levels as the wildtype PS1 protein. The current results suggest the PS1/GLT-1 interaction is disrupted in sporadic AD and is affected by PS1 conformational changes.

Disclosures: L.C. Anderson: None. **M. Maesako:** None. **N. Sekula:** None. **C. Zhang:** None. **S.L. Wagner:** None. **R.E. Tanzi:** None. **O. Berezovska:** None.

Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.05/D37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PAPIIT IN203616
CONACyT CB-255399

Title: Effect in weight, cogntion and serum glucose after the administration of intracerebroventricular streptozotocin injections during a 60 days period in CD1 mice. A model for sporadic Alzheimer's disease

Authors: *C. G. ESTRADA-GOMEZ, P. C. BELLO-MEDINA, S. DIAZ-CINTRA; Neurobiologia del Desarrollo y Neurofisiologia, Inst. De Neurobiologia. UNAM, Queretaro, Mexico

Abstract: Since diabetes has a factor which duplicates the risk of appearance of Alzheimer's disease (AD), however this mechanism remains unknown. In addition, the theory called "diabetes type 3" refers to an insulin resistance confined to the brain and that can be induced with the administration of intracerebroventricular (icv) streptozotocin (STZ). The aim of this study is to contribute to the characterization of the sporadic model of AD in male mice CD-1 of 8 weeks old. In order to evaluate it, we measured the changes in weigh, spatial memory and serum glucose during a 60 days period after an icv injection of STZ. These mice were housed randomly in an inverted cycle room, followed by a stereotaxic surgery. We injected (icv) 5 µl of artificial cerebrospinal fluid (vehicle group (VEH), n=30) or added STZ at a dose of 3 mg/kg (experimental group, n=30). The animals were let grow until 15, 30 and 60 days of age. During these times, all mice were weighed two times per week. When the time was fulfilled, all the animals were tested in the Morris Water Maze (filled with water at 22 ±1 °C) with a non-visible escape platform. In acquisition phase (8 trials/1 day), mice were given up to 60s per trial to find the platform, remained on the platform for 20s and then were placed in a holding cage for 20s until the next trial. Twenty-four hours later, retention test was conducted with one trial in the pool without the platform and starting in the first liberation point. On the next day, animals were sacrificed by cervical dislocation and its serum glucose was measured with a glucometer. We found in the animals that received STZ in three periods (15, 30 and 60 days) a decreased in their weights compared to the VEH groups. The STZ group showed a learning deficit in the Morris Water Maze task compared to the VEH groups. We didn't find significant differences between the serum glucose of VEH and STZ groups at the end of each period (15, 30 and 60 days), this finding is similar with other reports in which an icv injection of STZ did not pass the blood brain barrier because it does not alter the systemic metabolism. Thus, we found a model that resembles the cognitive deficit found in the AD. Also, the changes in weight as well as serum glucose help to understand that the insulin resistance effect by STZ occurs every time this drug is administrated directly in the brain due to it does not cross the blood-brain barrier. We thank to Azucena Aguilar Vazquez for her technical assistance. The present work was supported by PAPIIT (IN203616) and CONACyt (CB-255399)

Disclosures: C.G. Estrada-Gomez: None. **P.C. Bello-Medina:** None. **S. Diaz-Cintra:** None.

Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.06/D38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG055581
NIH Grant R01 AG056622 (T.M.)
Alzheimer's Association Grant NIRG-15-362799 (T.M.)
BrightFocus Foundation Grant A2017457S

Title: Characterizing Alzheimer's disease pathology and molecular signaling dysregulation in aged type 2 diabetic cynomolgus monkeys

Authors: *H. M. JESTER[1], S. GOSRANI[1], H. DING[2], M.-C. KO[2], H. ZIMMERMANN[3], X. WANG[3], X. ZHOU[3], T. MA[3];
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Abstract: Mounting evidence indicates type 2 diabetes mellitus (T2DM) as a risk factor for Alzheimer's disease (AD), the most common form of dementia syndromes. Consistently, multiple lines of studies suggest that T2DM and AD may share several pathophysiological mechanisms at the cellular/molecular level. It has been established that maintenance of long-term memory and synaptic plasticity requires *de novo* protein synthesis, and recent studies indicate AD-associated impairments in protein synthesis. Here we sought to explore AD-associated brain pathology and molecular signaling in aged (14-29-year-old), naturally occurring T2DM cynomolgus monkeys. We examined the prefrontal cortex, hippocampus, and cerebellum using western blot, ELISA, and immunohistochemistry. Our preliminary findings indicate that diabetic monkeys exhibited an increase in phosphorylated tau, compared to age-matched controls. Additionally, we observed in diabetic monkeys decreased phosphorylation of AMP-activated protein kinase (AMPK), a central molecular sensor to regulate energy metabolism. In agreement with recent studies in AD, T2DM monkeys also exhibited elevated phosphorylation of eukaryotic elongation factor 2 (eEF2), indicating repression of general protein synthesis. Taken together, our data provide further evidence to support a relationship between T2DM and AD-associated dementia syndromes.

Disclosures: H.M. Jester: None. **S. Gosrani:** None. **H. Ding:** None. **M. Ko:** None. **T. Ma:** None.

Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.07/D39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RGC Grant AoE/M-604/16 (Prof. Ip)
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HMRF Grant 05163736
Australia Grant NHMRC-APP1160691

Title: Full length APP regulates the size and cellular location of the axon initial segment with implications for Alzheimer's disease

Authors: *F. MA, K. HERRUP;
Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong

Abstract: The axon initial segment (AIS) is a specialized neuronal structure that is strategically located between the somatodendritic and axonal domains of a neuron where it serves as a barrier to membrane and protein trafficking. The AIS also serves as the initiation site for an action potential with its size and distance from the soma serving to dramatically alter action potential firing. These essential functions make the AIS a vulnerable feature of any neural network. Indeed, disruption of AIS structural integrity is closely connected to cognitive dysfunction in many neurodegenerative diseases. In the current study we probe the response of the AIS to the progression of Alzheimer's disease (AD). We examined the R1.40 transgenic mouse model of AD in which the entire human APP gene, with the Swedish mutation (APP_{Swe}), is inserted into the mouse genome. We found that in R1.40 neocortex and hippocampus the density AIS profiles (seen by immunostaining with ankyrin G or β4-spectrin) was reduced as was AIS length. Similarly, in primary cultures, R1.40 neurons had axons with shorter AIS located at an increased distance from the cell body. This is in part a developmental problem as the appearance of the AIS of R1.40 neurons in culture is delayed. To confirm that this was a direct effect of APP itself, we showed that overexpression of APP or APP_{Swe} in wild type neurons also reduced AIS length; the reduction was greater with APP_{Swe} than with wild type. By contrast, treatment with fibrillar Aβ had no effect on AIS properties. These effects appear to be partly calcium mediated. Blocking the calcium-dependent protease, calpain, improved but did not fully rescue the AIS phenotype following APP overexpression. To further explore the mechanistic basis of the APP/AIS interaction we showed with immunocytochemistry that APP localizes to the proximal end of the AIS. We also found that APP and Ankyrin G can be co-immunoprecipitated with each other from mouse brain (gray or white matter). Taken together our findings suggest that rather than merely being a source of Aβ, the APP holoprotein potentially fine tunes neuronal activity by dynamically regulating the length and position of the AIS. The implication is that in AD APP increases as neuronal damage increases dampening neuronal responsiveness. Finally, the AIS changes become irreversible and the resulting neuronal malfunctions contribute to the neurological symptoms of Alzheimer's.

Disclosures: F. Ma: None. **K. Herrup:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); the State Key Laboratory of Molecular Neuroscience.

Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.08/D40

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1 6934799
NIH R01 NS102730
Glenn Foundation for Medical Research

Title: Neuronal DNA double strand breaks in the CK-p25 mouse model of severe neurodegeneration

Authors: *G. M. WELCH[1], J. D. CHENG[1], Q. SU[2], M. KELLIS[1], A. PFENNING[2], L.-H. TSAI[1];
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Abstract: Failure to repair lesions in DNA is associated with neurodegenerative phenotypes. A particularly hazardous form of DNA damage is the DNA double strand break (DSB), which at pathological levels can induce cytotoxicity. The transcriptomic and epigenetic effects of DSBs in mature post-mitotic neurons in the context of neurodegenerative disease is currently undefined. We have previously shown increased levels of DSBs in neurons of the CK-p25 mouse model of severe neurodegeneration (Kim et al., Neuron, 2008). Presence of DSBs is concomitant with onset of an inflammatory response in microglia and precedes neuron cell death and cognitive decline in these mice (Fischer et al., Neuron, 2005; Cruz et al., Mathys et al., Cell Reports, 2017). In the present study, we hypothesized neuronal DSBs contribute to CK-p25 neurodegeneration. To address this hypothesis, we proposed this contribution could be traced through transcriptomic and epigenomic changes in neurons bearing the largest amount of DSBs. We used fluorescence-activated nuclei sorting (FANS) to isolate populations of neurons with high levels of γH2AX, a robust marker for DSBs, from the adult CK-p25 cortex. RNA sequencing of these nuclei revealed "γH2AX-positive" neurons show differential expression of a number of genes related the DNA damage response compared to "γH2AX-negative" neurons. To assess changes in chromatin associated with DSBs, we performed H3K27ac chromatin immunoprecipitation (ChIP) followed by next-generation sequencing. Differential analysis of these H3K27ac peaks revealed enrichment of peaks associated with p53 activity, a critical mediator of the DNA damage response, in γH2AX-positive neurons. Fluorescent in-situ hybridization and western blot analysis were used to confirm differential expression of genes of interest at the mRNA and protein level. We propose increased DNA damage response and p53 activity precede the onset of apoptotic activity in these cells. Indeed, γH2AX signal in the CK-

p25 cortex co-localizes with pyknotic nuclei by the time neuronal death and cognitive decline are observed (Kim et al., 2008). Combined, these data characterize a specific population of neurons with high levels of DSBs, and demonstrate a role for DSBs in the initiation of a neurodegenerative phenotype.

Disclosures: **G.M. Welch:** None. **J.D. Cheng:** None. **Q. Su:** None. **M. Kellis:** None. **A. Pfenning:** None. **L. Tsai:** None.

Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.09/D41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG017139
NIH Grant K02AG050767

Title: HCN channelopathy in the ventral hippocampus of Alzheimer's disease mouse models

Authors: ***A. ROGALSKY**[1], T. F. MUSIAL[1], E. MOLINA-CAMPOS[1], N. YBARRA[1], L. A. BEAN[1], Y. VOSKOBIYNYK[1], M. L. RUSSO[1], M. M. OH[2], R. J. VASSAR[2], J. F. DISTERHOFT[2], D. A. NICHOLSON[1];
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Abstract: Background
Alzheimer’s disease (AD) is marked by the accumulation of Amyloid beta peptides, neuronal cell death, and synapse loss. However, dysregulation of voltage gated ion channels may also contribute to AD progression. Our lab has shown that in the dorsal hippocampus of transgenic mouse models of AD, CA1 pyramidal neurons exhibit a channelopathy in which hyperpolarization-activated cyclic-nucleotide gated (HCN) channels are mislocalized. However, magnetic resonance imaging (MRI) studies have shown that the anterior hippocampus, corresponding to the ventral hippocampus of rodents, atrophies at a faster rate than the posterior. Additionally, the ventral hippocampus in AD mouse models appears to have more aggressive plaque pathology than the dorsal portion. Therefore, it is important to investigate the possibility that the HCN channelopathy observed in the dorsal hippocampus is also present in the ventral hippocampus.
Methods
Both 3xTg and 5xFAD mouse models were used. Immunogold electron microscopy was used to assess the relative quantity and location of HCN channels within neurons. And whole cell patch clamp was used to measure accommodation, sag, and rebound slope.
Results

Similar to the dorsal hippocampus, the ventral hippocampus exhibits altered physiology that suggests mixed HCN channelopathy. There was little to no evidence of exacerbated accommodation in the ventral hippocampus. However the transgenic mouse neurons showed both gain and loss of function indicated by altered sags and rebound slopes.
Preliminary experiments indicate that ventral HCN channels are mislocalized similarly to dorsal channels in transgenic mouse models of AD.
Conclusion
Besides the lack of exacerbated accommodation, the ventral and dorsal hippocampi exhibit similar patterns of channelopathy. Additionally, it appears likely that the measurably altered physiology from the wild type mice is due to mislocalization of HCN channel proteins.

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Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.10/D42

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH R01 AG058778

Title: Single particle tracking of functional mutants of PERK to understand cell specific activation mechanism under stress

Authors: *H. GUDLAVALLETI, R. VISWANATHAN, B. STOVEKEN, D. HOLSTEIN, P. MOZOLEWSKI, R. DOBROWOLSKI, J. LECHLEITER;
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Abstract: PKR like endoplasmic reticulum kinase (PERK) is a transmembrane protein in the ER that phosphorylates elf2α under cellular stress and inhibits protein translation. Neurodegenerative diseases that have been associated with the accumulation of unfolded proteins in brain cells have been shown to activate PERK mediated translational control. It has been proposed that the PERK luminal domain senses the misfolded proteins and it undergoes dimerization and oligomerization to activate PERK via autophosphorylation on its cytosolic domain. However, a detailed molecular understanding of PERK activation is unknown. By tagging PERK with a photoconvertible fluorescent protein (FC-FP) like mEos3.2, we can measure the diffusion rates and trajectories of PERK in a cell under normal physiological conditions and under stressed conditions using single particle super resolution microscopy. Our working hypothesis is that the diffusion rates decrease as PERK molecules form multimeric complexes with each other or with

other proteins under ER stress.
Our strategy to test this hypothesis is to introduce mutations in luminal domain of PERK to prevent oligomerization or in the cytosolic domain to inhibit kinase activity. All PERK variants will be fused with the FC-FP mEos3.2 for sptPALM and expressed at near endogenous levels in PERK knock out mouse embryonic fibroblasts (MEFs). In preliminary experiments, single particle tracking of PERK in MEFs revealed an average diffusion constant of 8.68 +/- 3.95 um/sec. After thapsigargin treatment (1 uM for 10 minutes), the diffusion rate of PERK was reduced to 6.83 +/- 2.76 um/sec, consistent with our hypothesis. Data were obtained from 2223 counts in 3 cells. Our plan is to use a similar strategy in human pluripotent stem cells (iPSCs) to test whether PERK activation differs in iPSC derived neurons and astrocytes.

Disclosures: H. Gudlavalleti: None. **R. Viswanathan:** None. **B. Stoveken:** None. **D. Holstein:** None. **P. Mozolewski:** None. **R. Dobrowolski:** None. **J. Lechleiter:** None.

Poster

378. Neurodegenerative Disorders and Injury II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 378.11/D43

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RO1 AG 058778

Title: Using super-resolution microscopy to study the role of PERK in neurodegenerative disorders

Authors: *R. VISWANATHAN[1], H. GUDLAVALLETI[1], D. HOLSTEIN[2], B. STOVEKEN[2], P. MOZOLEWSKI[2,3], R. DOBROWOLSKI[2,4], J. LECHLEITER[2];
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Abstract: Misfolded proteins are a common phenotype of many neurodegenerative diseases like Alzheimer’s, Frontotemporal dementia, and Progressive supranuclear palsy. When misfolded proteins accumulate in the endoplasmic reticulum (ER), cells respond to this stress through activation of the unfolded protein response (UPR). The UPR stress-sensor, PERK, is activated in the brains of patients with neurodegenerative tauopathies, and evidence indicates that both positive and negative modulation of its activity may be therapeutic. However, the exact mechanism of PERK activation and how it contributes to neurodegenerative diseases is poorly understood. It has been proposed that PERK activation requires assembly into homo-oligomeric complexes, but evidence of these structures *in situ* is scarce. Super-resolution optical techniques like photoactivated localization microscopy (PALM) and Stochastic optical reconstruction

microscopy (STORM) enable us to visualize and track PERK with nanometer resolution inside the cell and to better understand its basic biology and role in disease. However, the accuracy of PALM is limited by the detection efficiency of fluorescent proteins (e.g. the fraction of fluorescently-tagged PERK molecules detected in an experiment). We hypothesized that detection efficiency may be reduced by chemical fixatives such as formaldehyde and glutaraldehyde, which are commonly employed in PALM experiments. The goal of our study is to measure PERK at near-endogenous expression levels and to map its location throughout the ER with combined PALM/STORM.
Our first goal was to optimize fixative conditions required for STORM as this impacts the photo-convertible fluorescent protein (PC-FPs) efficiency and counting accuracy with PALM. Through fluorescence activated cell sorting (FACS), we have obtained data on the impact of fixation on the fluorescence of various PC-FPs. This information should enable us to optimized conditions to do concurrent PALM/STORM microscopy, which in turn, will permit us to measure and track the movement and diffusion rates of wild-type PERK in the ER in different cell systems, including undifferentiated IPSCs, differentiated neurons and astrocytes. Ultimately, this information on PERK dynamics will help us understand its mechanism of activation in both neurons and astrocytes as well as its dysregulation in neurodegenerative disorders.

Disclosures: R. Viswanathan: None. **H. Gudlavalleti:** None. **D. Holstein:** None. **B. Stoveken:** None. **P. Mozolewski:** None. **R. Dobrowolski:** None. **J. Lechleiter:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.01/D44

Topic: C.03. Parkinson’s Disease

Support: The Perry & Ruby Stevens Parkinson's Disease Center of Excellence Award
NIH Grant HL138093-01

Title: Role of parkin-mitofilin interaction in Parkinson’s disease stressor-induced neuron death

Authors: *A. IMAM ALIAGAN, M. DARAEI AHWAZI, N. MADUNGWE, L. LIU, N. TOMBO, Y. FENG, J. C. BOPASSA;
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Abstract: Parkinson’s disease (PD) is a neurodegenerative disease characterized by a gradual and preferential loss of dopaminergic neurons in the brain (substantia nigra). Genetic mutations or environmental toxins and drugs (PD) stressors have been found to cause mitochondrial dysfunction and parkin has been identified as a protein playing a critical role in this mechanism. However, the exact mitochondrial-dependent mechanisms resulting in PD stressor-induced

dopaminergic neuron death observed in PD remains elusive. Recently, we have shown that knockdown of the inner mitochondrial membrane (IMM) protein; mitofilin, which controls mitochondrial cristae morphology results in increased cell death in H9c2 myoblasts. In this work, we hypothesize that preferential dopaminergic neuron death induced by PD stressors might be initiated by the reduction in mitofilin protein level through parkin-mediated ubiquitination. Using N27-A+ dopaminergic neurons, we found that treatment with PD stressors (rotenone, oxidized dopamine (DA) and MPP+) led to: i) increased translocation of parkin into mitochondria where it ubiquitinates mitofilin and causes its degradation, ii) reduced mitochondrial membrane potential, increased oxidative stress (ROS), which is associated with abnormal mitochondrial structure and dysfunction as well as increased ER stress, iii) increased neuron apoptosis via AIF-PARP cleavage pathway. We identified the lysine residue of mitofilin that is ubiquitinated by parkin in response to PD stressors activation, and found that this ubiquitination is prevented when K◊R mutated mitofilin was expressed. Conversely, overexpressing the mitochondrial deubiquitinase, USP30, attenuated mitofilin loss induced by PD stressors leading to an increased survival of N27-A+ cells. In male wild type mice eliciting PD phenotype in response to rotenone, MPTP, or 6-OHDA unilateral administration, we found a remarkable loss of mitofilin, effect that was not present in male parkin knockout mice. Together, these results indicate that parkin translocates into mitochondria in response to PD stressors to promote the ubiquitination of mitofilin and its loss in the IMM resulting in mitochondrial structural damage and dysfunction that causes cell death via AIF-PARP pathway. Our results provide a better understanding of mitochondrial-dependent mechanism of neuronal death observed in PD and a novel potential therapeutic target in cases where mitochondrial dysfunction is implicated.

Disclosures: A. Imam Aliagan: None. **M. Daraei Ahwazi:** None. **N. Madungwe:** None. **L. Liu:** None. **N. Tombo:** None. **Y. Feng:** None. **J.C. Bopassa:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.02/D45

Topic: C.03. Parkinson’s Disease

Title: The adaptive defense mechanism associated with HFE genotype and its association with the vulnerability to paraquat-induced toxicity

Authors: *I. SONG, E. NEELY, A. SNYDER, S. LEE, J. CONNOR;
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Abstract: Parkinson’s disease, which over 10 million people suffer from worldwide, is characterized by motor impairment including bradykinesia. Past studies have identified the link between environmental toxins and mitochondrial dysfunction in Parkinson’s disease. Recently,

our laboratory reported protection from paraquat-induced motor impairment and cellular changes in HFE (H67D) mutant mice. The HFE protein regulates iron uptake by interacting with the transferrin receptor. Based on our previous study, we hypothesize that an antioxidant defense system, such as the Nrf2 pathway, is elevated in association with the HFE mutation. Because the Nrf2 pathway is preferably activated in astrocytes, we hypothesize that the HFE mutant astrocytes are responsible for the proposed neuroprotection. We performed immunostaining and immunoblotting on primary astrocytes in cell culture that revealed a visible increase in both Nrf2 and L-ferritin staining in the HFE mutant astrocytes prior to paraquat treatment. Moreover, we have found that the expression of Keap1, which is a negative regulator of Nrf2, was reduced in the HFE mutant astrocytes. Based on this data, we examined the difference in vulnerability to paraquat toxicity in association with HFE genotype. As we hypothesized, paraquat treatment induced a mitochondrial damage in the WT astrocytes only as shown by the changes in mitochondrial mass and loss of mitochondrial membrane potential. Furthermore, there was a significant increase in the astrocytic senescence marker ß-galactosidase in the WT after paraquat treatment, compared to that of H67D astrocytes. To further elucidate the role of HFE mutant astrocytes in neuroprotection against paraquat-induced toxicity, we have developed a SH-SY5Y neuroblastoma model that is stably transfected with WT or H63D HFE. With this model, we conducted a conditioned media experiment, in which the astrocytes were treated with a sublethal dose of paraquat and then the conditioned media from the paraquat treated astrocytes was added to the WT SH-SY5Y cells. A significant decrease in the viability of the WT SH-SY5Y cells was observed after treatment with WT astrocyte conditioned media in a dose-dependent manner but not with the H67D astrocyte conditioned media. By performing cytokine array, we have found that there was a significantly larger increase in IL-6 secretion in the WT astrocyte conditioned media compared to the H67D astrocyte conditioned media. The results of our study suggest that there is a difference in vulnerability to paraquat toxicity associated with HFE genotype, possibly due to a higher baseline activation of the antioxidant defense system in the HFE mutant astrocytes.

Disclosures: **I. Song:** None. **E. Neely:** None. **A. Snyder:** None. **S. Lee:** None. **J. Connor:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

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Program #/Poster #: 379.03/D46

Topic: C.03. Parkinson’s Disease

Support: USDPH NIH R21ES025920
the Center for Compulsive Behavior & Addiction

Title: Striatal alterations in mitochondrial function and α-synuclein in a rat model of methamphetamine-induced risk for Parkinson's disease

Authors: *I. CALMA, A. L. PERSONS, T. NAPIER;
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Abstract: Methamphetamine (meth) abusers have a greater risk to develop Parkinson's disease (PD) later in life than non-abusers. PD is characterized by motor disturbances, degeneration of nigrostriatal dopaminergic neurons, and emergence of α-synuclein rich protein aggregates. In meth self-administering (SA) male Sprague Dawley rats, we revealed an abstinence time-dependent bradykinesia that co-varied with reduced striatal tyrosine hydroxylase (TH) and mitochondrial electron transport proteins (ETP), increased mitochondrial membrane permeability, and decreased number of nigral TH+ neurons. These findings indicated that mitochondrial dysfunction is involved in protracted PD-like pathology initiated by meth abuse. To study this concept, we used osmotic minipumps to administer after meth SA a mitochondrial complex I inhibitor that can produce PD, rotenone. We determined that a mild treatment (1mg/kg/day X 6 days), which had no behavioral effects on its own, was able to exacerbate meth SA-induced bradykinesia, striatal TH loss and mitochondrial dysfunction. Here we extend these findings by evaluating the ETP, complex I, and α-synuclein, which normally binds to, and promotes complex I function. We tested four treatment groups: meth+rotenone (MR), meth+vehicle (MV), saline-yoked+rotenone (YR) and saline-yoked+vehicle (YV). Striatal tissues were collected 60 days after the SA task (40 days after rotenone). Complex I activity, measured with a colorimetric assay of the conversion of NADH to NAD, was significantly decreased in MR rats ($p<0.01$); activity in YV and MV rats was unchanged and YR was slightly (non-significantly) reduced. The ability of meth to potentiate the effects of rotenone on complex I activity implicates this enzyme in the persistent, pathogenic effects of meth. Optical density of immunohistochemical staining for striatal endogenous α-synuclein revealed a main ANOVA effect for rotenone ($p<0.05$), but this largely reflected a significant reduction in α-synuclein in MV rats. Thus, the proclivity of meth SA to promote PD-like pathology may involve striatal complex I processes that compensate for a meth-induced decrease α-synuclein, but succumb to the stress imposed by a subsequent exposure to even subthreshold doses of rotenone.

Disclosures: I. Calma: None. **A.L. Persons:** None. **T. Napier:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.04/E1

Topic: C.03. Parkinson's Disease

Support: F32 AG058396

JPB Foundation
NS 047085

Title: Estradiol lowers axonal mitochondrial oxidant stress in dopaminergic neurons

Authors: *K. STOUT[1], S. M. GRAVES[2], J. KONDAPALLI[1], D. SURMEIER[3];
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Abstract: Parkinson's disease (PD) is the most common neurodegenerative movement disorder, with cardinal motor symptoms arising from the progressive degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). PD risk is strongly affected by sex, as men have increased risk compared to women. How sex alters dopaminergic vulnerability is not currently known, but evidence suggests that the phenomenon is estradiol-dependent.
Previous work has shown that in dopaminergic neurons mitochondrial oxidative phosphorylation (OXPHOS) is coupled to activity in two ways. First, spiking increases the entry of Ca^{2+} into the mitochondrial matrix, accelerating OXPHOS. Second, activity-dependent elevation in cytosolic dopamine stimulates OXPHOS through mitochondrially anchored monoamine oxidase (MAO). While these measures ensure sustained activity and dopamine release in the short-term, both mechanisms increase mitochondrial oxidant stress, resulting in mitochondrial damage and increased vulnerability to other risk factors associated with PD, like age.
We hypothesized that estradiol diminishes mitochondrial oxidant stress, resulting in neuronal resilience. Indeed, axonal mitochondria in SNc DA neurons from intact female mice had lower oxidant stress than those from male or ovariectomized (OVX) female mice. Further, acute application of 17β-estradiol lowered axonal mitochondrial oxidant stress; this effect was eliminated by the estrogen receptor inhibitor, 4-hydroxytamoxifen. While 17β-estradiol did not affect cytosolic Ca^{2+} concentration in axons, it reduced mitochondrial Ca^{2+} entry in OVX female mice. We tested the functional consequences of these changes by measuring dopamine release. 17β-estradiol increased dopamine release by 50% in both male and female mice. To tax energy reserves, we used a 0.1 Hz stimulation train; this frequency causes a progressive drop in dopamine release, due in part to depletion of ATP. 17β-estradiol exaggerated this drop dopamine release with repetitive stimulation. Thus, our working hypothesis is that 17β-estradiol lowers axonal mitochondrial oxidant stress (and increases resilience) in SNc DA neurons by diminishing mitochondrial Ca^{2+} entry. Currently, we are exploring the possibility that 17β-estradiol also controls MAO activity.

Disclosures: K. Stout: None. **S.M. Graves:** None. **J. Kondapalli:** None. **D. Surmeier:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.05/E2

Topic: C.03. Parkinson's Disease

Support: NINDS Grant P50NS047085
JPB Foundation
MJF Foundation
IDP Foundation

Title: Spiking drives mitochondrial respiration through a calcium-dependent, anticipatory control system in substantia nigra dopaminergic neurons

Authors: *E. ZAMPESE[1], J. KONDAPALLI[1], D. L. WOKOSIN[1], D. SURMEIER[2];
[1]Physiol., Northwestern Univ., Chicago, IL; [2]Prof. Dept. Physiology/ NUIN, Northwestern Univ. Dept. of Physiol., Chicago, IL

Abstract: Mitochondrial dysfunction is widely thought to be a primary factor underlying the selective vulnerability of substantia nigra (SN) dopaminergic neurons in Parkinson's disease (PD). This dysfunction has been linked to sustained mitochondrial oxidant stress in SN dopaminergic neurons. Although the opening of plasma membrane Cav1 (L-type) Ca^{2+} channels is known to be necessary for mitochondrial to manifest oxidant stress, precisely how they are coupled to mitochondria is unclear.
To answer this question, electrophysiological and optical approaches were used to interrogate SN dopaminergic neurons in ex vivo brain slices from mice expressing genetically encoded sensors targeted to specific sub-cellular locations. These studies revealed that opening of plasma membrane Cav1 Ca^{2+} channels triggers a cascade of intracellular events leading to entry of Ca^{2+} into the mitochondrial matrix. A key step in this cascade is Ca^{2+}-induced Ca^{2+} release (CICR) from the endoplasmic reticulum that is mediated by ryanodine receptors (RYRs). CICR results in flux of Ca^{2+} through the mitochondrial Ca^{2+} uniporter (MCU) into the matrix, where it stimulates oxidative phosphorylation (OXPHOS). Stimulation of OXPHOS results in elevated production of reactive oxygen species and engagement of oxidant defenses in SN dopaminergic neurons.
A fundamental question is why this mitochondrial control mechanism is in place. Our working hypothesis is that the proteostatic burden created by their enormous axonal arbor and their sustained regenerative (spiking) activity poses a substantial bioenergetic challenge to SN dopaminergic neurons. To meet this challenge without compromising cellular function, SN dopaminergic neurons must anticipate bioenergetic demand, rather than rely upon feedback mechanisms that would allow cytosolic adenosine triphosphate (ATP) levels to drop. One anticipatory signal that would predict bioenergetic demand associated with transmitter release

and maintenance of ionic gradients is the spike. Indeed, spike rate was linearly related to mitochondrial matrix Ca^{2+} levels. Moreover, the slope of this relationship was dependent upon sub-cellular region, suggesting it was tuned to local bioenergetic demand.
Taken together, our results suggest that mitochondrial oxidant stress in SN dopaminergic neurons is a deleterious by-product of an adaptive, anticipatory bioenergetic control mechanism designed to meet the needs posed by their extraordinary design and role in basal ganglia function.

Disclosures: E. Zampese: None. **J. Kondapalli:** None. **D.L. Wokosin:** None. **D. Surmeier:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.06/E3

Topic: C.03. Parkinson’s Disease

Support: KBRI basic research program (ICT 19-BR-01-08)

Title: Analysis of mitochondria and endoplasmic reticulum contacts by genetically encoded tag and volume electron microscopy in neuron cell

Authors: *M. JUNG, J. MUN;
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Abstract: Cellular organelles, such as mitochondria and endoplasmic reticulum (ER), create a network to perform a variety of functions. Mitochondria and ER contact sites are very dynamic and specialized protein enriched region that determines their structure and functions. The contact sites play important roles in essential biological regulation of exchange of calcium (Ca^{2+}), lipids transfer between both organelles, autophagosome formation, and mitochondrial fission. Recently, many research has shown that disturbances to mitochondria and ER contacts occur in neurodegenerative diseases like as Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis. Visualization of this network between mitochondria and ER has been attempted using super-resolution fluorescence imaging and light microscopy; however, the limited resolution is insufficient to observe the membranes between the mitochondria and ER in detail. Transmission electron microscopy provides good membrane contrast and nanometer-scale resolution for the observation of cellular organelles. However, it is very difficult to distinguish between fragmented ER and other membrane structures (e.g. Golgi structure or vesicles) in electron micrographs. In addition, these highly curved cellular organelles are not easy to analyze because they exist in a three-dimensional structure throughout the cell. Therefore, we observed the morphology of mitochondria and ER via correlative light-electron microscopy (CLEM) and

volume electron microscopy techniques using enhanced ascorbate peroxidase 2 (APEX2) and horseradish peroxidase (HRP) staining. An en bloc staining method, ultrathin serial sectioning (array tomography), and volume electron microscopy were applied to observe the 3D structure. In this study, we suggest a combination of CLEM and 3D electron microscopy to perform detailed structural studies of mitochondria and ER in a whole neuronal cell.

Disclosures: M. Jung: None. **J. Mun:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.07/E4

Topic: C.03. Parkinson's Disease

Support: NIH (NS081746)

Title: Parkinson's disease protein DJ-1 interacts with f1fo ATP synthase to regulate protein synthesis

Authors: *R. CHEN[1], P. LICZNERSKI[1], M. GRAHAM[1], G. COSSU[2], W. MANDEMAKERS[3], V. BONIFATI[3], E. A. JONAS[1];
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). DJ-1 is a peptidase C56 family protein with known and uncharacterized cellular functions, mutations of which are linked to early onset familial PD. Skin fibroblasts are a good *in vitro* model to study the early pathological alterations present in the neurodegenerative patients' brains. Mitochondrial dysfunction is a hallmark of the development of PD. In this context, we studied mitochondrial functional properties (morphology and bioenergetics) in fibroblasts obtained from two different DJ1 PD patients and one aged-match healthy individual. We observed that the average mitochondrial electron density in the PD fibroblast cells that lack DJ-1 (Pt 1) is greater (or darker) than that in the normal mitochondria or in cells containing mutant DJ-1 (Pt 2). Both sets of PD patient fibroblast mitochondria carry dramatically low number of cristae, and most of these cristae are in an abnormal shape. Pt1 fibroblasts presented a significant reduction in mitochondrial length and an increased number of total mitochondria per measured area. Pt2 PD fibroblasts showed decreased proliferation, and the mitochondrial ATP synthase beta-subunit protein level was dramatically decreased in these cells, consistent with our previous studies showing a large inner mitochondrial membrane leak in DJ-1 KO neuronal mitochondria. In addition, we also noticed that Pt2 fibroblasts have much lower overall protein synthesis rates,

which were improved by overexpression of the ATP synthase β subunit, presumably because this enhanced mitochondrial inner membrane coupling. Attempting to close the leak with pharmacological reagents CsA, or Dex, however, was unsuccessful at rescuing the level of β subunit protein level itself in mitochondria, although the effect on overall rates of protein synthesis of these reagents has not yet been ascertained. Complementary to these changes, both PD fibroblasts showed dysfunctional mitochondrial bioenergetic profiles. This also differentiated these cells from aged-matched healthy control fibroblasts. These observations suggest a connection between ATP synthase complex stoichiometry, protein synthesis rate and PD. These findings comprise a promising new strategy to develop therapies for PD patients.

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Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.08/E5

Topic: C.03. Parkinson’s Disease

Support: NRF-2017M3A9G8084464
KGM5281921
KGM4621922
KGM4561912

Title: Abnormal mitochondria in a non-human primate model of MPTP-induced Parkinson’s disease: Drp1 and CDK5/p25 signaling

Authors: *J. PARK;
Natl. Primate Res. Ctr., Korea Res. Inst. of Biosci. & Biotechno, Cheongju, Korea, Republic of

Abstract: Mitochondria continuously fuse and divide to maintain homeostasis. An impairment in the balance between the fusion and fission processes can trigger mitochondrial dysfunction. Accumulating evidence suggests that mitochondrial dysfunction is related to neurodegenerative diseases such as Parkinson’s disease (PD), with excessive mitochondrial fission in dopaminergic neurons being one of the pathological mechanisms of PD. Here, we investigated the balance between mitochondrial fusion and fission in the substantia nigra of a non-human primate model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD. We found that MPTP induced shorter and abnormally distributed mitochondria. This phenomenon was accompanied by the activation of dynamin-related protein 1 (Drp1), a mitochondrial fission protein, through increased phosphorylation at S616. Thereafter, we assessed for activation of the components of the cyclin-dependent kinase 5 (CDK5) and extracellular signal-regulated kinase (ERK) signaling

cascades, which are known regulators of Drp1(S616) phosphorylation. MPTP induced an increase in p25 and p35, which are required for CDK5 activation. Together, these findings suggest that the phosphorylation of Drp1(S616) by CDK5 is involved in mitochondrial fission in the substantia nigra of a non-human primate model of MPTP-induced PD.

Disclosures: J. Park: None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.09/E6

Topic: C.03. Parkinson's Disease

Support: MJFF Grant 15984
NIH Grant F99NS108458

Title: Characterization of the *in vivo* effects of alpha-synuclein preformed fibrils on mouse brain mitochondria

Authors: R. B. CREED[1], A. A. MEMON[2], S. P. KOMARAGIRI[2], ***M. S. GOLDBERG**[3]; [1]Ctr. for Neurodegeneration and Exptl. Therapeutics, Dept. of Neurol, Univ. of Alabama At Birmingham, Birmingham, AL; [2]Neurol., Univ. of Alabama at Birmingham, Birmingham, AL; [3]Univ. Alabama At Birmingham, Birmingham, AL

Abstract: Alpha-synuclein pathology is one of the main pathological hallmarks of Parkinson's disease (PD). Mutations in the alpha-synuclein gene are causally linked to dominantly inherited forms of PD. Although the cause of PD remains uncertain, mitochondrial dysfunction is implicated in both familial and idiopathic PD. Some of the most widely used animal models of PD are based on toxins that inhibit mitochondrial complex I, such as MPTP and rotenone. There is substantial evidence that abnormal forms of alpha-synuclein cause mitochondrial dysfunction and this could be a common pathogenic mechanism for idiopathic and familial forms of PD. Recently, promising PD animal models have been generated by injection of pre-formed fibrils (PFF) of purified recombinant alpha-synuclein into the striatum or the substantia nigra of rats or mice. The effects of intracranial injection of alpha-synuclein PFFs on mouse brain mitochondria remains to be determined. To help fill this knowledge gap, we performed unilateral striatal injections of C57/BL6 mice with alpha-synuclein monomer or PFFs and euthanized the mice at 3 months and 6 months post injection to harvest brain tissue for analysis of mitochondrial function and abundance. In PFF but not monomer-injected mice, we observed prominent immunoreactivity using antibodies specific for alpha-synuclein phosphorylated at serine 129, which is a relatively selective marker of synuclein pathology in human postmortem brains and animal models. Functional analysis of striatal mitochondria revealed significantly altered

complex I activity in the PFF-injected mice at 6 months post-injection, but not at 3 months post-injection. These results indicate that the alpha-synuclein preformed fibril intracranial injection animal model of PD may be useful for mechanistic and therapeutic studies involving mitochondrial dysfunction caused by aggregated forms of alpha-synuclein.

Disclosures: R.B. Creed: None. **A.A. Memon:** None. **S.P. Komaragiri:** None. **M.S. Goldberg:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.10/E7

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS101958
Michael J. Fox Foundation for Parkinson Research

Title: Using mouse cell-type-specific expression patterns of GWAS-implicated genes to identify vulnerable cell types and circuits in Parkinson disease

Authors: *R. M. COWELL[1,2], S. BOAS[1,2], L. J. MCMEEKIN[1,2], S. N. FOX[1,2], K. JOYCE[1,2], M. SIMMONS[1], K. L. GAMBLE[2];
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Abstract: To accurately predict how genetic variation and/or cellular dysfunction influences disease etiology, one must consider the neuroanatomical location of the gene in a normal state. Until recently, this has been challenging to do in an unbiased way due to the magnitude of cellular heterogeneity in the brain, as well as the lack of cell-type-specific resolution in GTEx datasets. Recently, large efforts have been made to generate single-cell mRNA sequencing data to characterize transcriptional identity of brain cells in mice and humans. Here, we leverage publicly available mouse single-cell sequencing datasets to investigate the neuroanatomical distribution of GWAS-implicated genes for the purpose of exploring gene-gene interactions and convergent functional consequences of genetic variation. Principal component analyses of PD GWAS-implicated gene expression across nine regions of mouse brain revealed seven clusters of genes based on cell type and one set of genes with low to no brain expression. The largest set of genes showed enrichment of expression in neurons, with few genes being selectively enriched in dopaminergic neurons of the substantia nigra. Other neuroanatomical categories included polydendrocytes and oligodendrocytes, endothelial cells, astrocytes, and microglia. Interestingly, a cluster of genes was characterized by its enrichment in both prefrontal cortical and striatal neurons and relatively lower expression in dopaminergic neurons. Subsequent analyses of these seven gene clusters revealed unique information about functional categories, putative upstream

regulators of gene expression, and potential consequences of loss of function, with substantially greater statistical power than when all GWAS-associated genes were considered collectively. Additionally, analyses of the neuroanatomical location of genes associated with familial PD revealed previously unknown distributions of these genes in mouse brain, with differential association with the seven neuroanatomical clusters. Evaluation of cell-type-specific distribution of these genes in human midbrain revealed striking similarities between mouse and human cell-type-specific enrichment, suggesting that the seven neuroanatomical categories in mouse could represent distinct circuits and cell types relevant for understanding disease etiology. Altogether, these data suggest that PD etiologies may be defined by cellular identity and serve as a guide for choosing the appropriate cell types for investigating functional consequences of genetic variation.

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Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.11/E8

Topic: C.03. Parkinson’s Disease

Support: NIH Grant 1R43AG059509
University of Texas at Dallas

Title: Skilled motor assessment of the DJ-1 knockout rat

Authors: *C. A. SANCHEZ[1], C. A. THORN[2];
[1]Bioengineering, The Univ. of Texas At Dallas, Richardson, TX; [2]Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas, Richardson, TX

Abstract: DJ-1 knockout (KO) rats are one of several recently developed transgenic rat lines whose development was funded by the Michael J. Fox Foundation to improve the tools available to Parkinson’s disease (PD) researchers. DJ-1 KO rats have been shown to exhibit a motor phenotype, with deficits in some gross motor behaviors (e.g., rearing frequency, grip strength) appearing by ~6 months of age. In the current studies, we tested whether DJ-1 KO rats also exhibit deficits in motor skill learning or long-term performance, using a behavioral task previously demonstrated to be a sensitive assay of forelimb motor coordination and strength. Male DJ-1 KO rats (n = 8) and wild-type Long Evans controls (WT, n = 8) were trained on an isometric pull task that required subjects to reach for and pull a lever placed 1.5 cm outside the training box. Rats began training on the pull-bar task at 2 months of age, and received two 30-minute training sessions daily, 5 days per week. Rats also underwent weekly assessments of

general locomotor activity in an open field. Behavioral experiments were performed by personnel blinded to genotype. Preliminary results after 5 weeks of training indicate that DJ-1 animals do not exhibit deficits in acquisition or stable performance on the pull task prior to 3 months of age. Nor were differences between DJ-1 and WT groups observed in the open field in total distance traveled or in rearing frequency prior to 3 months of age. Behavioral assessments will continue in these animals up to at least 8 months of age, and further analysis of longitudinal motor performance will be presented. The results of these experiments will provide detailed longitudinal characterization of the skilled motor abilities of the DJ-1 KO rat model of PD.

Disclosures: C.A. Sanchez: None. **C.A. Thorn:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.12/E9

Topic: C.03. Parkinson’s Disease

Support: NIH grants NS100090, NS088206 and ES027245
Eugene and Linda Lloyd Endowment
Armbrust Endowment

Title: Gastrointestinal dysfunction in the MitoPark mouse model recapitulates the chronology of gut dysfunction in Parkinson's disease

Authors: *B. N. PALANISAMY[1], S. GHAISAS[4], M. LANGLEY[5], H. JIN[1], S. DUTTA[2], P. PLUMMER[3], K. NARAYANASWAMY[3], A. VELLAREDDY[3], A. KANTHASAMY[1], A. KANTHASAMY[1];
[1]Biomed. Sci., [2]Statistics, [3]Iowa State Univ., Ames, IA; [4]Univ. of Pennsylvania, Philadelphia, PA; [5]Mayo Clin., Wisconsin, WI

Abstract: Gastrointestinal (GI) disturbances are some of the earliest non-motor symptoms affecting most patients with Parkinson’s disease (PD). In many cases, these symptoms occur years before motor deficits become apparent, yet the molecular and cellular mechanisms that contribute to this early GI dysfunction remain poorly understood. The MitoPark model is a chronic, progressive mouse model that recapitulates several key pathophysiological aspects of PD. However, GI dysfunction has not been characterized in this model. Here, in comparison to age-matched littermate controls, we show that GI motility was one of the first non-motor symptoms to develop in MitoPark mice, and was detected as early as 8 weeks with significantly different transit times from 12 weeks onward. GI symptoms were observed well before motor symptoms develop, paralleling PD progression in humans. At age 24 weeks, we observed increased colon transit time and reduced fecal water content, indicating constipation. Intestinal

inflammation was also observed with increased expression of iNOS and TNFα in the small and large intestine. Specifically, iNOS was observed mainly in the enteric plexi of the MitoPark model. Also at 24 weeks, a pronounced loss of tyrosine hydroxylase-positive neurons was observed in enteric neurons, leading to a corresponding decrease in dopamine production in the intestinal tissue. We also observed decreased DARPP-32 expression in the colon, validating the loss of dopaminergic neurons in the gut. MitoPark mice also develop misfolded proteins as evidenced by positive oligomeric protein expression. Our 16s genomic analysis revealed changes in the fecal bacterial population, while fecal metabolites, including fatty acid glycerol and deoxycholic acid, showed significant changes among other metabolites. Together, our data shed more light on the GI pathology of the MitoPark mouse model of PD. Importantly, this model recapitulates the chronology and development of GI dysfunction, along with other non-motor symptoms, and offers an attractive model for pre-clinical assessment of the efficacy of new anti-Parkinsonian drugs for treating gut dysfunction in PD. [NIH
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Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.13/E10

Topic: C.03. Parkinson’s Disease

Support: R01NS076054 (D.K.)

Title: Parkin misregulation of clathrin-mediated synaptic vesicle recycling drives dopamine oxidation in Parkinson's disease patient neurons

Authors: *P. SONG[1], S. JEON[2], T. KRAINC[3], Y. C. WONG[1], D. KRAINC[4];
[1]Northwestern Univ., Chicago, IL; [2]Univ. of Illinois at Chicago, Chicago, IL; [3]Princeton Univ., Princeton, NJ; [4]Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Loss of function mutations in both the E3 ubiquitin ligase *Parkin* and the synaptic protein *Synaptojanin-1* lead to early-onset PD. However, whether they functionally act in the same cellular pathway to drive neurodegeneration of human dopaminergic neurons in PD remains unclear. Here, we identified a novel role for parkin in modulating the synaptic complex Synaptojanin-1/Dynamin-1/Endophilin A1 to regulate dopamine oxidation and downstream associated pathogenic phenotypes, using PD patient dopaminergic neurons with Parkin

mutations. We found that loss of parkin ubiquitination in patient neurons inhibited the synaptic membrane recruitment of Synaptojanin-1 and Dynamin-1 by Endophilin A1 during clathrin-mediated synaptic vesicle recycling. Moreover, parkin mutant neurons demonstrated defective clathrin uncoating and recycling of synaptic vesicles, leading to the subsequent accumulation of cytosolic oxidized dopamine and downstream pathogenic defects. Importantly, expression of Endophilin A1 in parkin mutant neurons was sufficient to rescue both DA oxidation and downstream dysfunction including defective glucocerebrosidase activity. This study thus highlights a role for synaptic vesicle recycling defects in driving oxidized dopamine accumulation and PD pathophysiology, and further suggests converging mechanisms for two PD genes Parkin and Synaptojanin-1 in the synaptic function of human dopaminergic neurons.
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Disclosures: **P. Song:** None. **S. Jeon:** None. **T. Krainc:** None. **Y.C. Wong:** None. **D. Krainc:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.14/E11

Topic: C.03. Parkinson’s Disease

Title: Domain specific roles of α-synuclein in mitochondrial fragmentation and oxidation

Authors: *S. GUNAWARDENA[1], T. J. KRZYSTEK[2], R. BANERJEE[3], L. THURSTON[1], J. Q. HUANG[1], K. SWINTER[1];
[2]Biol. Sci., [1]The State Univ. of New York at Buffalo, Buffalo, NY; [3]State Univ. of New York at Buffalo, Buffalo, NY

Abstract: The balance between fission via dynamin-related protein (DRP1) and fusion via mitofusin (MFN) is critical for mitochondrial health in axons. Defects in fission-fusion and disruption of mitochondrial movement within axons have been linked to mitochondrial dysfunction and neurodegeneration. While α-synuclein (α-syn) has been proposed to influence mitochondrial dynamics within axons the mechanistic details of how α-syn effects mitochondrial homeostasis remains unknown. We found that excess α-syn causes mitochondrial fragmentation, similar to what is observed with excess DRP1, but in contrast to excess MFN2, which causes elongated mitochondria. Intriguingly, co-expression of DRP1 rescues α-syn-mediated mitochondrial fragmentation, indicating that α-syn and DRP1 likely have synergistic functions. Deletion of the acidic C-terminal domain of α-syn (α-syn1-120) which is thought to prevent membrane binding, or the NAC domain (α-synΔNAC) which is thought to promote self-aggregation did not affect α-syn-mediated mitochondrial fragmentation. Further, α-syn-mediated mitochondrial fragments were oxidized, while α-syn 1-120-mediated fragmented mitochondria

were healthier. Together our *in vivo* observations suggest that mitochondrial size is independent of mitochondrial health, and proposes domain specific roles for α-syn during mitochondrial homeostasis. These findings uncover a novel role for α-syn during mitochondrial homeostasis and highlight a common pathological mechanism for targeted therapeutics early before neuronal loss or clinical manifestation of Parkinson's disease.

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Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 379.15/E12

Topic: C.03. Parkinson's Disease

Support: Collaborations internationales 2018

Title: Parkin loss-of-function *Drosophila* dopaminergic neurons show signs of mitochondrial dysfunction during pupal development

Authors: *L. M. BUHLMAN[1], G. BERTOLIN[3], G. B. CALL[2];
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Abstract: The search for the mechanism by which parkin loss of function causes neurodegeneration has led to discoveries that parkin-mediated maintenance of mitochondrial function and turnover may be critical for neuronal survival. Parkinson's disease clinical trials in which mitochondrial function is targeted have been unsuccessful, possibly because intervention occurs after substantial degeneration has taken place. Most cells are not susceptible to degeneration in the absence of parkin; however, parkin-null *Drosophila* have selective degeneration in protocerebral posterior lateral 1 (PPL1) dopaminergic neurons, which are functionally homologous to those of the mammalian substantia nigra. In order to identify unique properties of vulnerable dopaminergic neurons, we express fluorescent protein markers and use confocal microscopy to measure mitochondrial size, morphology, turnover, motility and network volume in PPL1 in *park*$^{25/25}$ (parkin-null) flies. We have previously reported that adult *park*$^{25/25}$ PPL1 mitochondrial networks have increased protein oxidation, fragmentation and transiently decreased mitophagy initiation - all of which are undetectable in non-degenerating dopaminergic neurons. In order to determine whether onset of mitochondrial pathology can be detected during development, we used super resolution confocal microscopy and 3D digital image processing to measure mitochondrial morphology, fusion/fission events and motility in PPL1 of living *park*$^{25/25}$ pupa. While fusion/fission events and mitochondrion size are unaffected, mitochondria have

decreased motility and network volume. Future studies will address whether changes in mitophagy initiation can be detected in living pupa PPL1. Our results demonstrate that vulnerable dopaminergic neuron pathology can be detected during *park*$^{25/25}$ development, and support earlier administration of potential therapeutic interventions with the goal of preventing neurodegeneration.

Disclosures: L.M. Buhlman: None. **G. Bertolin:** None. **G.B. Call:** None.

Poster

379. Parkinson's Disease: Mitochondrial Mechanisms and Genetics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

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Title: GBA1 depletion compromises mitochondrial metabolism and sensitises neurons to calcium overload: From neuronal models to new therapeutic strategies

Authors: *N. PLOTEGHER[1,2], D. PEROCHEAU[3], R. FERRAZZA[5], G. MASSARO[4], A. A. RAHIM[4], G. GUELLA[5], S. N. WADDINGTON[3], G. SZABADKAI[2], M. BISAGLIA[1], L. BUBACCO[1], E. GREGGIO[1], M. R. DUCHEN[2];
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Abstract: Homozygous mutations of the lysosomal enzyme glucocerebrosidase (GBA1) cause Gaucher disease (GD), a lysosomal storage disorder which includes a severe neuronopathic form. Heterozygous mutations of GBA1 are the major genetic risk factor for Parkinson's disease (PD). GBA1 depletion and defects were associated with impaired autophagy and mitochondrial dysfunction by us and others.
We have now explored the impact of mitochondrial dysfunction on cell physiology in primary neurons from a *gba1*$^{-/-}$ mouse model of neuronopathic GD. Our data reveal dysregulated calcium handling and decreased bioenergetic reserve, which increased vulnerability to otherwise innocuous glutamate concentrations. Strikingly, *gba1*$^{+/-}$ neurons showed a behaviour that was

more similar to *gba1*$^{-/-}$ than to *gba1*$^{+/+}$, consistent with a role of these mechanisms in the pathogenesis of PD. *Gba1*$^{-/-}$ neurons also showed increased reactive oxygen species production that may explain the difference between *gba1*$^{+/-}$ and *gba1*$^{-/-}$ mice in the disease phenotype. These data reveal the fundamental role of bioenergetic capacity in protecting cells from dysregulation of calcium homeostasis.
We are now investigating the role of GBA1 trafficking and stability in PD models, also in relationship to the other PD-related protein Leucine-Rich Repeat Kinase 2 (LRRK2), which may affect the proper functioning or localization of GBA1. The idea of stabilizing GBA1 to improve its delivery to the lysosomes and/or its activity may be crucial in the development of new therapeutic strategies.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 380.01/E14

Topic: C.03. Parkinson’s Disease

Support: Neurological Foundation of New Zealand (3712814)
Brain Research New Zealand (3709826)

Title: Changes in dopamine signaling and responses to drugs affecting dopaminergic neurotransmission in striatal and ventral midbrain slices from DAT-KO rats

Authors: J. T. LLOYD[1], P. KALLINGAPPA[2], A. G. YEE[2], P. Y. CHEUNG[2], R. N. KARUNASINGHE[2], A. JABED[2], P. S. FREESTONE[2], *J. LIPSKI[3];
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Abstract: Cessation of dopamine (DA) transmission largely depends on reuptake by the DA transporter (DAT) encoded by the Slc6a3 gene. DAT expression/activity is reduced in several neurological disorders and after exposure to drugs of abuse (e.g. cocaine, methylphenidate, amphetamine). Our aim was to characterize behavioral, neurochemical and electrophysiological effects of eliminating DAT activity in a novel DAT knockout rat generated using CRISPR/Cas9. As expected, DAT-KO rats displayed no DAT immunoreactivity in the striatum, increased basal locomotor activity, and paradoxical calming by amphetamine. Fast-scan cyclic voltammetry (FSCV) in brain slices demonstrated a large decrease in the clearance of electrically stimulated DA release in the dorsal striatum and to a lesser extent in the Substantia Nigra *pars compacta*

(SNc). Cocaine increased the amplitude of DA release and slowed its clearance in slices from wild-type (WT), but not DAT-KO rats. Basal extracellular DA concentration ($[DA]_{out}$), measured with fast-scan controlled-adsorption voltammetry (FSCAV; Burrell et al., ACS Chem. Neurosci. 2015, 6:1802), was higher in DAT-KO rats compared to WT littermates, and was enhanced by L-DOPA, showing that DA release after L-DOPA is not due to DAT reversal. Baseline firing frequency of SNc neurons and $GABA_B$-mediated inhibition were similar in DAT-KO and WT rats. However, D_2-mediated inhibition (by quinpirole, amphetamine and L-DOPA) was blunted in DAT-KOs, likely due to downregulation of D_2 receptors previously reported in DAT-KO mice. Amphetamine increased $[DA]_{out}$ in the dorsal striatum of WT rats, and this effect was strongly attenuated in DAT-KOs. Surprisingly, amphetamine increased $[DA]_{out}$ in the SNc of DAT-KOs, which did not differ to the response seen in WTs. The mechanism of this release is likely through reversal of low-affinity, high capacity uptake-2 transporters. These results not only validate our DAT-KO model, but also provide novel insights into the mechanism of DA releasing agents and form the basis for our future *in vivo* studies.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 380.02/E15

Topic: C.03. Parkinson’s Disease

Support: MOST105-2314-B-016-001-MY3

Title: Forced, but not spontaneous behaviors are initially upheld as dopamine transmission declines in Parkinson model

Authors: *Y.-H. CHEN[1], B. J. HOFFER[2], L. OLSON[3];
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Abstract: To determine the role of reduced dopaminergic transmission for declines of forced versus spontaneous behavior, we used a model of Parkinson’s disease with progressive degeneration of dopamine (DA) neurons, the MitoPark mouse. Mice were subjected to rotarod tests of motor coordination, and open field and cylinder tests for spontaneous locomotor activity and postural axial support. To measure DA release in dorsal striatum and the shell of Nucleus Accumbens (NAc) we used ex vivo fast-scan cyclic voltammetry (FSCV) in 6 to 24 week old mice. To determine decline of DA transporter function we used 18FE-PE2I PET. We show here

that FSCV is a sensitive tool to detect evoked DA release dysfunction in MitoPark mice and that electrically evoked DA release is affected earlier in nigrostriatal than mesolimbic DA systems. DA reuptake was also affected more slowly in NAc shell. PET data showed DA uptake to be barely above detection level in 16 and 20 weeks old MitoPark mice. Rotarod performance was not impaired until mice were 16 weeks old, when evoked DA release in striatum had decreased to ≈ 40% of wild type levels. In contrast, impairment of open field locomotion and rearing began at 10 weeks, in parallel with the initial modest decline of evoked DA release. We conclude that forced behaviors, such as motivation not to fall, can be partially maintained even when DA release is severely compromised, whereas spontaneous behaviors are much more sensitive to impaired DA release, and that presumed secondary non-dopaminergic system alterations do not markedly counteract or aggravate effects of severe impairment of DA release.

Disclosures: Y. Chen: None. **B.J. Hoffer:** None. **L. Olson:** None.

Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Program #/Poster #: 380.03/E16

Topic: C.03. Parkinson’s Disease

Support: SUNY Research Foundation

Title: Determining the role of the dorsal striatum in D1R-D3R cooperativity in dyskinesia: Implications for L-DOPA-induced dyskinesia

Authors: *K. E. LANZA[1], K. CHEMAKIN[1], N. E. CHAMBERS[1], J. SERGIO[1], C. BISHOP[2]; [2]Psychology, [1]Binghamton Univ., Binghamton, NY

Abstract: Although L-DOPA remains the gold standard pharmacotherapy to treat most motor symptoms of Parkinson’s Disease (PD), the majority of patients will develop L-DOPA-Induced dyskinesia (LID) characterized by Abnormal Involuntary Movements (AIMs). There are many pre and postsynaptic factors that contribute to LID. Among these, postsynaptic sensitization of the dopamine D1 receptor (D1R) and upregulation of the dopamine D3 receptor (D3R) have been identified as key mechanisms in LID manifestation. D3R upregulation occurs primarily on striatal D1R-bearing cells where D1R-D3R physically and functionally interact. This interaction facilitates D1R-D3R cooperativity to augment LID severity. We previously demonstrated that systemic pharmacological co-stimulation of D1R and D3R results in synergistic dyskinesia that is correlated with enhanced downstream phosphorylation of striatal ERK1/2 (pERK1/2), a known substrate of LID. Although the striatum is a potential site of action of D1R-D3R synergy, D1R-D3R may interact extrastriatally to modulate circuit-level effects. Therefore, the present study sought to localize the striatal contributions of D1R-D3R cooperativity on the expression of

dyskinesia. To this end, Sprague Dawley rats were rendered hemi-parkinsonian with 6-hydroxydopamine into the medial forebrain bundle. Three weeks later, rats underwent chronic L-DOPA administration (6 mg/kg; s.c.) for 14 days. LID was quantified on days 1,7, and 14 using the AIMs scale. Dyskinetic animals entered into microinjection experiments. In two separate cohorts, intrastriatal doses were established for both the D1R agonist SKF38393 (0, 1.0, or 5.0 μg/μl) and the D3R agonist PD128907 (0, 0.5, or 5.0 μg/μl). Agonists were microinjected in a within-subject counterbalanced design. After threshold doses were established, a third cohort was tested for the presence of striatal D1R-D3R synergy. After each microinjection, AIMs were monitored for a period of 3 hours. Dose response data revealed dose-dependent effects of D1R and D3R agonists on dyskinesia. Preliminary data of D1R-D3R co-administration point to striatal specific contribution to D1R-D3R synergy and by extension, implicate aberrant D1R-D3R interactions as treatment targets for LID management.

Disclosures: K.E. Lanza: None. **K. Chemakin:** None. **N.E. Chambers:** None. **J. Sergio:** None. **C. Bishop:** None.

Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Program #/Poster #: 380.04/E17

Topic: C.03. Parkinson’s Disease

Support: ISCIII-Sara Borrell (CD15/00092)

Title: Resting state connectivity changes in hypersexual impulse control disorders in Parkinson's disease

Authors: D. MATA-MARÍN[1], J. PINEDA-PARDO[1], F. ALONSO[1,2], J. MOLINA[3], J. OBESO[1], L. VELA[1,4], ***I. OBESO**[1];
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Abstract: Dopaminergic medication to treat motor signs in Parkinson´s disease (PD) may come at a cost inducing non-motor complications such as impulse control disorders (ICD). ICD is a side-effect of anti-parkinsonian medication whereby patients experience enhanced desire towards natural rewards resulting in uncontrolled actions. Yet, the precise neural and behavioural mechanisms associated to ICD and of each specific subdomain are unclear. The objective was to decipher the network and neural connectivity change in one specific ICD (i.e. hypersexuality). Eighteen PD-ICD and 16 PD+ICD patients were scanned while on and off medicated for 10-minute resting period. Behavioural assessments done on medication included a stop signal task

(with erotic images), the Frontal Assessment Battery (FAB), Stroop test and the Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease (QUIP). A dual-regression analysis was performed to extract four independent components representing the salience, sensoriomotor, default-mode and central executive networks per subject. Also a seed-based connectivity analysis was performed using as subcortical seeds. Two-way ANOVA comparison was performed to identify drug (on vs. off) or disease (PD-ICD vs. PD+ICD) main effects (uncorrected *P*-value of 0.001 using $P_{FWE}<0.05$ at the cluster level). Finally, correlations between networks and neuropsychological variables were performed.
The right inferior frontal gyrus (IFG) had a significant disease-effect within the salience network, being higher the connectivity for the PD+ICD group. Seed-based connectivity analyses showed main effects from motor striatum (drug-effect) to the temporal pole that correlated with the $QUIP_{sex}$ ($p<0.01$) for the PD+ICD group (while medicated). The connectivity with the executive striatum showed a significant disease-effect in the precuneus and superior parietal cortex, both correlating in the PD+ICD group with $QUIP_{total}$ ($p<0.02$) and $QUIP_{sex}$ ($p<0.03$), respectively. Finally, the limbic striatum showed a significant drug-effect in the parahipocampal gyrus which correlated with stop signal RT in ICD+PD ($p<0.01$) and controls ($p<0.01$).
The deficit in activating the salience network (including the right IFG) in PD+ICD is indicative of a probable dysfunction in stimulus detection, necessary for adaptive and controlled behaviour. Striatal seed-based connectivity indicates significant changes in frontal, parietal and temporal changes associated to hypersexual PD+ICD.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Topic: C.03. Parkinson's Disease

Support: Chair UAM-Fundación Tatiana Pérez de Guzmán el Bueno
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Title: Noradrenaline axons and alpha adrenoceptors in human and macaque thalamic nuclei involved in basal ganglia circuits

Authors: *I. PÉREZ-SANTOS[1], N. PALOMERO-GALLAGHER[2,3], K. ZILLES[2,3], C. CAVADA[1];

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Abstract: There is a significant loss of noradrenaline (NA) in the thalamus of Parkinson's disease patients and MPTP parkinsonian monkeys. NA depletion mainly affects the ventral motor nuclei, both in humans and monkeys; in humans, there is also significant NA reduction in other thalamic nuclei, including the intralaminar nuclei. In this work, we studied the normal distribution of NA axons and two types of Alpha-adrenoceptors in thalamic regions of humans and macaques which are connected directly or indirectly with the striatum, i.e. the ventral anterior (VA), ventral lateral (VL), intralaminar, and midline nuclei. We used immunohistochemistry against dopamine beta-hydroxylase and the noradrenaline transporter to reveal NA axons; and quantitative autoradiography to detect Alpha-1 and Alpha-2 adrenoceptors, using tritiated Prazosin and UK-14,304, respectively, as ligands. NA axonal maps were created using Neurolucida software, and Alpha receptor maps using a Matlab-based in-house developed software. The patterns of axonal distribution were comparable in the human and macaque thalamus. VA and VL exhibited a moderate NA innervation. The intralaminar nuclei exhibited higher NA axonal densities. Within the posterior intralaminar centromedian-parafascicular complex, NA axons were more abundant in parafascicular than in the centromedian nucleus. Humans appear to have a denser NA innervation than macaques in the centromedian nucleus. The midline nuclei were the most densely innervated. Alpha-1 receptor densities were higher than Alpha-2 densities, with the exception of the centromedian nucleus in the macaque thalamus. This particularity was not present in the human thalamus. The parafascicular nucleus exhibited high Alpha-1 and Alpha-2 receptor densities. Anterior intralaminar nuclei held low Alpha receptor concentrations, despite the dense NA innervation present in the axonal maps. This could be due to the presence of other receptors not analyzed (e.g. Beta family). VA and VL held a moderate density of Alpha-1 and a low density of Alpha-2 receptors, with higher densities in some regions, such as the ventromedial subdivision of VA (VAvm) and area X in macaques, and the magnocellular subdivision of VA (VAmc) in humans. The midline nuclei held the highest receptor densities. The thalamic regions with the most outstanding NA system are mainly connected to the caudate nucleus and ventral striatum. We conclude that the complex distributions of axons and receptors in thalamic nuclei involved in basal ganglia circuits suggest an important role for NA signaling, particularly in associative and limbic thalamo-striatal circuits.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Program #/Poster #: 380.06/E19

Topic: C.03. Parkinson's Disease

Support: American Parkinson's Disease Association
Center for Development and Behavioral Neuroscience at Binghamton University

Title: Chemogenetic and pharmacological interrogation of pedunculopontine nucleus cholinergic neuron involvement in L-DOPA-induced dyskinesia and gait deficits in hemi-parkinsonian rats

Authors: *N. E. CHAMBERS[1], J. SERGIO[1], A. MCLUNE[1], K. E. LANZA[2], C. R. BISHOP[2]; [2]Psychology, [1]Binghamton Univ., Binghamton, NY

Abstract: Parkinson's disease (PD) is a movement disorder characterized by loss of nigrostriatal dopamine neurons and brainstem acetylcholine (ACh) neurons in the pedunculopontine nucleus (PPN). Recent evidence suggests that PPN ACh neurons contribute to PD gait deficits and L-DOPA-induced dyskinesia (LID), a debilitating side effect of chronic L-DOPA-administration. To date, bimodal effects of PPN ACh neurons on LID and gait are unknown. Furthermore, although M4R positive allosteric modulators (PAM) show efficacy as an anti-LID therapeutic, the role of the PPN in M4R effects is not established. Rats in the current study were rendered hemiparkinsonian through unilateral infusion of 6-OHDA. In experiment 1, ChAT-cre+ Long-Evans rats received PPN infusions of excitatory (hM3dq), inhibitory (hM4di), or control (mCherry) designer receptors exclusively activated by designer drugs (DREADDs) viral vectors for selective modulation of PPN ACh neurons. Thereafter, rats received the DREADD ligand Compound 21 to determine chemogenetic effects on motor behaviors. L-DOPA was then administered for 2 weeks to elicit stable LID. Next, rats received C21 30 min before L-DOPA to examine the effects of chemogenetic modulation on LID and L-DOPA's motor efficacy. In experiment 2, wild-type Long-Evans rats received unilateral PPN-targeted cannulae. Following establishment of baseline motor deficits and stable LID, rats received a PPN microinfusion of M4R PAM VU0467154, followed 5 min later by L-DOPA. In both experiments, after L-DOPA administration LID was tested for 3h and gait and motor efficacy were assayed at the 30- and 60 min time points, respectively. At study end, lesion efficacy (TH-ir), successful transfection (PPN ChAT and mCherry co-expression), cellular activation (PPN ChAT + c-fos co-expression) and cannula placement were verified. Overall, chemogenetic stimulation of PPN cholinergic neurons appeared to improve movement, increase LID severity, and elevate ChAT and c-fos co-expression. Conversely, inhibition appeared to worsen motor performance, reduce LID severity, and decrease ChAT and c-fos co-expression. Finally, VU0467154 dose-dependently reduced

LID and c-fos-ChAT co-expression without affecting L-DOPA's motor efficacy, suggesting PPN cholinergic neurons as potential targets to reduce LID and PD motor deficits.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Program #/Poster #: 380.07/E20

Topic: C.03. Parkinson's Disease

Support: Chair in Neuroscience UAM-Fundación Tatiana Pérez de Guzmán el Bueno.

Title: Thalamic dopamine changes in Parkinsonism: Insight into novel pathogenic mechanisms

Authors: *M. H. G. MONJE[1,2], J. BLESA[3], M. GARCIA-CABEZAS[4], J. A. OBESO[3], C. CAVADA[5];
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Abstract: Dopamine loss in Parkinson's disease (PD) affects most intensely the mesostriatal system. In order to understand a series of ill-defined pathogenic mechanisms and clinical manifestations, it is relevant to understand how dopamine changes affects brain structures beyond the striatum. The primate thalamus is densely innervated with dopaminergic axons that express the dopamine transporter (DAT); they have a diverse and complex origin and distribution. We hypothesized that dopamine changes may be present in the thalamus of the parkinsonian brain. Also, given the heterogeneity of the thalamus, differences might be present among the different nuclei. The toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was administered to adult macaque monkeys using a slow intoxication protocol. The intoxicated monkeys were classified into two groups by motor tests: non-symptomatic and parkinsonian. Dopaminergic innervation was studied by immunohistochemistry against DAT. The total length and length density of DAT-ir axons were estimated with stereology using a 3D fractionator. We also generated maps of the distribution of the DAT-ir axons. Compared to control animals, parkinsonian monkeys exhibited less DAT-ir axonal length density in the mediodorsal (MD) and centromedian-parafascicular (CnMd-Pf) nuclei. In MD, the dopamine denervation was present early, that is, in monkeys non-symptomatic at the time of sacrifice and with moderate substantia nigra cell loss (40-60%); MD dopamine denervation was greatest in the most severe parkinsonian animals. In the reticular (R) nucleus, DAT-ir axonal length density exhibited an

inverse pattern, with a progressive increase up to a maximum density in parkinsonian animals. DAT-ir axonal length density in the ventral thalamic nuclei did not show differences between groups. DAT-ir axons maps supported the quantitative findings. These results show that the dopaminergic axons innervating the thalamus show a heterogeneous reaction to MPTP, present even in the presymptomatic stages of the parkinsonism. Changes in the dopaminergic thalamic system may result in dysfunction of thalamocortical, thalamostriatal and intrathalamic transmission, and may contribute to motor and non-motor manifestations of PD, in particular attention and sleep disturbances.

Disclosures: M.H.G. Monje: None. **J. Blesa:** None. **M. Garcia-Cabezas:** None. **J.A. Obeso:** None. **C. Cavada:** None.

Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Program #/Poster #: 380.08/E21

Topic: C.03. Parkinson’s Disease

Support: Supported by the Chair in Neuroscience UAM-Tatiana Pérez de Guzman el Bueno.

Title: Compartments of dopaminergic neurons within the macaque monkey ventral substantia nigra have differential vulnerability to MPTP degeneration

Authors: J. BLESA[1], C. GIMÉNEZ-RODRÍGUEZ[2], P. ANDRÉS-CAMAZÓN[2], M. H. G. MONJE[2], N. LÓPEZ-GONZÁLEZ DEL REY[1], L. JIMÉNEZ-SÁNCHEZ[2], J. A. OBESO[1], ***C. CAVADA**[2];
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Abstract: Human dopaminergic neurons within the calbindin-poor nigrosomal compartment of the substantia nigra (*SN*) are distinctly prone to degeneration in Parkinson’s disease (PD). One study (n= 5) indicated that those neurons die earlier and in larger numbers than the neurons within the calbindin-rich matrix. No longitudinal study, particularly in the early period of neurodegeneration (pre-symptomatic), is available. We have re-examined the macaque monkey *SN* using immunohistochemistry for calbindin-D28k. We confirmed that dorsal tier neurons express calbindin while ventral tier neurons do not. We also observed two compartments within the ventral tier of the *SN pars compact*a: calbindin-poor neuropil surrounded by a calbindin-rich zone. We propose to name these compartments nigrosomes and matrix, respectively, by analogy with the compartments of the human *SN*. We then verified that the nigrosomes and the matrix are present in the *SN* of monkeys rendered parkinsonian with the neurotoxin MPTP. We additionally

tested if the macaque monkey nigrosomes and matrix hold dopaminergic neurons with differential vulnerability to neurodegeneration. We used sixteen brains of diversely affected MPTP-treated monkeys (pre-symptomatic and symptomatic) and four control brains from non-MPTP-treated monkeys. We estimated the numbers and densities of tyrosine-hydroxylase-immunoreactive neurons (TH-ir) using stereology. In control monkeys, the densities of TH-ir neurons are 2.5 times higher in the nigrosomes than in the matrix. In MPTP-treated monkeys, TH-ir cell loss is higher in the nigrosomes than in the matrix. When parkinsonian symptoms are present, the numbers and densities of TH-ir neurons are similar or lower in the nigrosomes than in the matrix. These results indicate that the dopaminergic neurons within the monkey nigrosomes, known to project to the sensory-motor striatum, are the most vulnerable in the MPTP model of parkinsonism. The present observations confirm and expand earlier findings in humans and strengthen the value of the MPTP monkey model of PD to explore novel paths of research into this condition.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Topic: C.03. Parkinson’s Disease

Support: National Natural Science Foundation of China 81671109
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Title: Inhibition of striatal dopamine D5 receptor-mediated pathway attenuates levodopa-induced dyskinesia in a rat model of Parkinson’s disease

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Abstract: Levodopa-induced dyskinesia (LID) is experienced by most patients of Parkinson’s disease (PD) upon the long-term use of the dopamine precursor levodopa. Dopaminergic signaling plays a critical role in the pathogenesis of LID through its interactions with dopamine receptors in striatum. The specific roles of striatal D5 receptor in the pathophysiological process of LID is still poorly established. In the study, we used PD rats with or without dyskinetic symptoms after chronic levodopa administration. The results showed that the expression level of D5 receptors in the sensorimotor striatum of dyskinetic rats are significantly higher than that of

the non-dyskinetic controls. The administration of levodopa increased c-Fos expression in a subpopulation of sensorimotor striatum neurons of dyskinetic rats, but not in non-dyskinetic rats. The majority of the c-Fos+ neurons activated by levodopa in striatum are positive for D5 receptor staining. Intrastriatal injection of D1-like (D1 and D5) dopamine receptor antagonist, R(+)-SCH-23390, significantly inhibited dyskinetic behavior and c-Fos expression in striatal D5 receptor positive neurons in dyskinetic rats after the injection of levodopa. Intrastriatal perfusion of small interfering RNA directed against DRD5 downregulated D5 receptors expression and moderately inhibited dyskinetic behavior of dyskinetic animals. The results suggested that striatal dopamine D5 receptor might play a novel role in the pathophysiology of LID.

Disclosures: **G. Zhang:** None. **Y. Wang:** None. **L. Yao:** None. **J. Feng:** None. **Y. Sun:** None.

Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 380.10/E23

Topic: C.03. Parkinson’s Disease

Title: Perturbations of the dopaminergic pathway in the olfactory bulb may contribute to prodromal Parkinson's disease-related hyposmia

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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder that is aligned with an extensive prodromal phase defined by the presence of an array of non-motor symptoms, including hyposmia. Disruptions in hyposmia are a well characterised feature of PD. Understanding the biological basis for this symptom should assist in the creation of early diagnostic criteria and potentially inform future therapeutic trials.
The protein tau has been identified as a genetic risk factor in the development of sporadic PD. The tau knockout (KO) mouse has been reported as an age-dependent model of PD and we have shown previously that these animals display *pre-motor* hyposmia, making them a model of interest for investigations of prodromal disease. We examined the tissue of these animals, including the olfactory bulb, to elucidate hyposmia-related changes and compare these to post-mortem tissue from PD patients.
One of the major findings from this study was a significant increase in the level of tyrosine hydroxylase (TH) in the olfactory bulb of 7-month-old tau KO mice (n=6) compared to wildtype (WT) littermate controls (n=6). TH is the rate limiting enzyme in dopamine synthesis and this increase was recapitulated in the human PD olfactory bulb tissue, suggesting that modulation of the dopamine pathway may related to PD hyposmia. Initially we examined if modulating

dopamine levels had a functional effect on olfaction in healthy animals by dosing 7-month-old WT mice with either vehicle (n=10), 20 mg/kg cocaine (dopamine agonist) (n=9), or 0.33 mg/kg haloperidol (dopamine receptor antagonist) (n=10) and measured olfaction using the odour detection test (ODT). WT mice treated with either cocaine or haloperidol demonstrated significant hyposmia on the ODT, whereas vehicle treated animals had preserved olfaction. Given the increase of TH in both the tau KO mice and human tissue, it was hypothesised that an increase in the level of TH was reflective of dopaminergic changes that are underlying PD-related hyposmia. As such, 7-month-old tau KO animals underwent the same drug trial. Tau KO mice dosed with vehicle (n=10) or 20 mg/kg cocaine (n=10) had no change in the presentation of their hyposmia. Tau KO animals treated with 0.33 mg/kg haloperidol (n=11) had significantly restored olfaction.
These data suggest that the olfactory system is sensitive to perturbations of dopamine metabolism and antagonism of dopaminergic receptors in tau KO mice rescues the pre-motor hyposmic phenotype. The refelective increase of TH in human PD post-mortem olfactory bulb suggests that hyposmia in PD may be a dopamine-mediated phenomenon.

Disclosures: L.C. Beauchamp: None. **L.J. Vella:** None. **A.I. Bush:** None. **K.J. Barnham:** None.

Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 380.11/E24

Topic: C.03. Parkinson’s Disease

Title: The effects of acute and chronic L-DOPA on sensorimotor gating in hemi-Parkinsonian rats

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Abstract: Parkinson’s disease (PD) is a neurodegenerative movement disorder characterized primarily by motor impairment that results from nigrostriatal dopamine cell loss. However, several non-motor symptoms associated with PD severely impact patients’ and caregivers’ quality of life yet remain understudied and/or poorly treated. Prominent among these symptoms is psychosis, which affects the majority of patients within 20 years of diagnosis. Unfortunately, clinical evidence suggests that L-DOPA, the gold standard for treatment of PD may increase risk of psychosis, particularly hallucinations. Thus, this study investigated the effects of L-DOPA in rat PD model on prepulse inhibition (PPI), a measure of sensorimotor gating often disrupted in neuropsychiatric disorders and preclinical models of psychosis. To test this, we rendered rats

hemi-parkinsonian with a 6-OHDA lesion to the left medial forebrain bundle. These rats were split into two groups: half received a single injection of either L-DOPA (6 mg/kg) or vehicle 20 minutes prior to PPI testing, and half underwent a 14 day regimen of daily L-DOPA injections and received either L-DOPA or vehicle 20 minutes prior to PPI testing. The results revealed that although DA lesion alone was not sufficient to alter PPI, chronic administration of L-DOPA and on treatment day significantly decreased PPI. Interestingly, this same group of rats also developed moderate to severe L-DOPA-induced dyskinesia during repeated L-DOPA treatment. This suggests that the DA loss and chronic L-DOPA treatment together exacerbate dysfunction in sensorimotor gating neurocircuitry that elevates risk for psychosis and also implicate treatment strategies for complications.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 380.12/E25

Topic: C.03. Parkinson’s Disease

Title: Pharmacological blockade of monoacylglycerol lipase reduces L-DOPA-induced dyskinesia in MPTP-lesioned macaques

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Abstract: In patients with Parkinson’s disease, long-term dopamine-replacement therapy with L-DOPA invariably leads to the development of disabling involuntary movements, L-DOPA-induced dyskinesia (LID). LID can be as troublesome as the underlying disease itself and can take the form of dance-like movements, chorea, or sustained, abnormal postures, dystonia. Although, the precise mechanisms that contribute to the expression of LID are not fully understood, dysregulation of dopaminergic and nondopaminergic neurotransmitter systems in the basal ganglia have been implicated. The endocannabinoid system (ECS) mediates multiple forms of synaptic plasticity in the basal ganglia. In LID, disruption of endocannabinoid plasticity in the striatum leads to overactive cortico-striatal glutamate signaling and may contribute to the motor fluctuations associated with this disease. We propose that enhancing levels of the principal endocannabinoid, 2-arachidonoylglycerol (2-AG), in the brain through inhibition of its enzymatic hydrolysis by monoacylglycerol lipase (MGLL) would normalize dysregulated cortico-striatal neurotransmission and improve symptoms of LID. To test our hypothesis, we

evaluated the potential of the selective MGLL inhibitor, ABD-1970, to reduce established LID in MPTP-lesioned macaques. Following oral administration, ABD-1970 produced a significant reduction in peak LID (including both dystonia and chorea) with LID typically being reduced from disabling to absent or non-disabling levels. The reduction of LID by ABD-1970 (up to 45%), was greater than the clinically used reference treatment, amantadine (29%). Importantly, ABD-1970 was not associated with a decrease in the anti-parkinsonian benefit of L-DOPA or the total duration or quality of associated on-time. The study demonstrates, in a well-validated non-human primate model, the potential of MGLL as a therapeutic target for LID.

Disclosures: **J.R. Clapper:** A. Employment/Salary (full or part-time):; Abide Therapeutics. **T.H. Johnston:** 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.; Atuka Inc. **J.M. Brotchie:** 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.; Atuka Inc. **S.H. Fox:** 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.; University of Toronto. **J.L. Blankman:** A. Employment/Salary (full or part-time):; Abide Therapeutics. **A. Viader:** A. Employment/Salary (full or part-time):; Abide Therapeutics. **A.B. Ezekowitz:** A. Employment/Salary (full or part-time):; Abide Therapeutics. **G.P. O'Neill:** A. Employment/Salary (full or part-time):; Abide Therapeutics. **C.R. Beals:** A. Employment/Salary (full or part-time):; Abide Therapeutics.

Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 380.13/E26

Topic: C.03. Parkinson’s Disease

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Title: Impulse control disorder and sub-second dopamine fluctuations during risky decision-making in Parkinson’s disease

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Abstract: Impulse Control Disorder (ICD) secondary to Parkinson's disease (PD) treatment is a devastating non-motor morbidity and possible side-effect of effective motor symptom treatment. Dopamine agonist therapy is the most common culprit for medication-induced ICD. Understanding ICD propensity and development represents an unmet need in the increasingly recognized demand for greater treatment of non-motor morbidity in PD. PD patients with (n = 3) and without (n = 3) ICD secondary to dopamine agonist therapy performed a gambling task incentivized by monetary rewards while we monitored dopamine release using human voltammetry (i.e., elastic net electrochemistry). We assess the hypothesis that ICD positive individuals have a higher propensity to gamble corresponding to differences in sub-second dopamine fluctuations during risky decision-making. Both groups used a video game interface to manage their investments and receive feedback on their results real-time. Data analyses comprised of a fixed effect analysis to measure the differences in investment behavior between patients with and without ICD. First, we show that the distribution of all investment levels in ICD positive participants shows a rightward skew towards investments of greater monetary value versus a more uniform distribution across all bet sizes in the ICD negative participants. Indeed, on average ICD positive participants invested at a significantly higher level (risked more) than ICD negative participants (p-value = 3.8133e-11). ICD positive participants also expressed a lower magnitude change in bet when increasing (p = 6.4979e-05) or decreasing (p-value = 4.5731e-06) investment levels compared to ICD negative participants. This suggests that ICD positive individuals enter the market with bets of high monetary value and maintain these high bets in the face of losses, a characteristic pattern that contrasts ICD negative individuals who may consider investment gains and losses when selecting their bet sizes. Future directions include analyzing human voltammetry data collected during the task to assess the role of dopamine in these decisions, and to increase sample size to make results more widely applicable to the PD population.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 380.14/E27

Topic: C.03. Parkinson's Disease

Title: The endocannabinoid system in Parkinson's disease

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Abstract: The endocannabinoid system is an important modulator of a number of physiological processes. Endocannabinoids and cannabinoid receptor signaling have been linked to inhibition of excitatory transmission via cannabinoid receptor 1 (CB1) and modulation of immune responses via cannabinoid receptor 2 (CB2) and might therefore be promising targets in neurodegeneration and neuroinflammation. Changes in this system have been seen in several disease states including Parkinson's disease. The endocannabinoid (eCB) system and the monoaminergic system interact specifically within the basal ganglia. Here, CB1 receptors co-localize with dopamine D1/D2 receptors in dopamine projecting areas. Acute blockade of CB1 receptor potentiates the activating role of dopamine D2 receptor agonists on locomotor activity. While CB1 stimulation results in sensitization of the motor effects of indirect dopaminergic agonists. Moreover, pre-clinical research suggests that modulation of eCB signaling could improve motor symptoms. The CB2 receptor has been reported to be upregulated in neurodegenerative and neuroinflammatory diseases. An upregulation of CB2 receptors implicates activation of microglia, which can produce a variety of cytokines. Using microdialysis, it is possible to determine the effect of different modulators of the eCB system on the monoaminergic system, the immune system and also on the eCB system itself. Levels of the eCB's: anandamide (AEA), 2-arachidonoylglycerol (2-AG) are difficult to determine due to the lipophilic properties of these fatty acids. We have optimized both the eCB sampling technique to allow for microdialysis and the analytical method. Optimization of the analytical method allows us to combine high sensitivity and low LLOQ with good specificity (separation of 2-AG and its isomer 1-AG). Using a dual probe approach it is even possible to simultaneously evaluate neuroinflammation by analysing these microdialysate samples for a variety of cytokines. We use the above described method to demonstrate effects of a highly selective FAAH and a MAGL inhibitor on levels of AEA, 2-AG and 1-AG in microdialysate from the nucleus accumbens of freely moving rats. These data show that our optimized experimental design and sensitive bio-analytical methods allows better examination of the effects of potential new medication on the interaction between different neurotransmitter systems, the immune system and the endocannabinoid system.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Topic: C.03. Parkinson's Disease

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Title: Effects of 6-hydroxydopamine lesions of the substantia nigra, the dorsal striatum, the medial accumbens and the lateral accumbens on free-choice high-fat high-sugar diet component preference

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Abstract: Dopamine (DA) signaling regulates feeding-related behavior. Conversely, caloric intake, especially of palatable dietary items, can modulate DA-related brain circuitries. For example, it has been observed that increased intake of dietary fat results in blunted DA signaling, and, to compensate for this lowered DA function, caloric intake may subsequently increase. To determine how DA signaling regulates food preference, we utilized 6-hydroxydopamine (6-OHDA)-mediated lesioning to deplete DA-related signaling in specific brain regions of male Sprague Dawley rats. Food preference was assessed by providing the rats with free-choice access to control diet (CD), fat, tap water, and 30% sucrose solution. Rats with lesions of the substantia nigra pars compacta (SNc; which also offers a model of Parkinson's disease) consumed less calories ($P< 0.05$), as reflected by a decrease in CD intake ($P< 0.05$), but displayed an increase in fat intake ($P< 0.05$), without change in sucrose solution intake compared to non-lesioned controls. To determine which of the SN projection areas contributed to these effects, we next compared 6-OHDA lesions targeted to DA neuron terminals in the dorsal striatum, the medial nucleus accumbens (NAc) or the lateral NAc. We observed that 6-OHDA lesioning of the lateral NAc, but not the dorsal striatum or medial NAc, led to increased fat intake ($P< 0.05$). These

findings indicate a role for DA signaling in the lateral NAc in regulating food preference, in particularly the intake of fat. This work was supported by the Centre National de la Recherche Scientifique (UPR3212), the University of Strasbourg, the Agence Nationale de la Recherche (ANR-15-CE37-0005-02; EUR Euridol ANR-17-EURE-0022), the Fondation pour la Recherche Médicale (FDT20170437322), the NeuroTime Erasmus Mundus Joint Doctorate, the University of Amsterdam.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Program #/Poster #: 380.16/E29

Topic: C.03. Parkinson’s Disease

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Title: PET imaging studies of PDE10A in a nonhuman primate model of Parkinson disease

Authors: H. LIU[1], Z. LUO[1], J. GU[1], Y. YU[1], H. WHITE[1], H. FLORES[1], Y. ZHOU[1], V. CARROLL[2], G. D. TAMAGNAN[3], J. S. PERLMUTTER[1], ***Z. TU**[1];
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Abstract: Objectives Phosphodiesterase 10A (PDE10A) is located almost exclusively in striatal medium spiny neurons and hydrolyzes cAMP and cGMP. PDE10A levels reflect post-synaptic activity of the nigrostriatal dopaminergic (DA) pathway. Thus, PET radioligands for PDE10A have potential to investigate pathophysiology, disease progression or drug effects for diseases with striatal pathologies such as Parkinson disease (PD). Since the change of pre-synaptic biomarkers of DA pathway do not fully reflect the severity of motor parkinsonism and the loss of nigral DA cell bodies, PDE10A could potentially serve as a key biomarker to assess PD severity. Herein we reported our initial evaluation of two PDE10A PET radioligands (^{11}C-TZ1964B and ^{18}F-MNI659), along with dopaminergic D1 and D2 receptor radioligands (^{11}C-NNC and ^{11}C-NMB), in an MPTP-induced PD model in nonhuman primates (NHPs). **Methods** Six male adult

macaques underwent a series of 2-hour dynamic PET scans (59 scans in total) before and after unilateral internal carotid MPTP (0.25 mg/kg). Post-MPTP scans were done at week 14-16 to detect nigrostriatal injury and changes in DA pathway including PDE10A. A simplified reference tissue model was used to analyze PET data and generate binding potential (BP) values, with cerebellum as the reference. **Results** Test-retest variability analysis of baseline scans of ^{11}C-TZ1964B, ^{18}F-MNI659, ^{11}C-NNC and ^{11}C-NMB revealed good reproducibility. Right-to-left ratio of striatum BP values for each tracer is approximately 1. The tracer uptake is 7-15 % higher in caudate than putamen for these 4 tracers. Ipsilateral nigrostriatal injury was confirmed by a 77% reduction in ipsilateral striatal ^{11}C-DTBZ PET at week 14 post-MPTP. The tracer uptake of PDE10A radioligands in right striatum (ipsilateral side) was 96% (^{11}C-TZ1964B) and 82% (^{18}F-MNI659) of baseline levels, while the uptake of ^{11}C-NNC and ^{11}C-NMB dropped more dramatically to 61% and 59% of baseline levels. The contralateral side (left striatum) showed no change of PDE10A level, but modest decline of D1 and D2 tracer uptake (72% and 70% relative to baseline, respectively). **Conclusion** The four radioligands, targeting PDE10A, D1 and D2 receptors respectively, showed small test-retest variability and responded differently to MPTP treatment. The change of PDE10A PET measures is more modest than that of D1 and D2 PET, demonstrating indirect regulation of PDE10A levels via DA pathway. Larger sample size and statistical analysis are warranted to explore the relationship between striatal tracer uptake of each tracer with motor behavior and *in vitro* measure of nigrostriatal and striatal neurons.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Topic: C.03. Parkinson’s Disease

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Title: L-tryptophan is a biomarker for Parkinson's disease

Authors: *P. A. BRODERICK, J. R. HORDOF, M. CABRERA;
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Abstract: Globally, the catecholamine neurotransmitter dopamine is well known as a biomarker for Parkinson's (PD) disease. But, we have found that the indoleamine, serotonin (5-HT) may well be a more sensitive biomarker for Parkinson's than dopamine. Here, we show that L-Tryptophan (L-TP) is indeed a unique biomarker for PD as well! Thus, we set about to study the effects of L-3,4-dihydroxyphenylalanine (L-DOPA), the main stay treatment for PD, with Neuromolecular Imaging, (NMI) and BRODERICK PROBE® nanobiosensors. This nanobiotechnology for the first time demonstrates the ability to observe in real-time the release and quantification of neurochemicals in pursuit of unknown biomarkers in neurodegenerative disease to find a more suitable treatment with less adverse effects. Moreover, these nanobiosensors live streams directly online and originates from precise neuroanatomic brain sites such as, in this case, the dorsal striatum in basal ganglia. Thus, the nanobiotechnology herein imaged neuromolecules with and wihout (L-DOPA) in dorsal striatal basal ganglia neurons. PD and non-PD animals were videotracked, and images were readily seen on a laptop via a potentiostat using a semiderivative electrical circuit. Administered L-DOPA doses were 50 and 100 mg/kg intraperitoneally (ip); the same experimental paradigm was used to image and then contrast data. Results show that the baseline release of biogenic amine molecules was significantly above detection limits in non-PD animals and after administration of L-DOPA, biogenic amines increased significantly in these same animals. Nevertheless, it is intriguing to see that L-DOPA could not enable synaptic DA release in PD animals, thus confirming biogenic amines as biomarkers for PD. Importantly, there were other significant biomarkers present in PD animals and absent in non-PD animals in the brain; peptide neurotransmitters including dynorphin and somatostatin, which also increased following L-DOPA. However, to treat the PD brain, it is the L-TP biomarker that must first be metabolized by Indoleamine 2,3-dioxygenase I,II (IDO) in the Kynurenine pathway (KP). It has been shown that through KP in the brain, 5% of L-TP remains with potential for synthesis of 5-HT. This is a good starting point for novel drug administration to increase 5-HT release without hindrance of the blood-brain barrier. Alternatively, medications that increase5-HT release *a priori*, gives us an effective way to treat PD (Piercey, MF, Svensson, KA, Lin, CH, Haadsman-Svensson, SR, McCall, RB, Smith, MW, Broderick, PA & Carlsson, A, SFN,1993).(Tribute to Arvid, in memoriam).

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Topic: C.03. Parkinson's Disease

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Title: Extra-striatal dopamine in Parkinson's disease with rapid eye movement sleep behaviour disorder

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Abstract: Rapid eye movement sleep behaviour disorder (RBD) is a common condition found in 50% of Parkinson's disease (PD) patients. Molecular imaging evidence shows that PD with RBD (PD-RBD+) has a distinct striatal dopamine phenotype compared to PD without RBD (PD-RBD-) where PD-RBD+ show lower dopamine transporter activity within the caudate and putamen. However, the characterization of the extra-striatal dopamine within the mesocortical and mesolimbic pathways remains unknown. Therefore, we aim to elucidate this with PET imaging in PD patients with and without RBD, while having healthy older adults as controls (HC). A total of 45 participants were enrolled in the imaging study: 15 were PD-RBD+ (mean age = 68.1 ± 6.48 years), 15 were PD-RBD- (70.7 ± 5.67 years) and 15 were HC (67.1 ± 5.14 years). Each participant underwent a single PET scan with [^{11}C]FLB 457 to detect the D2 receptors within the extra-striatal regions of interest (ROI), including the prefrontal and temporal areas. They also underwent a single MRI scan to rule out structural lesions and to provide anatomical reference for the parametric PET image analysis. [^{11}C]FLB 457 retention was expressed as the non-displaceable binding potential (BP_{ND}) using the simplified reference tissue model 2 with the cerebellum as a reference region. Analysis of variance was used to compare the BP_{ND} between the three groups for each ROI. Post hoc independent sample *t* tests were used to test for differences between conditions and were corrected for multiple comparisons using false discovery rate. The main effect of group condition on [^{11}C]FLB 457 binding was tested and revealed significant effect within the superior temporal region on the right [$F(2, 42)=3.98$, $p=0.026$] and left [$F(2, 42)=4.29$, $p=0.02$] hemisphere; and left uncus para-hippocampus [$F(2, 42)=4.43$, $p=0.018$]. Specifically, we found that PD-RBD- binding was lower than HC in the superior temporal region in both right ($p=0.039$) and left ($p=0.021$) hemispheres. However, the PD-RBD+ binding was found to be lower relative to HC ($p=0.045$) on the right side only, and slightly higher than PD-RBD-, but this did not reach significance. For the left uncus para-hippocampus, both PD-RBD+ ($p=0.027$) and PD-RBD- ($p=0.027$) binding was lower than HC, but PD-RBD+ was slightly higher than PD-RBD-, which did not reach significance. Our results implicate that relative to HC, PD-RBD+ has lower levels of D2 receptor availability within the left uncus para-hippocampus, a region involved in the limbic system, which influences sleep; and the right superior temporal region. Results imply that extra-striatal dopaminergic system may play a role in contributing to the RBD in PD patients.

Disclosures: M. Valli: None. **S. Cho:** None. **J. Kim:** None. **M. Diez-Cirarda:** None. **A. Mihaescu:** None. **A. Strafella:** None.

Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 380.19/E32

Topic: C.03. Parkinson’s Disease

Support: Parkinson Canada

Title: CRISPR Cas9 mouse model to study glutamate co-transmission by sertonin neurons of the dorsal raphe nucleus

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Abstract: Ascending serotonin (5-hydroxytryptamine, 5-HT) projections arise mainly from the dorsal raphe nucleus (DRN). The vast majority of 5-HT DRN neurons express the atypical type III vesicular glutamate transporter (VGluT3), allowing co-release of glutamate and 5-HT by their axons terminals. The aim of this study is to generate and characterize a new mouse model to determine the role of glutamate co-transmission by 5-HT neurons of the DRN. To do so, we used CRISPR/Cas9 technology, allowing region and neuron-specific conditional knock-out, in adult mice. To knock-out the expression of VGluT3 specifically in 5-HT neurons of the DRN, an AAV encoding a guide RNA for the *vglut3* gene was injected in the DRN of transgenic ePet-cre+/Cas9flox+ mice. RT-qPCR assay, RNAscope and immunohistochemistry confirm the depletion of VGluT3 in AAV-infected 5-HT neurons. Moreover, high-resolution confocal analysis reveals a decrease in the number of axon varicosities in VGluT3-depleted 5-HT neurons. In open-field behavioral test, VGlut3-depleted mice tend to show an increase in spontaneous motor activity as well as to remain at the periphery rather than the center of the arena. The shredding nestlet test also supports a high level of anxiety in our model. Moreover, VGluT3-depleted mice have a lack of interest for sweetened liquid reward. Using this model, we were able to highlight the involvement of VGluT3 in the regulation of anxious and spontaneous motor behaviors as well as in behaviors involving the reward system. In addition, our preliminary results suggest the involvement of VGluT3 in the maintenance of 5-HT axon morphology.

Disclosures: L. Saidi: 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.; Parkinson Canada. **M. Parent:** None. **C. Proulx:** None.

Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 380.20/E33

Topic: C.03. Parkinson's Disease

Support: The Grainger Foundation

Title: Quantification of subthalamic nucleus deep brain stimulation evoked changes in tonic dopamine levels *in vivo* using multiple cyclic square wave voltammetry

Authors: *A. S. BARATH[1], A. RUSHEEN[2], H. SHIN[4], C. D. BLAHA[2], D. JANG[5], K. E. BENNET[3], K. H. LEE[2], Y. OH[2];
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Abstract: Introduction: Deep brain stimulation (DBS) is a standard of care for patients with advanced Parkinson's disease. More than 30 years since its introduction in its current form, the mechanism of action of DBS remains elusive. It is well known that patients who respond favorably to levodopa also respond favorably to DBS. Also, it has been observed that the therapeutic benefits of DBS are maximal in the off phase of medication when the drug concentration falls below a therapeutic level. Therefore, we hypothesize that DBS in Parkinson's disease may act via modulation of striatal tonic dopamine concentration. Recently, we developed multiple cyclic square wave voltammetry (MCSWV) technique for measuring tonic levels of dopamine. In this study, we investigated how the tonic dopamine levels were modulated during subthalamic nucleus electrical stimulation using MCSWV for elucidating our hypothesis. **Method:** Normal male, Sprague Dawley rats weighing between 250-315 grams were anesthetized with urethane (1.5 g/kg i.p.) and administered buprenorphine (0.1 mg/kg s.c.) for pain control. Concentric bipolar stimulation electrodes were placed in the subthalamic nucleus (STN; ML:+2.5, AP:-3.8, DV:-7.6 in mm), a common DBS target for Parkinson's disease. Carbon fiber microelectrodes were placed in the dorsal striatum (ML:+2, AP:+1.2, DV:-3.5 to -4.5 in mm) for recording dopamine concentration. MCSWV was used to measure stimulation-evoked tonic level changes in dopamine. Fast-scan cyclic voltammetry was used to measure stimulation-evoked phasic changes in dopamine concentrations. Pharmacological confirmation of the dopamine responses was performed with nomifensine (20 mg/kg i.p.), a dopamine reuptake inhibitor. **Results:** STN electrical stimulation (0.4 mA, 90 Hz, 2 ms biphasic pulse width) for 2 sec resulted in stimulation time-locked phasic increase in oxidation current at 0.6 V. Tonic dopamine levels, recorded over a period of 3-4 hours, increased in response to 5 min of

STN stimulation with therapeutic DBS parameters. Increase in the tonic dopamine signal post nomifensine injection confirmed that the electroactive species being measured was dopamine. **Discussion:** These results demonstrate that modulation of tonic dopamine levels could be a possible mechanism of therapeutic effect of STN DBS in Parkinson's disease. Tonic dopamine levels measured using MCSWV may be used for a feedback biomarker in development of closed-loop DBS system. This would be anticipated to optimize symptom control in Parkinson's disease subjects, and prolong battery life. Also, MCSWV could potentially be used for elucidation of mechanism of action of DBS in other diseases.

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Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: Hall A

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Program #/Poster #: 380.21/E34

Topic: C.03. Parkinson's Disease

Support: 2018CXGC1502
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109115

Title: Electrophysiological and neurochemical considerations of distinct neuronal populationsin the rat pedunculopontine nucleus and their responsiveness following 6-hydroxydopamine lesions

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Abstract: Pedunculopontine nucleus (PPN) is composed of a morphologically and neurochemically heterogeneous population of neurons, which is severely affected by Parkinson's disease (PD). However, the role of each subtype neuron within the PPN in the pathophysiology of PD has not been fully elucidated. In this paper, we present the discharge profiles of three classified subtypes of PPN neurons and their alterations treated by 6-hydroxydopamine (6-OHDA) lesion. Following 6-OHDA lesion, spike timing of Type III neuron (glutamatergic) had a tight coordination with the oscillations in the 12-30 Hz frequency range in PPN. We also provide a morphological evidence showing either loss of neurons or altered somatic cell size occurred on the glutamatergic neurons in PD cases. These findings showed electrophysiological detection methods combined with immunohistochemistry have revealed that Parkinsonian variation in distinct firing characteristics has a close relationship with the neuronal morphology, which would make contributions to the divergence dysfunctions in Parkinsonism.

Disclosures: **X. Wang:** None. **M. Li:** None. **D. Chen:** None. **M. Wang:** None.

Poster

380. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Title: Glutamatergic neurons in the subthalamic nucleus regulate mechanical and thermal pain thresholds in Parkinsonian mice

Authors: *C. ZHOU, Y. LUAN, D. TANG, H. WU, W. GU, C. XIAO;
Xuzhou Med. Univ., Xuzhou City, China

Abstract: In PD patients, pain is a prevalent non-motor symptom. Poor understanding of its underlying mechanisms posits difficulty in optimizing its therapy. Deep brain stimulation (DBS) in the subthalamic nucleus (STN) of PD patients has been reported to relieve motor deficits, and pain symptoms, as well, suggesting that the STN could be a promising therapeutic target for PD pain. Parkinsonian mouse models were established with unilateral injection of 6-OHDA in the medial forebrain bundle to lesion DA neurons (by 60% in two weeks) in the substantia nigra pars compacta (SNc), and exhibited hypoactivity in the open field arena and apomorphine-induced contralateral rotations. From 7 days after 6-OHDA lesion, the mouse models displayed reduced mechanical and thermal pain thresholds in both hind paws. Meanwhile, the STN neurons on the lesion side fired twice as fast as those on the non-lesion side, and optogenetic inhibition of hyperactive STN glutamatergic neurons on the lesion side significantly elevated pain threshold. Direct stimulation of both STN glutamatergic neurons and their axonal projections to the substantia nigra pars reticulata (SNr) and internal segment of the globus pallidus (GPi) / ventral pallidum (VP) sufficiently reduced thermal and mechanical pain thresholds in healthy mice. Furthermore, selective inhibition of STN projections to the SNr and GPi/VP with optogenetics differentially increased mechanical and thermal pain thresholds in PD. Our c-fos staining data show that in control mice, optogenetic stimulation of STN glutamatergic neurons caused

hyperactivity in multiple nuclei implicated in pain perception and modulation, while in PD mice, optogenetic inhibition of the STN neurons reversed hyperactivity in ascending pain pathway, in contrast, enhanced neuronal activity in descending pain pathway. Our results support that STN glutamatergic neurons are projection-specifically implicated in pain hypersensitivity in PD models via regulating ascending and descending pain pathways, and can be a therapeutic target for pain symptoms in PD.

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Poster

381. Parkinson's Disease Oscillations

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Program #/Poster #: 381.01/E36

Topic: C.03. Parkinson's Disease

Support: NIH NINDS grant R01 NS058945
NIH NINDS grant P50 NS098573

Title: Changes in beta oscillatory activity within and across the basal ganglia-thalamocortical network in a progressive MPTP non-human primate model of Parkinson's disease

Authors: *Z. T. BUSBY[1], Y. YU[1], J. ZHANG[1], W. C. FRYLING[2], D. ESCOBAR SANABRIA[1], M. D. JOHNSON[3], L. A. JOHNSON[1], J. L. VITEK[1];
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Abstract: Alterations in beta band oscillatory activity and synchronization in the basal ganglia-thalamocortical (BGTC) network have been linked to the development motor signs in Parkinson's disease (PD). Although these changes are thought to occur throughout the BGTC network, most previous studies have only examined individual nodes in the circuit. Our understanding of how the progression of PD motor signs is associated with changes in oscillatory activity across multiple nodes in the BGTC network simultaneously is limited. We addressed these issues in this study by examining low and high beta band activity in local field potentials (LFP) recorded simultaneously across cortical and subcortical areas in a progressive non-human primate (NHP) model of PD.LFP activity was recorded with a 96 channel Utah array in primary motor cortex (M1) and two DBS leads targeting the subthalamic nucleus (STN) and globus pallidus internus (GPi). After normal state recordings, the animal was given intramuscular injections of MPTP weekly or biweekly. Following each injection, the severity of parkinsonism was evaluated using a modified clinical rating scale (mUPDRS) and spontaneous resting state LFPs were obtained. Beta band power within each site and coherence across sites were

calculated in the low (10-20 Hz) and high beta band (21-35Hz). Within several days after the second injection and the emergence of parkinsonian motor signs we observed an increase in low beta band activity in the STN and GPi but a decrease in M1. In the STN and GPi low beta power continued to increase coincident with increasing severity of PD. High beta band activity also changed in the parkinsonian condition, increasing early in M1 but only in more severe stages in STN and GPi. We observed an increase in coherence across M1-GP and STN-GP, but not in M1-STN. These changes occurred early in the low beta band although less consistently in the the high beta band.These results suggest that alterations in beta band activity occur early in the parkinsonian condition across both subcortical and cortical structures, and include both low and high beta bands. The differences between changes in low and high beta band as motor signs progressed suggests the possibility of distinct roles for low and high beta power changes in the development of parkinsonism. The significant correlation of low but not high beta band activity to the severity of parkinsonism, and the stronger and more consistent relationship between changes in the STN and GPi and motor signs would suggest that low beta band activity in these sites might be a better signal for biomarker-based stimulation strategies (e.g. closed-loop DBS).

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Poster

381. Parkinson's Disease Oscillations

Location: Hall A

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Topic: C.03. Parkinson’s Disease

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MnDRIVE (Minnesota's Discovery, Research and Innovation Economy) Brain Conditions Program
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Title: Oscillatory activity in the pallidum is subject and location specific in Parkinson’s disease

Authors: *L. A. JOHNSON[1], J. E. AMAN[1], D. ESCOBAR SANABRIA[1], J. WANG[1], R. PATRIAT[2], M. E. HILL[1], S. E. COOPER[1], L. E. SCHROCK[1], N. HAREL[2], M. C. PARK[3], J. L. VITEK[1];
[1]Neurol., [2]Radiology, [3]Neurosurg., Univ. of Minnesota, Minneapolis, MN

Abstract: Background: Local field potential (LFP) recordings from recently introduced directional deep brain stimulation (dDBS) leads may provide insights into the pathophysiology of Parkinson’s disease (PD) and be a useful tool to inform programming strategies that optimize

clinical benefit. There is little data, however, regarding LFP activity in the globus pallidus internus (GPi), a target increasingly used for DBS in PD. The goal of this study was to utilize dDBS leads to characterize the spectral characteristics and spatial topography of LFP activity in the GPi in patients with PD.

Methods: Data were collected from externalized dDBS leads of three patients with idiopathic PD after overnight withdrawal of parkinsonian medication. Bipolar LFPs were created by subtracting recordings from vertically adjacent contacts, and spectral analysis was performed using customized scripts in MATLAB. Oscillatory activity across lead segments was examined in the context of lead locations and segment orientations determined using co-registered preoperative 7-T MRI and postoperative CT scans, as well as clinical DBS settings chosen in subsequent patient programming visits.

Results: We found that each of the three patients had a unique topography of oscillatory activity in the pallidum, with prominent peaks in the 5-35Hz range occurring at different frequencies and spatial locations. Despite subject-specific spectral profiles, a consistent finding across patients was that oscillatory power was strongest in LFPs recorded from segments facing the posterior-lateral "sensorimotor" region of GPi, whereas medial segmented contacts facing the internal capsule had the weakest LFP activity. Interestingly, the clinically chosen contact configurations for optimizing benefits in the three subjects aligned with the contact pairs showing the largest amplitude of LFP oscillations relative to the other contacts within the same lead.

Conclusions: These data support the hypothesis that optimal improvement in motor signs is location specific and localized to the sensorimotor territory of the GPi and corresponds to the site of maximal power in LFP activity within frequency spectrums that is patient specific. These data also provide compelling evidence for the use LFP activity obtained perioperatively for the development of local predictive stimulation models that may optimize patient benefits while reducing clinic time needed for programming.

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Poster

381. Parkinson's Disease Oscillations

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Topic: C.03. Parkinson's Disease

Support: NIH R01 NS037019
NIH R01 NS058945
NIH R01 NS077657
NIH P50 NS098573

Title: Intermittent delivery of coordinated reset deep brain stimulation is more effective than continuous delivery

Authors: *S. P. FERGUS[1], K. M. SWITALLA[1], J. ZHANG[1], L. A. JOHNSON[1], M. D. JOHNSON[2], J. WANG[1], J. L. VITEK[1];
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Abstract: Parkinson's disease (PD) has been closely linked to abnormal neuronal synchrony in the basal ganglia-thalamocortical network. A novel deep brain stimulation (DBS) technique, termed coordinated reset (CR), was developed to disrupt this pathological synchrony. CR DBS randomly delivers low intensity bursts across multiple contacts of the DBS lead. Modeling studies have shown that intermittent CR might be more effective at inducing carryover benefits than continuous delivery of CR DBS. In this study, we tested this hypothesis by comparing the effect of CR DBS delivered continuously and intermittently. A within-subject experiment design in a nonhuman primate (NHP) model of PD was used to evaluate the relative effect of CR DBS delivered continuously versus intermittently. The NHP was made parkinsonian using the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and implanted with an 8-contact DBS lead in the subthalamic nucleus. With the same total amount, i.e. 48 hours, of stimulation, continuous CR was delivered for two days while intermittent CR was delivered over four days with a 2-hour ON: 2-hour OFF cycle. Motor assessments including a clinical rating scale modified for NHPs (mUPDRS) and a reach task were performed daily on treatment days and for six days after treatment. We observed that intermittent CR produced greater acute and carryover motor benefit than continuous CR. The percent improvement in mUPDRS scores during intermittent CR (23.5%) was significantly higher than during continuous CR (14.1%). Therapeutic effects of intermittent CR persisted after stimulation cessation, with up to 20.7% mUPDRS improvement through the fourth day after treatment, while continuous CR did not induce any carryover improvement. Intermittent CR also produced greater acute and subacute improvement in movement velocity. These findings support our hypothesis that CR DBS

delivered intermittently will be more effective in alleviating PD motor signs with a longer carryover effect. Although additional studies in more subjects will be needed for further validation of this finding, these data provide the rationale for further innovation in how we can modify DBS approaches to optimize its benefit for patients with PD.

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Poster

381. Parkinson's Disease Oscillations

Location: Hall A

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Program #/Poster #: 381.04/E39

Topic: C.03. Parkinson’s Disease

Support: NIH NINDS grant R01 NS058945
NIH NINDS grant P50 NS098573

Title: Task-related modulations of beta oscillatory activity in the basal ganglia and motor cortex are altered in Parkinsonism

Authors: *Y. YU[1], D. H. ROSS[1], S. NEBECK[1], J. ZHANG[1], M. D. JOHNSON[2], L. A. JOHNSON[1], J. L. VITEK[1];
[1]Dept. of Neurol., Univ. of Minnesota Dept. of Neurol., Minneapolis, MN; [2]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Beta band oscillatory activity in the basal ganglia-thalamocortical (BGTC) network is known to play an important role in motor control, and excessive beta activity has been suggested to be associated with the development of motor signs in Parkinson's disease (PD). The nature of how parkinsonism might disrupt the temporal dynamics of beta band oscillatory activity across the BGTC network during motor tasks, however, is not well understood. Using a within subject design, in this study we recorded local field potentials (LFPs) from basal ganglia and cortical sites simultaneously during a reaching task to investigate how the induction of parkinsonism alters movement-related modulations of beta oscillatory activity in the BGTC.One non-human primate (rhesus macaque) was trained to perform a food reach and retrieval task while LFP data was simultaneously collected from a microelectrode array in the primary motor cortex (M1) and macroelectrodes in the subthalamic nucleus (STN) and internal globus pallidus (GPi) in the normal, mild and moderate parkinsonian state. Parkinsonism was induced by sequential injections of MPTP. Perievent spectrograms were calculated to investigate the changes in low (10-20 Hz) and high (21-30 Hz) beta band LFP activity that occurred during the reaction and reaching time periods on a trial by trial basis relative to the pre-movement baseline period.

Increasing parkinsonian severity was associated with higher pre-movement baseline levels of low beta activity in the STN, GPi and M1, which was significantly reduced during movement. Similar changes were observed for high beta activity in the STN and M1 but not GPi. In the parkinsonian condition during the reaction time period (following cue presentation prior to movement) low beta was reduced across all three sites while high beta was increased in the STN and M1.These data support the hypothesis that the coordination of oscillatory activity across multiple power spectrums are required for normal movement and that the abnormal movement observed in parkinsonism likely results from a disruption in the temporal dynamics of this activity across nodal points in the BGTC circuit.

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Poster

381. Parkinson's Disease Oscillations

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Title: Longitudinal analysis of local field potentials recorded from deep brain stimulation leads in the subthalamic nucleus

Authors: *A. BRINDA[1], A. M. DOYLE[2], J. D. KRIEG[1], J. ALISCH[1], L. K. WILMERDING[3], M. BLUMENFELD[1], J. DAO[1], M. D. JOHNSON[1];
[1]Biomed. Engin., [2]Neurosci., Univ. of Minnesota - Twin Cities, Minneapolis, MN; [3]Boston Univ., Boston, MA

Abstract: The electrode-tissue interface around a deep brain stimulation (DBS) lead is known to be highly dynamic and spatially heterogeneous following implantation, which may have implications on interpretation of recorded local field potentials (LFPs) and use of these signals in the context of closed-loop DBS therapies. Electrochemical impedance spectroscopy (EIS) is a useful tool for characterizing the formation of a fibrous tissue encapsulation layer around a DBS lead during the post-implant immune reaction. In this study, we longitudinally tracked the resting-state LFP spectral power beginning right after lead implant and ending two weeks post-implant. This time period captures the acute microvasculature disruption and immune reaction, consisting of vasogenic and cellular edema, leading to a chronic tissue response in which fibrous tissue encapsulation layers begin to stabilize. Two drug-naive, non-human primates (*macaca*-

mulatta, female) were each implanted with a directional DBS lead in the subthalamic nucleus. In parallel to LFP recordings, EIS was collected each day and analyzed using an equivalent circuit model to aid in characterizing the electrode-tissue interface dynamics. Significant increases in both electrode site impedances (1kHz and 20Hz) and LFP spectral power were observed for both animals in the period between putative vasogenic edema (low tissue impedances, days 1-2) and cellular edema (high tissue impedances, days 7-9) (paired, two-sided Wilcoxon signed-rank test, p=0.03, n=6 and n=12). In subject A, these changes in average impedance and spectral power are both confirmed to have occurred after days 2 and 8 by a change-point analysis (CPA). For subject B, the average impedances directly correlated with the average LFP spectral power over the 15 days after surgery (Pearson Correlation, p=1.1e-5 and p=3.7e-4, n=15). Interestingly, CPA reveals a major time point in equivalent circuit model parameters, but not in LFP spectral power, after day 2 in subject B. Both impedance and LFP data show a significant change after day 5, suggesting the cellular edema phase progressed at a faster rate than in subject A. These results suggest that LFP spectral power is relatively weak in the peri-implant stage as compared with the chronic, post-implant stage in which electrode site impedance has significantly increased. Not only do these results show dramatic changes in LFPs recorded post-implant, they additionally suggest that electrochemical impedance spectroscopy can be helpful in relating electrode-tissue interface dynamics to interpretation of LFPs.

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Poster

381. Parkinson's Disease Oscillations

Location: Hall A

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ControlExtraData.DynamicPosterDisplay:
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Parkinson Study Group and the Parkinson’s Disease Foundation’s Advancing Parkinson’s Treatments Innovations Grant

Title: Dynamic changes in oscillatory activity in the pallidum during motor tasks in Parkinson's disease

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Abstract: Background: Parkinson's disease (PD) has been associated with exaggerated low frequency (~5-35 Hz) oscillatory activity in the subthalamic nucleus (STN). Relatively few studies, however, have examined local field potential (LFP) activity in the internal segment of the globus pallidus (GPi) in PD patients, particularly its relationship to movement and severity of PD motor signs. The goal of this study was to characterize the dynamic changes in LFP activity in the GPi during motor tasks in PD patients with externalized directional DBS leads. **Methods**: Three PD patients were implanted with DBS leads in the GPi. LFPs were recorded from the externalized DBS leads in the patients' off medication and off DBS state at rest, during a cued reach-to-target task, and during a passive movement task designed to measure rigidity of the forearm. Lead location and orientation were determined using pre-operative high resolution 7T MR images registered to post-operative CT scans. **Results**: During the active reaching task subject-specific modulations of both high (>20Hz) and low (<20Hz) beta band oscillatory activity were observed. High and low beta oscillatory activity desynchronized coherently during movement in one subject, while in a second low beta desynchronized during movement while high beta power was reduced between each reach and return movement. A third subject showed only low beta modulation. The passive motor task revealed modulation of beta oscillatory activity in specific frequency bands that was also subject specific. **Conclusions**: Our results show that GPi oscillatory activity in the 5-35 Hz range is modulated during movement, and is both patient, location and task specific. Optimization of closed loop DBS algorithms will likely need to address these variables in order to develop customized algorithms based on each patient's unique physiological profile.

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Poster

381. Parkinson's Disease Oscillations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.07/E42

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01-NS094206
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Title: Subject-specific computational models of subthalamic nucleus deep brain stimulation with pallido-pedunculopontine axis fibers of passage

Authors: *M. GOFTARI[1], J. KIM[2], M. LIVANOS[3], R. PATRIAT[4], E. JOHNSON[5], L. SCHROCK[5], N. HAREL[4], S. E. COOPER[5], M. D. JOHNSON[1];
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Abstract: Deep brain stimulation (DBS) targeting the subthalamic nucleus (STN) can be an effective neurosurgical treatment for the motor signs of Parkinson's disease (PD). While parkinsonian tremor, rigidity, and bradykinesia are often readily straightforward to treat, STN-DBS typically has more mixed effects on parkinsonian gait. Given that levodopa therapy exhibits similar mixed effects, parkinsonian gait is thought to involve network dysfunction beyond the nigrostriatal dopaminergic pathway. The pedunculopontine nucleus (PPN) within the brainstem is known to be involved in gait, has connections to and from the basal ganglia, and has axons that course through and/or adjacent to the STN. In this study, we developed subject-specific computational models built from a combination of segmented 7T patient imaging data, patient lead localization, finite element models predicting DBS-induced extracellular tissue voltages and multi-compartment biophysical neuron models predicting the degree to which neuronal pathways are modulated by a given STN-DBS set of stimulation settings. The models consisted of nine pathways in and around the STN, including (1) a pallidofugal pathway that projected along the lenticular fasciculus and to motor thalamus and pedunculopontine nucleus, and (2) pedunculopontine efferents that coursed through the STN en route to the globus pallidus via the ansa lenticularis. The models showed activation (~40-80%) of the pedunculopontine pathway for most clinically-optimized STN-DBS settings across subjects (n=5 hemispheres). The activation of the pallidofugal pathway at these settings was robust for only 1/3 subjects, in which ~70% activation of the pallidofugal pathway was observed. The models also suggest that it is possible to design stimulation settings that can more selectively activate pathways in and around the STN. These results have important implications for better treatment of individual motor signs of Parkinson's disease.

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Poster

381. Parkinson's Disease Oscillations

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Topic: C.03. Parkinson's Disease

Support: Udall grant P50-NS098573
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Title: Investigating the role of the centromedian nucleus in gait

Authors: *A. M. DOYLE[1], J. D. KRIEG[2], L. K. WILMERDING[2], K. BRACKMAN[1], A. R. RUPPERT[1], A. JACOBSON[1], A. GRAY[1], D. BOUDANI[1], J. L. VITEK[3], M. D. JOHNSON[2]; [1]Neurosci., [2]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN; [3]Dept. of Neurol., Univ. of Minnesota Dept. of Neurol., Minneapolis, MN

Abstract: Parkinson's disease has been characterized by degeneration in the centromedian-parafascicular nucleus (CM-PF) of the thalamus. Changes in CM-PF activity are known to reflect arousal and abrupt changes in behavior, and may have relevance to several parkinsonian motor signs including challenges with selecting new motor actions in changing environments and potential contribution to freezing-of-gait. In this study, we investigated how firing rate, firing patterns, and oscillations changed with the onset of parkinsonism and how these changes relate to gait behaviors including gait initiation and reaction to new obstacles or environmental stimuli. A non-human primate was implanted with a 96-channel microdrive that targeted the CM-PF amongst other brain regions. Wide-band wireless recordings were collected from the microdrive channels while the subject ambulated through a custom-designed habitrail enclosure. The habitrail included two boxes, in which food rewards were administered, that were connected through an 9'-long walkway with a pressure mat to rapidly quantify the spatial and temporal dynamics of gait. Obstacles were introduced into the habitrail walkway periodically during the course of the subject navigating the habitrail. Drug-naive spike recordings from CM-PF cells showed no clear modulation to individual limb movements; however, cells were robustly modulated in response to gait cessation, gait initiation, and the presentation of novel stimuli in the habitrail environment. These results suggest that the degeneration within the CM-PF in Parkinson's disease may be responsible in part to motor signs that are triggered by environmental cues including freezing of gait. Funding acknowledgments: Udall grant P50-NS098573 and NIH Grant 1F31NS108625-01

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Poster

381. Parkinson's Disease Oscillations

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.09/E44

Topic: C.03. Parkinson's Disease

Support: NIH T-32

Title: Thalamic white matter investigated with serial PS-OCT imaging

Authors: ***M. YEATTS**[1], C. LIU[1], S. R. HEILBRONNER[2], M. D. JOHNSON[3], T. AKKIN[1]; [1]Univ. of Minnesota, Minneapolis, MN; [2]Neurosci., Univ. of Minnesota, New Brighton, MN; [3]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Introduction: Deep brain stimulation (DBS) targeting neural pathways within the thalamus has been used to treat individuals with essential tremor, epilepsy, and schizophrenia, among others. The precise neural pathways underlying the therapy are often difficult to disentangle since the thalamus has heterogeneous axonal tractographies that are not readily visible with traditional imaging techniques such as diffusion tensor imaging (DTI). In this study, we developed a polarization-sensitive optical coherence tomography (PS-OCT) approach to visualize axonal pathways within and adjacent to non-human primate (NHP) thalamus with 10-micron lateral resolution.
Methods: Two perfused and paraformaldehyde-fixed rhesus macaque brains were blocked about the right thalamus. The initial exposed face was scanned in a tiled array using PS-OCT before removing a 100-micron thick slice to access the deeper regions. One thalamus was imaged in the sagittal plane, and the other was imaged in the coronal plane using repeated imaging and slicing procedures. For each exposed face, the reflectance, retardance, and cross polarization images were collected. The images were stitched, aligned, and stacked into a three-dimensional block to render thalamic axonal tracts.
Results: PS-OCT enabled visualization of both afferent and efferent pathways that across multiple thalamic nuclei relevant to DBS therapy. For example, distinct tracts of differing orientations in the region of the anteriomedial and centromedian thalamic nuclei, the mamillothalamic tract, the habenulopeduncular tract, and separable bundles of the internal capsule. The stria medularis and fornix were also visualized with highly aligned and dense white matter tracts.
Conclusions: Improved understanding of axonal tractography within thalamus will be important to optimize DBS therapy for a range of existing and emerging clinical indications. This imaging

approach is poised to enable rendering of axonal tracts in three dimensions and help guide future DBS lead designs and programming algorithms.

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Poster

381. Parkinson's Disease Oscillations

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Topic: C.03. Parkinson's Disease

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Title: Comparison of therapeutic efficacy between multiple current sources and multiple stimulation sets

Authors: *J. D. S. KRIEG[1], A. M. DOYLE[2], J. SLOPSEMA[1], F. AGNESI[3], M. D. JOHNSON[1];
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Abstract: Deep brain stimulation (DBS) is a well-established surgical therapy for a growing list of neurological and neuropsychiatric disorders. With this fully implantable therapy, electrical pulse trains are delivered to key nodal points within the brain, and aim to disrupt the propagation of pathological information throughout the larger brain network. Successful therapy relies heavily on the precision by which stimulation through one or more electrodes along the DBS lead can disrupt this pathological information. One approach to improve targeting precision has been leveraging technology from the cochlear implant field that integrates multiple independent current sources (MICS) into the neurostimulator hardware. However, the utility of such technology comes at the cost of hardware design complexity. In this study, we investigated a novel sequential, multiple stimulation set (MSS) approach using a single current source that rapidly switches between electrodes along a DBS lead. We specifically compared these MICS and MSS approaches in computational models and *in vivo* in two non-human primates, who were implanted with a 6-channel directional DBS lead in the subthalamic nucleus. The models suggest that while stimulation thresholds between MICS and MSS (evaluated at 0.5, 1.0, 1.5, 2.5, and

3.75 ms switching delays) were not significantly different overall, there are likely to be subtle differences in axonal orientation selectivity as the delay between pairs of MSS pulses increases. In the *in vivo* study, as proof of principle, there were no significant differences in stimulation thresholds for inducing motor contraction side effects during STN-DBS. The results suggest that MSS at short switching delays has promise to augment single current source stimulation paradigms and refine targeting of DBS therapy in cases when neurostimulator hardware is limited to a single current source.

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Poster

381. Parkinson's Disease Oscillations

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.11/F2

Topic: C.03. Parkinson’s Disease

Support: NINDS Grant 5 R21 NS096398-02

Title: Limited trunk motion and variability during gait contribute to FOG and improve during STN DBS

Authors: *J. O'DAY[1], C. ANIDI[2], R. W. ANDERSON[3], J. PARKER[1], M. N. PETRUCCI[4], R. NEUVILLE[1], H. BRONTE-STEWART[5];
[2]Neurol. and Neurolog. Sci., [1]Stanford Univ., Stanford, CA; [3]Neurol., Stanford Univ., San Francisco, CA; [4]Neurol., [5]Dept. of Neurol., Stanford Univ., Stanford, CA

Abstract: Objective: Limited truncal motion during forward walking may contribute to freezing of gait (FOG) in PD but has not been extensively investigated. Lower (60Hz) and high (140Hz) subthalamic deep brain stimulation (STN DBS) may improve FOG (Xie 2015, Anidi 2018) but whether this is associated with changes in lumbar motion during gait is not known. We hypothesized that PD freezers would exhibit restricted lumbar motion and variability during a turning and barrier course (TBC) known to elicit FOG (Syrkin-Nikolau 2017) and that these would improve during STN DBS.
Methods: Twelve PD subjects (7 male), off medication, received randomized presentations of no, 60 Hz, and 140 Hz STN DBS, while walking in the ellipses and figures of eight through narrow openings of the TBC. Nine age-matched healthy controls completed the TBC. A validated logistic regression model identified periods of FOG based on shank inertial measurement unit (IMU) data. The root mean square angular velocity (Vrms) of trunk motion and the standard deviation of the trunk’s linear acceleration (SD_{LA}) was calculated from a lumbar IMU, in the anterior-posterior (AP) and medio-lateral (ML) directions during the TBC.

Results: Freezers (N = 7) had reduced trunk Vrms (AP and ML) and SD_{LA} (AP) while OFF therapy during the TBC compared to controls ($p < 0.02$). Both 60Hz and 140Hz DBS improved freezers' percent time freezing ($p < 0.05$) to that of non-freezers. Freezers' lumbar AP Vrms increased on 140 Hz DBS compared to OFF ($p < 0.05$), and to a value similar to that of controls and non-freezers. Freezers' lumbar ML Vrms also increased during both 60 Hz and 140 Hz DBS ($p < 0.05$). Freezers' AP SD_{LA} increased during 60 Hz and 140 Hz DBS, ($p < 0.03$). Freezers' normal ML SD_{LA} showed no change during DBS. Non-freezers' AP and ML SD_{LA} increased during 140 Hz DBS ($p < 0.05$) toward control levels, though non-freezers' lumbar AP and ML Vrms did not change during DBS.
Conclusions: During the TBC, freezers demonstrated FOG and reduced truncal motion and variability compared to that of non-freezers and controls. STN DBS at 60Hz and 140Hz resolved FOG and improved truncal motion and variability in freezers. These results support our hypotheses that freezers exhibit reduced truncal motion and variability during gait, which improve during STN DBS, in association with resolution of FOG. This suggests that reduced truncal motion and variability contribute to FOG and that lumbar kinematics may be useful control variables for closed loop DBS systems aimed at improving FOG. To our knowledge this is the first study to investigate truncal kinematics during a gait task that mimics real life situations that elicit FOG and during lower and high frequency STN DBS.

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Poster

381. Parkinson's Disease Oscillations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.12/F3

Topic: C.03. Parkinson's Disease

Support: National Natural Science Foundation of China 61701323

Title: Alternations in M1 LFP oscillations of Parkinsonian mice during different movement states

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Abstract: Neural oscillation impairment is thought to be a candidate pathophysiological mechanism underlying movement disorders such as Parkinson's disease. There remains, however, a pressing need to carefully characterize neural oscillations in PD during different movement states. At present, the research in humans and rats has become increasingly rich, but

the research in mice is poor. To address this, we simultaneously recorded local field potentials (LFPs) from M1 of mice (control and 6-OHDA group) when they performed an open field test, to investigate the properties of neural oscillatory activity. The behavioral results showed that the 6-OHDA mice spent significantly less amount of time moving, but more time freezing compared with the control group. And we detected that in 6-OHDA, the power of M1 LFP oscillations at the beta (11-30Hz) and gamma (30-55Hz) range frequencies were stronger than those of control mice during resting state. The opposite was observed for moving state. Furthermore, we investigated whether the two oscillations would change between the different behavioral states. In the control mice, the oscillations at beta frequencies diminished considerably in moving states compared to resting. And the opposite was found for the gamma oscillations. It is worth noting that the 6-OHDA mice didn't display the similar trends when a change in behavioral state occurred. The results show that the oscillatory activity across the M1 in the normal and 6-OHDA states reveals two characteristic frequency bands which may be associated with movement. Thus, our findings suggest that pathological beta and gamma oscillations might be the much more sensitive neurophysiological markers for Parkinson' mice.

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Poster

381. Parkinson's Disease Oscillations

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Topic: C.03. Parkinson's Disease

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Medtronic Inc.

Title: A comparison of methods for quantifying subthalamic beta burst dynamics in people with Parkinson's disease

Authors: *R. W. ANDERSON, R. S. NEUVILLE, C. M. ANIDI, M. N. PETRUCCI, J. E. PARKER, A. VELISAR, H. M. BRONTE-STEWART;
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Abstract: Parkinson's disease (PD) is a progressive neurological disorder, the neural signature of which includes an increase in synchronization of neuronal activity in the beta frequency range (13-30 Hz) in the basal ganglia and motor cortex. Increases in neural synchrony have been measured by averaging local field potential (LFP) power in overlapping bins and representing this in power spectral density (PSD) analysis and time frequency spectrograms. These techniques are well suited to examine the average power in the signal but are less sensitive to the temporal fluctuations in neural (or beta) synchrony ('beta burst' dynamics) that may be more relevant to distinguish pathological from physiological neural activity. Recently, four methods (termed Anderson1, Anderson2, Feingold, and Tinkhauser) have been proposed to quantify beta burst dynamics, all of which use bandpass filtering and rectification but which differ in the calculations of the threshold, critical in determining the duration of elevated LFP power. In addition it is not known whether the width of the frequency band chosen for analysis affects burst dynamics.
In this study we compared the four methods of beta burst analysis using chronic LFP recordings in the resting state from PD subjects implanted with an investigative sensing neurostimulator (Activa PC+S, Medtronic Inc.). Each method was sensitive to different aspects of the LFP and the choice of method affected the range of beta burst durations captured and mean beta power. Using 1000 trials of 1/f pink noise, it was evident that the choice of filter bandwidth affected burst duration. We also compare the four methods in the analysis of beta burst dynamics in the akinetic rigid and tremor dominant phenotype of PD. The choice of burst analysis method will be important for different objectives, such as when focusing on pathological (longer) burst dynamics or when examining both physiological and pathological burst dynamics, and when designing closed loop neurostimulation algorithms.

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Poster

381. Parkinson's Disease Oscillations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

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Helen M. Cahill Award for Research in Parkinson's Disease

Medtronic Inc.

Title: The relationship between beta burst durations and the conversion to freezing of gait in Parkinson's disease

Authors: *M. N. PETRUCCI[1], R. S. NEUVILLE[1], R. W. ANDERSON[1], J. E. PARKER[1], C. M. ANIDI[1], J. J. O'DAY[2], A. VELISAR[1], H. M. BRONTE-STEWART[1];
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Abstract: Freezing of gait (FOG) is a debilitating symptom of Parkinson's disease (PD) that worsens with disease progression and impacts quality of life. Increased burst durations in the beta band of local field potentials (LFPs) in the subthalamic nucleus (STN) have been observed in people with PD and FOG compared to those without FOG, and burst durations are longer during a FOG episode (Anidi et al., 2018). The purpose of this study was to investigate if burst durations in the beta band are associated with the development of FOG symptoms in people with PD. Participants that had FOG, did not have or develop FOG, or developed FOG, performed a stepping in place (SIP) and a rapid alternative finger tapping task (RAFT) with simultaneous bilateral recording of STN LFPs using a fully implanted neurostimulator (Medtronic Activa® PC+S system). Burst durations within the high and low beta bands during the SIP task were compared between two time points 35 ± 3 months apart. Burst durations in the same bands during the RAFT task performed within 1-3 months of the first time point were compared to the SIP task at the second time point. Kolomogrov-Smirnov tests were used for all comparisons between tasks within each band. Preliminary results suggest that FOG may be associated with a significantly broader distribution of bursts (towards longer durations) in the low and high beta bands in participants that had or developed FOG. This increase in beta bursts was associated with an increase in arrhythmicity and percent time freezing within the SIP task. Furthermore, the distribution of burst durations within the RAFT task was not different than the SIP task performed at the second time point for these participants, suggesting that beta burst associated with freezing behavior in the RAFT task may precede the development of FOG. No difference in burst durations or movement kinematics in the SIP task was observed in the participant without FOG between time points. Overall, an increase in the amount of longer burst durations in the beta band and worsening movement was found in participants with FOG or who developed FOG in the SIP and RAFT tasks, potentially providing an additional biomarker for predicting the conversion to FOG.

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Poster

381. Parkinson's Disease Oscillations

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Title: Neuromodulatory effects of electroacupuncture on corticostriatal beta synchronization and motor dysfunction in Parkinsonian rats

Authors: *J. JIA, X. JIANG, Y. YAN, K. WANG, J. WEI;
Dept. of Physiol. and Pathophysiology, Capital Med. Univ., Beijing city, China

Abstract: Exaggerated beta synchronized oscillation in the cortico-basal ganglia circuit is a hallmark of disease-specific motor symptoms in Parkinson's disease (PD). Series of studies have shown that Electro-acupuncture (EA) could improve the motor symptoms of PD, yet the potential mechanism remains unclear. In the present study, injection of 6-hydroxydopamine (6-OHDA) into the right medial forebrain were used as parkinsonian model. And the electrical stimulation at 100 Hz was administrated on the specific acupoints "Baihui" and "Dazhui" (GV20 and GV14, respectively). So the effects of EA on the motor symptoms, the striatal glutamate levels and corticostriatl beta synchronized oscillations were investigated. Four weeks EA treatment significantly alleviated the motor signs of 6-OHDA lesioned rats including the decreased latency of fall in the rotarod and the reduced movement distance in the open-field test. Notably, EA exhibited a time-dependent effect on the improvement of motor symptoms. However, neither the dopaminergic neurons in the substantia nigra nor the dopamine levels in the striatum were restored by the EA stimulations. Conversely, the increased release of glutamate in the striatum of 6-OHDA rats was inhibited by the EA stimulation in a time-dependent manner. Enhanced high beta (25–40 Hz) oscillations and coherence between the motor cortex and dorsolateral striatum were found in 6-OHDA lesioned rats. EA treatment obviously reduced the beta oscillations and desynchronization of corticostriatal circuit in a time-dependent manner. In additional, selectively inactivation of glutamatergic projection with the chemogenetical strategy reduced the beta oscillatory synchronization in the corticostrital circuit and alleviated motor symptoms in 6-OHDA lesioned rats. Thus, these data suggested that chronic EA treatment produced the dynamic modulatory effects on the striatal glutamate release, the synchronized beta oscillations of coritcostriatal circuit and the motor behaviors in 6-OHDA-lesioned rats.

Glutamate transmission probably mediated the alternation of the corticostriatal coordination in the pathophysiology of parkinsonism.

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Poster

381. Parkinson's Disease Oscillations

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Topic: C.03. Parkinson's Disease

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Title: Freezing of gait and subthalamic neural dynamics during stepping are modulated when altering optic flow in a virtual environment in Parkinson's disease

Authors: ***J. E. PARKER**[1], J. O'DAY[2], R. S. NEUVILLE[1], M. N. PETRUCCI[1], R. W. ANDERSON[1], A. VELISAR[1], H. M. BRONTE-STEWART[1];
[1]Neurol. and Neurolog. Sci., [2]Bioengineering, Stanford Univ., Stanford, CA

Abstract: Freezing of Gait (FOG) in Parkinson's disease (PD) is exacerbated when optic flow during forward motion is limited. Congruent optic flow in normal or virtual environments may improve/minimize FOG. We recently reported that subthalamic (STN) beta (movement) band burst dynamics differentiated PD freezers from non-freezers during non-freezing gait (Anidi 2018). In this study we investigated whether altered optic flow in a virtual environment during a stepping in place (SIP) task changed gait/FOG and/or STN neural dynamics.
Seven PD subjects (14 STNs), off therapy, performed 100s of SIP on dual forceplates with a visual surround (Bertec Corp., OH, USA) with no optic flow (nOF), and with a virtual optic flow environment not linked to their cadence (altered optic flow, aOF). STN local field potentials (LFPs) were recorded from an implanted, sensing neurostimulator (Activa® PC+S, Medtronic, Inc.) and synchronized with kinetics. Band burst dynamics (mean duration, normalized burst power) were analyzed from the alpha (8-12 Hz) and the beta movement band (4Hz band around the peak frequency during SIP) and compared between SIP/nOF and SIP/aOF. Comparisons with a forward walking task with optimized congruent optic flow will be investigated.
Gait impairment and FOG were worse during SIP/aOF: frequency of stepping decreased in 100%

of subjects and arrhythmicity increased in 86% of subjects; the percent time freezing increased in 4 subjects, who already exhibited FOG in the SIP/nOF condition, and FOG appeared during SIP/aOF when not apparent during SIP/nOF in one subject. Neural dynamics of the movement band (MB) became more pathological: during SIP/aOF, MB normalized mean burst power increased in 69% of STNs, and MB mean burst durations increased in 54% of STNs. Alpha band mean burst power increased in 71% of STNs and alpha mean burst durations decreased in 57% of STNs during SIP/aOF.
Gait/FOG and STN MB neural dynamics (mean burst duration and power) became worse during stepping with altered optic flow in the majority of PD subjects. Alpha band burst power increased in the majority of subjects and burst durations decreased in 57% of subjects; whether this was related to cognitive impairment will be investigated. While some studies have shown the positive impact of virtual reality on FOG, this virtual environment was not linked to the subject-specific cadence and acted as incongruent optic flow, disrupting FOG and STN neural dynamics. These results demonstrate that STN neural signatures of FOG are sensitive to altering the congruency of optic flow during motion, substantiating their role in the pathogenesis of FOG in PD.

Disclosures: **J.E. Parker:** None. **J. O'Day:** None. **R.S. Neuville:** None. **M.N. Petrucci:** None. **R.W. Anderson:** None. **A. Velisar:** None. **H.M. Bronte-Stewart:** F. Consulting Fees (e.g., advisory boards); Dr. Bronte-Stewart is a member of the clinical advisory board for Medtronic Inc..

Poster

381. Parkinson's Disease Oscillations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.17/F8

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation
NINDS Grant 5 R21 NS096398-02
Robert and Ruth Halperin Foundation
John A. Blume Foundation
Helen M. Cahill Award for Research in Parkinson's Disease
Medtronic Inc.

Title: Beta peak frequency and burst dynamics are conserved across different movements in Parkinson’s disease

Authors: R. S. NEUVILLE, R. W. ANDERSON, M. N. PETRUCCI, J. E. PARKER, A. VELISAR, ***H. BRONTE-STEWART**;
Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA

Abstract: Adaptive or closed-loop deep brain stimulation (aDBS) driven by STN beta band power when PD subjects are seated works; it is unknown if the same neural control variable(s) will be relevant when subjects are performing different movements, such as typing, eating, or walking. To investigate whether an aDBS system would have to continually update its classifier algorithms when subjects performed different movements, we compared the beta movement band peak frequency and burst metrics during fine, limb, and axial movement tasks in freely moving PD subjects. STN local field potentials (LFPs) were recorded from a sensing neurostimulator (Activa® PC+S, Medtronic, Inc.) in 11 PD subjects (22 STNs), during thirty seconds of repetitive alternating finger tapping, wrist-flexion extension, stepping in place, and free walking; subjects were off medication/off DBS, 1-2 months after DBS implantation. A peak detection algorithm determined and compared the peak frequency in the beta band during each movement. Subject-specific, movement bands were ± 3 Hz around the averaged peak frequency of the four movement tasks. Movement band burst dynamics were calculated and compared among tasks and to a gamma band in which there was no peak detected above the 1/f curve. The peak frequency was similar among the four movement tasks ($p=0.588$). There was no difference between mean movement band burst duration, burst peak power, and burst average power among tasks ($p=0.192$, 0.464, 0.433 respectively). There was no difference within these burst metrics between tremor dominant and akinetic rigid subjects, regardless of task ($p=0.286$, 0.205, and 0.196 respectively). Mean burst duration, peak power, and burst average power were longer/greater in the movement band than in the gamma band, regardless of the task ($p=0.003$,<0.001,<0.001 respectively). The peak frequency of exaggerated beta synchrony (beta peak) was conserved among fine, limb, and axial movements. The movement band mean burst duration, peak and average power were also conserved among tasks. The conservation of a PD subject's neural profile during different movements supports the feasibility of aDBS using the same beta band power or burst metrics during different activities of daily living. Burst durations and power were longer/greater in a band with elevated power over the 1/f curve compared to one without, supporting the premise that prolonged LFP burst durations are a feature of a pathological rather than physiological neural network.

Disclosures: R.S. Neuville: None. **R.W. Anderson:** None. **M.N. Petrucci:** None. **J.E. Parker:** None. **A. Velisar:** None. **H. Bronte-Stewart:** F. Consulting Fees (e.g., advisory boards); Clinical advisory board for Medtronic Inc.

Poster

381. Parkinson's Disease Oscillations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.18/F9

Topic: C.03. Parkinson's Disease

Support: APDA

NINDS-NS 1276394
GAANN

Title: Reinforcement learning requires balanced activation of dopamine and sonic hedgehog pathways in striatal cholinergic interneurons

Authors: *S. URIBE-CANO[1], D. R. ZUELKE[3], L. B. MALAVE[4], A. H. KOTTMANN[2];
[1]Psychology, [2]Physiology, Pharmacol. and Neurosci., City Univ. of New York, New York, NY;
[3]CUNY Sch. of Med., New York, NY; [4]CUNY Sch. of Medicine, CCNY, New York, NY

Abstract: Cholinergic interneurons (CIN) in the striatum act as critical circuit integrators of the model free- and procedural- learning machine housed in the basal ganglia. CIN activity is modulated by mesencephalic-dopamine (DA) neuron (DAN) projections which, according to reinforcement learning theories, provide a "teaching signal" that can modulate the strength of glutamatergic synapses in the striatum. The mechanisms of DAN action on CINs and how CIN activity mediates reinforcement learning are not completely understood. In particular, the finding that DANs communicate with their targets not only via DA but through multiple factors suggests that the "teaching signal" they produce may be a composite of several signaling molecules. We previously found that in the adult brain all DANs selectively signal to striatal CINs and fast spiking interneurons via the secreted cell signaling molecule Sonic hedgehog (Shh). Conditional ablation of Shh from DANs results in decreased cholinergic tone, a selective loss of cortical glutamatergic inputs onto CINs, progressive CIN degeneration in aged mice, and slowed reinforcement learning with precocious motor habit formation in young adult mice in a conditional T-Maze paradigm (Gonzalez Reyes et al., 2012). As proof of principle, we found that the pharmacological activation of Smoothened (Smo) on CINs, a GPCR required for Shh signal transduction, in parkinsonian rodent models can ameliorate L-Dopa induced dyskinesia, a form of aberrant motor learning (Malave et al., 2019, preprint at BioRX). Based on this data we hypothesize that successful reinforcement learning requires balanced Smo and DA receptor activation on CINs. To test this hypothesis, we produced an allelic series of recombinant mice with ablated, partially reduced, and constitutively activated Smo activity in CINs. These mouse lines are currently being tested in motor habit learning paradigms using a conditional T-maze. Further, we have produced a series of chimeric Melanopsin-Smo GPCRs hoping to gain optical control over Smo signaling selectively in CINs. These recombinant receptors are being tested *in vitro* with assays for Smo specific G protein recruitment and phospholipase A2 activation. Lastly, we have begun identifying protein targets of Smo signaling in the intact striatum through metabolic labeling of the CIN specific proteome. Analysis of protein abundance and modifications among enriched samples of striatal CIN synaptosomes will be carried out via mass spectrometry.

Disclosures: S. Uribe-Cano: None. **D.R. Zuelke:** None. **L.B. Malave:** None. **A.H. Kottmann:** None.

Poster

381. Parkinson's Disease Oscillations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.19/F10

Topic: C.03. Parkinson's Disease

Title: Elevated thalamocortical synchronization in Parkinson's disease assessed using magnetoencephalography

Authors: R. F. H. CASH[1,2], *K. ZENG[2], L. G. DOMINGUEZ[3], R. WENNBERG[3], D. CHEYNE[4], M. J. N. BROWN[2,5], R. CHEN[2];
[1]Departments of Biomed. Engin. and Psychiatry, Univ. of Melbourne, Victoria, Australia; [2]Div. of Neurology, Dept. Medicine, Univ. of Toronto and Krembil Brain Inst., Toronto, ON, Canada; [3]Mitchell Goldhar MEG Unit, Clin. Neurophysiol. Laboratory, Toronto Western Hospital, Univ. of Toronto, Toronto, ON, Canada; [4]Dept. of Med. Imaging, Univ. of Toronto, Toronto, ON, Canada; [5]Dept. of Kinesiology and Hlth. Science, California State Univ., Sacramento, CA

Abstract: *Introduction*: Parkinson's disease (PD) is associated with excessive beta-gamma phase amplitude coupling (PAC) within thalamocortical motor loops. Recent research suggests that this coupling is linked to sharper beta band waveforms, and is normalised by DBS. Here we employed magnetoencephalography (MEG) and beamforming to non-invasively measure thalamocortical PAC, waveform sharpness and their relationship in individuals with predominantly akinetic rigid PD (on and off medications) compared the results to healthy controls (HC). *Methods*: Individuals with PD (n = 16, 10 men, mean age 63.4 years) and age matched HC (n = 17, 8 men, mean age 63.3 years) were recruited. PD individuals attended two sessions(ON and OFF dopaminergic medications); HC attended a single session. MEG comprised a 4.5 minute scan (eyes closed) with head tracking sensors. MEG was registered to individual anatomical MRIs. Clinical severity was assessed using the Unified Parkinson's disease Rating Scale (UPDRS). PAC across beta (13-30 Hz) and gamma (50-150 Hz) bands and beta band waveform sharpness were measured. PAC was calculated in motor cortex, thalamus and occipital cortex (control region), and between thalamus and motor cortex or occipital cortex. *Results*: PAC was significantly elevated in both the more and less affected motor cortices and between thalamus (beta) and motor cortex (gamma band) in the PD OFF compared to PD ON condition and to HC. Beta band waveform sharpness was modestly higher in PD OFF compared to PD ON ($p = 0.027$) and HC ($p = 0.029$) in the motor cortex but not in thalamus in the more affected hemisphere only. There was a non-significant ($p=0.086$), positive, association between PAC and waveform sharpness in PD OFF in the more affected motor cortex. Control analyses in the occipital cortex indicated that neither PAC nor waveform sharpness were elevated in PD ON or OFF medication conditions relative to HC. *Discussion*: Our data provide the first noninvasive demonstration of elevated thalamocortical PAC in motor regions but not in control regions in

PD. Our findings of elevated cortical PAC in PD OFF and its normalization by dopaminergic medication are consistent with previous reports. In addition, our findings suggest that increased PAC in PD cannot be explained by high waveform sharpness, but PAC and sharpness may be related biological phenomena.

Disclosures: R.F.H. Cash: None. **K. Zeng:** None. **L.G. Dominguez:** None. **R. Wennberg:** None. **D. Cheyne:** None. **M.J.N. Brown:** None. **R. Chen:** None.

Poster

381. Parkinson's Disease Oscillations

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.20/F11

Topic: C.03. Parkinson's Disease

Support: NINDS - NS1276394
APDA

Title: Transcriptional profiling of striatal cholinergic interneurons with and without dopamine neuron provided sonic hedgehog

Authors: *D. R. ZUELKE[1,2], L. B. MALAVE[1,3], P. BARKAUS[1], S. MAHARJ[1], K. KENNEDY[1], A. H. KOTTMANN[1,4];
[1]CUNY Sch. of Med., New York, NY; [2]Biol., [3]Neurosci., [4]CUNY Grad. Ctr., New York, NY

Abstract: Sonic hedgehog (Shh) competent cholinergic interneurons (CINs) in the striatum are key members of the striatal microcircuits involved in movement and procedural learning. Aberrant CIN activity is implicated in progressive neurodegenerative diseases involving the basal ganglia like Parkinson disease (PD). Previously we showed that conditional ablation of Shh from DAN (Shh_{DAN}) results in decreased cholinergic tone, a selective loss of cortical glutamatergic inputs onto CINs, and progressive CIN degeneration in aged mice (Gonzalez-Reyes et al., 2012). Recently, we implicated activation of the Shh pathway in the attenuation of L-Dopa induced dyskinesia in parkinsonian rodent and macaque models (Malave et al., 2019, BioRX). The Shh pathway is well known to regulate gene expression in target cells. The transcriptional changes in CINs with and without Shh_{DAN} has not been explored. As CINs comprise only 1-2% of striatal neurons, we utilized translating ribosome affinity purification to isolate mRNA for RNAseq. Analysis revealed differentially expressed genes involved in G-protein signaling, glutamate signaling, transcriptional control, and neurodegeneration. Among these targets of Shh, hedgehog interacting protein (Hhip), a factor modulating Shh signaling strength, was significantly downregulated in CINs lacking Shh_{DAN}. Here we validate the differential expression of Shh regulated candidate genes using a combination of qRT-PCR, *in situ* hybridization, and immunohistochemistry. Our work will contribute to defining the Shh/Smo

based connectome within the basal ganglia and the functional consequences of disturbed Shh/Smo signaling in basal ganglia diseases.

Disclosures: D.R. Zuelke: None. **L.B. Malave:** None. **P. Barkaus:** None. **S. Maharj:** None. **K. Kennedy:** None. **A.H. Kottmann:** None.

Poster

381. Parkinson's Disease Oscillations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.21/F12

Topic: C.03. Parkinson's Disease

Support: Anandamahidol Foundation Scholarship

Title: Distinct subpopulations of parvalbumin-expressing neurons in the external globus pallidus (GPe) mediate different behavioral functions

Authors: *V. LILASCHAROEN, E. H. WANG, N. DO, A. N. TRAN, X. WANG, B. LIM; UCSD, La Jolla, CA

Abstract: The external segment of the globus pallidus (GPe), a subcortical nucleus centrally located in the indirect pathway of the basal ganglia, plays a pivotal role in processing and broadcasting information received from the striatum and the subthalamic nucleus through its widespread projections across major basal ganglia nuclei. One of the largest neuronal populations of the GPe expresses the Ca^{2+}-binding protein parvalbumin (PV) and projects to multiple nuclei of the basal ganglia and the thalamus and has been shown to be involved in movement disability in Parkinson's disease (PD). However, most studies to date considered GPe PV neurons as a homogeneous population. We found that the GPe PV neurons can be further subdivided into two non-overlapping populations based on their projections to either the substantia nigra pars reticulata (SNr) or the parafascicular thalamus (PF) and their intrinsic electrophysiological properties. We further investigated the circuit-specific roles of these subpopulations in locomotion and reversal learning as well as their contributions to the impairments of motor and cognitive flexibility in a PD mouse model. Our findings establish the behavioral significance of two distinct GPe PV neuronal populations embedded in discrete neural pathways and their differential contributions to specific subdomains of Parkinsonian-like behaviors.

Disclosures: V. Lilascharoen: None. **E.H. Wang:** None. **N. Do:** None. **A.N. Tran:** None. **X. Wang:** None. **B. Lim:** None.

Poster

381. Parkinson's Disease Oscillations

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Program #/Poster #: 381.22/F13

Topic: C.03. Parkinson's Disease

Support: RISE NIGMS R25GM056833
APDA
NINDS NS 1276394

Title: Sonic Hedgehog signaling from mesencephalic and cortical projections stimulate striatal cholinergic and fast spiking interneurons altering L-Dopa induced dyskinesia formation and expression

Authors: *L. B. MALAVE[1], D. R. ZUELKE[2], S. URIBE-CANO[3], A. H. KOTTMANN[4]; [1]CUNY Sch. of Medicine, CCNY, New York, NY; [2]CUNY Sch. of Med., New York, NY; [3]CUNY City Col. of New York, New York, NY; [4]Physiology, Pharmacol. and Neurosci., City Univ. of New York, New York, NY

Abstract: Cholinergic (CIN) and fast spiking (FSI) interneurons of the striatum are critical components of the striatal microcircuit that integrate cortical and thalamic information streams onto medium spiny output neurons. Both populations of neurons have become implicated in L-Dopa induced dyskinesia (LID), a severe complication of DA substitution therapy in Parkinson's disease (PD), which is caused by the degeneration of dopamine neurons (DAN). While all neurons of the striatum express various receptors for the neurotransmitters dopamine (DA), acetylcholine (ACh), GABA, and Glutamate, CIN and FSI are the only neuronal subtypes that express the signal transduction components of the secreted cell signaling protein Sonic Hedgehog (Shh). All mesencephalic DAN and pyramidal tract (PT) neurons, which project collateral axons into the striatum express Shh throughout life. We previously showed that conditional ablation of Shh from DAN (Shh_{DAN}) results in decreased cholinergic tone, a selective loss of cortical glutamatergic inputs onto CINs, progressive CIN degeneration in aged mice, and slowed reinforcement learning with precocious motor habit formation in young adult mice in a conditional T-Maze paradigm. Further, the pharmacological activation of Smoothened (Smo) on CINs, a GPCR required for Shh signal transduction, in parkinsonian rodent and macaque models can ameliorate LID. We find that Smo agonist treatment blunts the DA mediated inhibition of CIN. Interestingly, D2R as well as Smo couple to Gα/i pointing to the possibility that these pathways share downstream effectors. Conditional ablation of Smo from CINs results in progressive CIN degeneration, and reduced p-rpS6 levels, a neuronal activity marker, which cannot be rescued by Smo agonist treatment. Interestingly, the conditional ablation of Shh from PT neurons, in contrast to the ablation of Shh from DAN, increases neuronal activity of CIN as

measured by p-rsP6 levels. This observation suggests that Shh_{PT} might signal selectively to FSI, which provide negative control over CIN. To further investigate this phenomenon we produced allelic series of recombinant mice with ablated, partially reduced, and constitutively activated Smo (SmoM2) activity in CIN and FSI to test whether graded Smo activity will correlate with p-rpS6 levels, degree of neuroprotection and reduction in the formation and display of LID. Our results reveal that Shh_{DAN} is an important regulator of neuroplasticity of CIN, further suggests that Shh_{PT} signals selectively on FSI, and demonstrate, that dysregulation of Shh signaling in the striatum is a significant contributor to primary and therapy induced pathology in PD.

Disclosures: L.B. Malave: None. **D.R. Zuelke:** None. **S. Uribe-Cano:** None. **A.H. Kottmann:** None.

Poster

381. Parkinson's Disease Oscillations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.23/F14

Topic: C.03. Parkinson's Disease

Support: NSF-CAREER 1351112 (Dorval)
NIH NINDS 2R37NS033123-14A1 (Pulst)
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Utah Neuroscience Initiative Collaborative Pilot Project Award (Pulst, Dorval)
NIH P41GM103545 (Butson)
NSF US IGNITE 10037840 (Butson)
National Ataxia Foundation Postdoctoral Fellowship (C. Anderson)

Title: Neural selectivity, efficiency, and dose equivalence in deep brain stimulation through pulse width tuning and segmented electrodes

Authors: *C. J. ANDERSON[1], D. N. ANDERSON[2], S. M. PULST[5], C. R. BUTSON[3], A. D. DORVAL[4];
[1]Neurol., [3]Scientific Computing & Imaging Inst., [4]Dept. of Bioengineering, [2]Univ. of Utah, Salt Lake City, UT; [5]Univ. of Utah Clin. Neurosciences Ctr., Salt Lake City, UT

Abstract: Background: Achieving deep brain stimulation (DBS) dose equivalence is challenging, especially with pulse width tuning and directional contacts. Further, the precise effects of pulse width tuning are unknown, and recent reports of the effects of pulse width tuning on neural selectivity and side effects seemingly contradict classic biophysical studies.
Methods: We created multicompartment neuron models for two axon diameters and used finite element modeling to determine extracellular influence from standard and segmented electrodes. We analyzed axon activation profiles and calculated volumes of tissue activated.

Results: We find that long pulse widths focus the stimulation effect on small, nearby fibers, suppressing distant white matter tract activation (responsible for some DBS side effects) and improving battery utilization. Directional leads enable similar benefits to a greater degree. We derive equations for equivalent activation with pulse width tuning and segmented contacts. Reexamining previous reports of short pulse stimulation reducing side effects, we find that short pulse stimulation was not dose equivalent and consistently featured substantially less spread of neural activation.
Interpretations: We find agreement with classic studies and reinterpret recent articles concluding that short pulse widths focus the stimulation effect on small, nearby fibers, decrease side effects, and improve power consumption. The DBS field may need to reconsider the use of shortened pulse widths. Future work examining pulse width tuning must ensure dose equivalence rather than stimulating with energy- or charge-equivalent settings.

Disclosures: C.J. Anderson: None. **D.N. Anderson:** None. **S.M. Pulst:** None. **C.R. Butson:** None. **A.D. Dorval:** None.

Poster

381. Parkinson's Disease Oscillations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 381.24/F15

Topic: C.03. Parkinson’s Disease

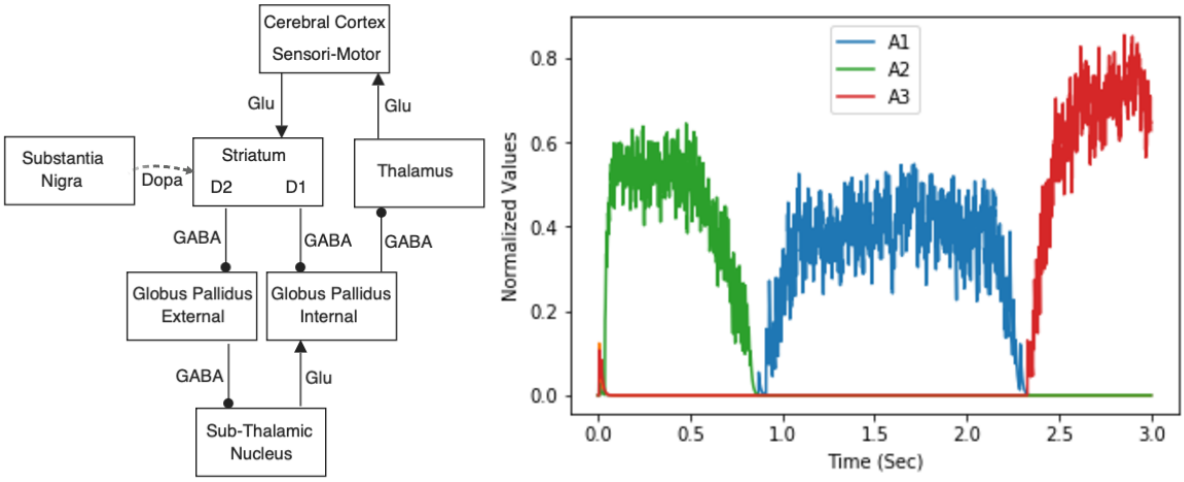
Support: UConn/UTC-IASE Startup Grant

Title: Dynamic spiking network model of basal ganglia for movement disorders

Authors: *A. DUTTA;
Electrical and Computer Engineering, Biomed. Engin., Univ. of Connecticut, Storrs, CT

Abstract: Basal ganglia dysfunction is a problem with the deep brain structures that help start and control movements, most likely caused by a degeneration of neurons in the BG and is responsible for a variety of neurological disorders. This necessitates the development of a spiking network model of BG-Thalamus-Cortex loop based on the interplay of excitatory, inhibitory and modulatory dynamics leading to decision making and execution, hopefully leading to new therapies.
The Cortex makes/receives excitatory/glutamatergic connections to/from Striatum (D1, D2) and Thalamus respectively. The Striatum projects inhibitory/GABAergic connections to Globus Pallidus (GP) extending to Sub-Thalamic Nucleus (STN) which makes a reciprocal inhibition to GP in turn exciting Thalamus. The Substantia Nigra (SN) projects neuro-modulatory/Dopaminergic connection to Striatum.
A representative population of spiking neurons are used to model each region of BG as show in

the figure. Rate coding is used to interconnect with appropriate excitatory/inhibitory synaptic weights. The SN though is used for neuromodulation of Striatum's synaptic strength. The Cortical neurons encode states and the BG does sequential decision-making based on rewards modulated by Dopamine and communicate back the decoded actions through Thalamus.



A degeneration of neural cells in Striatum, SN is known to cause Huntington's and Parkinson's disease respectively. A sequence of actions with maximum rewards in the order of 2, 1, 3 during 1, 2, 3 secs is fed into BG modeled with lower neuro-modulatory Dopamine levels and the results from the Thalamus are plotted. It is evident that actions are triggered with hesitation. This can cause trouble in sequential decision making of cognitive actions possibly explaining Parkinson's and its mechanics.This spiking neural network model based on population rate coding and interconnected by excitatory, inhibitory and modulatory synapses is shown to be a valuable aid in analyzing and treating neurological disorders.

Disclosures: A. Dutta: None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.01/F16

Topic: C.03. Parkinson's Disease

Support: Dept of Veterans Affairs Merit Review Program

Title: Differential ultrastructural alterations in the Vglut2 (STN) glutamatergic input to the substantia nigra pars compacta/pars reticulata following nigrostriatal dopamine loss in a progressive mouse model of Parkinson's disease

Authors: *C. K. MESHUL[1,2], C. MOORE[3], M. XU[4], J. K. BOHLEN[5];
[1]Res. Services/VA Med. Ctr., VA Med. Ctr., Portland, OR; [2]Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [3]Res. Services/VA Med. Ctr., Cindy Moore, Portland, OR; [4]Res. Services/VA Med. Ctr., Mo Xu, Portland, OR; [5]Res. Services/VA Med. Ctr., Jerry Bohlen, Portland, OR

Abstract: Parkinson's disease (**PD**) is the second most common neurodegenerative disease. Loss of nigrostriatal dopamine (**DA**) results in the over-activation of the subthalamic nucleus (**STN**). The STN sends axonal projections to both the substantia nigra pars compacta (**SNpc**) and pars reticulata (**SNpr**). The vesicular glutamate transporter 2 (**Vglut2**) is localized within STN terminals synapsing within the SN. A lesion of the nigrostriatal pathway results in a decrease in the extracellular striatal glutamate, which is inversely associated with an increase in the density of nerve terminal glutamate immuno-gold labeling. We have developed a chronic/progressive mouse model of PD where MPTP, or vehicle (**VEH**), is administered weekly at increasingly higher doses for 4 weeks. The aim of the current study was to determine if there were differential changes in either the density of Vglut2+ nerve terminal glutamate immuno-gold labeling within the SNpc/SNpr and/or in the proportion of Vglut2+ terminals synapsing on tyrosine hydroxylase (**TH**) positively or negatively labeled dendrites. Within the SNpc, there was no change in the density of nerve terminal glutamate immuno-gold labeling within Vglut2+ terminals. There was a significant shift in the percentage of Vglut2+ terminals contacting TH+ vs TH- labeled dendrites (GABA?) in the VEH vs MPTP treated group, possibly due to DA cell loss: TH+ dendrites: VEH: 81.6% vs MPTP: 50.2%; $p = 0.01$; TH- dendrites: VEH: 21.7% vs MPTP: 51.8%, $p = 0.005$. Within the SNpr, there was a significant decrease in the density of glutamate immuno-gold labeling (#/um^2) in Vglut2+ terminals contacting TH+ and TH- labeled dendrites: TH+ dendrites: VEH vs MPTP: 74.9 vs 47.6, $p = 0.03$; TH- dendrites: VEH vs MPTP: 87.4 vs 53.7, $p = 0.02$. Within the SNpc, there was no change in the density of glutamate immuno-gold labeling within Vglut2+ terminals following MPTP treatment, suggesting no change in glutamate release. Within the SNpr, the decrease in the density of glutamate immunogold within Vglut2+ terminals suggests an increase in glutamate release, a finding consistent with the model of basal ganglia function in PD. We conclude that there is a differential effect of nigrostriatal DA loss on the glutamate input from the STN synapsing either within the SNpr and SNpc.

Disclosures: C.K. Meshul: None. **C. Moore:** None. **M. Xu:** None. **J.K. Bohlen:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.02/F17

Topic: C.03. Parkinson's Disease

Title: Unravelling the neural circuits related to movement disorders: Pedunculopontine nucleus

Authors: *M. RIDDER[1], H. INAGAKI[2], L. XU[1], K. SVOBODA[2], P. SAH[1];
[1]The Queensland Brain Inst., Univ. of Queensland, St Lucia, Australia; [2]HHMI / Janelia Farm Res. Campus, Ashburn, VA

Abstract: Parkinson's disease (PD) is a progressive neurological disorder that results from loss of dopaminergic neurons in the midbrain. Freezing of gait (FOG) and postural instability are often seen in patients with advanced PD. FOG is defined as a brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk. It is one of the most debilitating motor symptoms in patients with PD as it may lead to falls and a loss of independence. Deep brain stimulation of the pedunclopontine nucleus (PPN) offers relief of FOG for some patients, yet its mechanism is unknown.
Persistent activity in a thalamocortical loop involving anterior lateral motor cortex (ALM) and parts of VM, VAL, MD) underlies motor planning. To initiate movements, the brain converts motor plans into movement commands, which is associated with large and rapid changes in activity in the thalamocortical loop. Ascending projections from PPN to thalamus could play a key role in terminating motor planning and movement initiation. Indeed, optogenetic stimulation of PPN ascending pathway triggers movement initiation in mice trained on a delayed-response task. Here we mapped the connectivity of PPN projection neurons to the thalamus. We expressed channelrhodopsin 2 (ChR2) in PPN neurons using viral transduction. Whole-cell recording were made from VM and MD neurons in brain slices while PPN axons were stimulated in the vicinity of the recording. In some cases neurons projecting to ALM were identified by retrograde labeling. Stimulation of PPN axons produced robust glutamatergic responses in a majority of thalamic neurons, including those that project to ALM. The responses were mediated by AMPA and NMDA-receptors. We found little evidence for cholinergic or GABAergic input from PPN to VM and MD. A better understanding of PPN neural circuits in relationship to motor thalamocortical loop may explain pathophysiology of FOG and the mechanism of DBS treatment.

Disclosures: M. Ridder: None. **H. Inagaki:** None. **L. Xu:** None. **K. Svoboda:** None. **P. Sah:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

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Program #/Poster #: 382.03/F18

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Support: NINDS/NIA (NS075321)

American Parkinson Disease Association (APDA) Advanced Research Center for PD at WUSTL
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Title: Multimodal PET and fcMRI reveals regional modulation by STN DBS correlating to motor outcomes

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Abstract: STN DBS is an effective treatment for Parkinson disease (PD), but predictors of clinical response are limited. ^{15}O PET reveals changes in brain activity in DBS without many of the methodological difficulties found in fMRI related to device safety and unpredictable MRI artifact. Regional blood flow responses to STN DBS have been observed with ^{15}O PET and in some cases correlate with clinical outcomes. A multimodal approach associating blood flow responses during STN DBS and motor outcomes with preoperative functional connectivity (BOLD fMRI) may permit improved prediction of clinical response to DBS. Forty-one participants with STN DBS had ^{15}O PET in OFF, unilateral dorsal STN stimulation ON, and unilateral ventral STN stimulation ON conditions, and we compared rCBF using paired T-tests. We used a whole-brain approach in addition to an *a priori* set of regions to identify responses to STN DBS. We then used the sites of regional responses for seed-based correlation in 80 participants with STN DBS and preoperative resting state fcMRI, as well as 300 seeds divided into 17 canonical networks. Connectivity of each PET-defined region to STN and intra-network seeds were correlated to change in UPDRS after DBS. DBS produced significant changes in 13 regions in the PET analysis. Connectivity of each region to canonical networks using fcMRI revealed that these activations related to basal ganglia, thalamic, dorsal sensorimotor, salience, parietal memory, and frontoparietal networks. Connectivity of left STN to ipsilateral internal globus pallidus strongly correlated with motor outcomes ($R = -0.41$, $p < 0.001$), with higher connectivity being associated with greater improvement in motor outcomes. Primary motor cortex connectivity to sensorimotor network was the network-level connectivity most correlated with change in motor scores after DBS. We conclude that DBS modulates a diverse set of brain regions. These regions not only pertain to networks typically associated with motor activity, e.g. basal ganglia, thalamic, and sensorimotor networks, but also memory, executive and associative functions. Motor outcomes are highly associated with STN connectivity to particular regions modulated by DBS.

Disclosures: **J.R. Younce:** None. **A. Tanenbaum:** None. **M.C. Campbell:** None. **J.S. Perlmutter:** None. **S.A. Norris:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.04/F19

Topic: C.03. Parkinson's Disease

Support: NINDS IRP Support

Title: Inhibitory and excitatory DREADD-induced modulation of parafascicular thalamic nucleus projections to the basal ganglia alters motor function in hemiparkinsonian rats

Authors: N. NOVIKOV, E. BRAZHNIK, A. J. MCCOY, M. W. PRESTON, ***J. R. WALTERS**;
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Abstract: The parafascicular thalamic nucleus (Pf) receives inputs from the basal ganglia (BG), cortex, and cerebellum and provides segregated projections to the striatum (STR) and subthalamic nucleus (STN). However, the Pf's impact on the pathophysiology of Parkinson's disease (PD) is unclear. Data from PD patients and animal models suggest that emergence of excessive beta oscillations in BG circuit activity contributes to PD motor dysfunction. Our previous studies in hemiparkinsonian rats support the view that synchronized activity in the substantia nigra pars reticulata (SNr) promotes the transmission of 25-35 Hz high beta oscillations throughout BG-thalamocortical circuits. Yet, these excessive oscillations are not evident in recordings from the Pf in PD rats. The current study uses the hemiparkinsonian rat to gain insight into the potential role of the Pf in modulating motor circuit activity and motor function after loss of dopamine, and explores the hypothesis that reduction in Pf activity has a therapeutic effect on motor deficits in PD. Three groups of rats received infusion of the inhibitory Cre-dependent DREADD viral vector AAV2/8-hSyn-HA-KORD-IRES-mCitrine (KORD) into the Pf. Cre-recombinase was delivered to Pf projecting cells via infusion of AAV pmSyn1-EBFP-Cre directly to the Pf (group 1), or retrogradely from STR (group 2) and STN (group 3) to selectively inactivate Pf projections to these nuclei. Also, the effect of activation of Pf neurons with the excitatory viral vector AAV2/5-hSyn-hM3-DIO-mCherry (hM3-DIO) was examined. Electrodes were implanted in the motor cortex (MCx), dorso-lateral STR and SNr. Histology revealed modest DREADD expression in Pf. High beta power in the MCx, STR and SNr local field potential (LFP) activity and coherence between the regions were observed during walking in a circular treadmill. At 4-6 weeks post-surgery, stimulation of the novel inhibitory receptors expressed in KORD-transfected Pf neurons with salvinorin B (SalB, 1 mg/kg) substantially improved clockwise walking in all three groups. Notably, high beta LFP

power/coherence in motor circuits was not altered. In contrast, stimulation of novel excitatory receptors expressed in hM3-DIO-transfected Pf neurons with clozapine-N-oxide (CNO, 5 mg/kg) increased bradykinesia. Results support the view that SalB-mediated reduction of activity in Pf projections to the BG modulates activity in BG output in a manner which reduces motor deficits, while CNO-mediated activation enhances deficits. Ongoing DREADD experiments explore how changes in Pf activity may affect the pattern of high beta bursts as well as spiking activity in the STR, SNr, and MCx during bradykinesia.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

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Program #/Poster #: 382.05/F20

Topic: C.03. Parkinson's Disease

Support: Canadian Institutes of Health Research (Vanier CGS)
Canada Research Chair

Title: Right arm flexion during magnetoencephalography demonstrates more focal and robust sensorimotor beta attenuation in Parkinson's patients versus healthy controls

Authors: ***I. E. HARMSEN**[1], N. C. ROWLAND[3], L. G. DOMINGUEZ[2], A. M. LOZANO[1]; [1]Neurosurg., [2]Neurol., Univ. of Toronto, Toronto, ON, Canada; [3]Neurosurg., Med. Univ. of South Carolina, Charleston, SC

Abstract: *Background:*
Parkinson's disease is a neurodegenerative movement disorder that is characterized by abnormal local field potential beta (13-30 Hz) activity in the basal ganglia that correlates with reduced voluntary movement. In contrast, our prior study using electrocorticography (ECoG) suggests the sensorimotor cortex of patients with Parkinson's has an oscillatory profile that may act to promote movement.
Objective:
To more clearly define the role of oscillatory activity in Parkinson's, we conducted whole-head magnetoencephalography (MEG) recordings to measure oscillatory changes in brain regions beyond the reach of ECoG.
Methods:
MEG recordings were performed in 5 Parkinson's patients (1 female, ages 52-71) with deep brain stimulation (DBS) in the off state. We analyzed MEG signals during a right arm flexion (RAF) motor task and compared findings to similar recordings in 9 healthy controls (4 females,

ages 17-79). MEG signals were analyzed in sensor- and source-space to assess whole-brain as well as motor-specific oscillatory changes.
Results:
We show that (1) the sensorimotor cortical region involved in beta desynchronization is more focal and robust in Parkinson's patients during RAF compared to controls (p=0.039) and (2) within the Parkinson's cohort, disease severity as measured by tremor score is inversely correlated with the ability to attenuate sensorimotor beta power (Pearson's correlation coefficient = 0.952, p=0.048).
Conclusions:
Our findings suggest that voluntary movement modulates beta activity within a more restricted cortical region in Parkinson's patients than in healthy controls, and within that more focal region the beta attenuation during movement is more robust. This supports the conclusion from our prior study that the Parkinsonian cortex instantiates compensatory changes in order to preserve voluntary movement. Characterizing these neurophysiological differences improves our understanding of Parkinson's pathophysiology and lays the foundation for understanding the modulatory effects of DBS.

Disclosures: I.E. Harmsen: None. **N.C. Rowland:** None. **L.G. Dominguez:** None. **A.M. Lozano:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.06/F21

Topic: C.03. Parkinson's Disease

Support: National Natural Science Foundation of China 81171193
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Title: Pathological response of striatal projection neurons to dopamine is associated with abnormal involuntary movements in Parkinsonian rats

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Abstract: L-DOPA-induced dyskinesia (LID) is a major complication of long-term dopamine replacement therapy in Parkinson's disease. Dysregulation of striatal projection neurons (SPNs) is thought to play a role in the mechanisms underlying LID development. However, functional changes in the SPN response to dopamine have not been fully profiled in rodent models of LID.

This study was aimed at examining the SPN activity changes in response to dopamine during the development of abnormal involuntary movements (AIMs, the equivalent of the primate LID) in hemi-parkinsonism rats. Fifteen rats with unilateral 6-OHDA-lesion were injected with L-DOPA (12 mg/kg plus benserazide 6 mg/kg, i.p.) for 7 days to induce stable AIMs. On L-DOPA treatment days 1 and 7, motor behavior (AIMs scores) was correlated with in vivo electrophysiology analyzing LFP and single cell activity in both primary motor cortex and dorsolateral striatum. The pharmacological response to the classic antidyskinetic agent amantadine (60 mg/kg, i.p.) or a metabotropic glutamate group II receptors 2/3 (mGluR2/3) agonist LY354740 (12 mg/kg, i.p.) was used to assess the specificity of physiological changes. As AIMs established during L-DOPA treatment for one week, high γ (hγ) oscillation predominated during the motor response in cortex and striatum, the number of unstable SPN responses to dopamine increased, and the coherence between these patterns of oscillation and spiking activity also increased. Amantadine significantly reduced AIMs scores, in parallel to reduction of hγ oscillation, and more markedly to a decrease in unstable SPN responses to dopamine. In contrast, LY354740 significantly shortened LID duration, but was weaker in diminishing the strength of LID or decreasing the proportion of unstable SPN responses. In conclusion, the development of AIMs in the rat model of PD is associated with the typical changes in corticostriatal network and SPN activity that characterize altered dopamine responses. The physiological correlation of amantadine or LY354740's antidyskinetic effect supports a key role of unstable SPN responses in the generation of the rodent LID.

Disclosures: X. Cao: None. **C. Zheng:** None. **G. Chen:** None. **W. Zeng:** None. **Y. Xu:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.07/F22

Topic: C.03. Parkinson's Disease

Title: Modulation of D3 receptor expression by D3nf isoform in striato-nigral projections of the 6-OHDA denervated rat

Authors: *B. CAMPOS[1], J. AVALOS-FUENTES[2], C. PIÑA LEYVA[2], H. CORTÉS[3], F. PAZ-BERMUDEZ[2], B. FLORÁN GARDUÑO[2];
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Abstract: The functional response to dopamine D3 receptors (D3R) activation in striato-nigral projections can be mediated by two different signaling pathways, one designated as "typical" in which D3R are coupled to Gi proteins inhibiting adenylyl cyclase activity and in consequence cAMP formation. A second called "atypical" in which D3R stimulates Gs proteins when D1R

are co-activated, increasing adenylyl cyclase activity. It has been suggested that the abundance of the non-functional isoform of the D3R called D3nf determines the density of D3R at the plasma membrane level. In striato-nigral projections, signaling of D3R normally “atypical” becomes “typical” during experimental Parkinson. In the present study we determined whether the two modalities of signaling are correlated with the relative expression of D3R and its isoform during experimental Parkinson. We induced hemiparkinsonism in adult male Wistar rats by the unilateral administration of 6-OHDA in the middle forebrain bundle. Using RTq-PCR we found a decrease in D3nf’s mRNA expression in the lesioned striatum. Western Blot determinations showed a decreased in protein expression levels of D3nf isoform in striatum and substantia nigra reticulata (SNr) of the lesioned hemisphere. This decrease in the relative expression of D3nf isoform was accompanied by an increased in the Bmax of D3R in the striatum of the lesioned hemisphere as indicated by binding with [^{3}H] 7-OH-DPAT. Further, there was a change in the functional response to D3R activation; in the control hemisphere, D3R activation potentiated cAMP formation and [^{3}H]-GABA release induced by high K^+ with D1R co-activation, i.e. an “atypical” response in striatum as well as SNr. In contrast, in the lesioned hemisphere, D3R activation inhibited cAMP formation and [^{3}H]-GABA release induced by D1R activation, i.e. a “typical” response was observed. Furthermore, *in vivo* intranigral co-activation of D3R in the lesioned hemisphere has opposing effects over D1R activation, evaluated by contralateral rotational behavior, this “typical” response was changed to “atypical” once we pharmacologically eliminated the D3R signaling pathway with N-Ethylmaleimide (alkylating agent of Gi proteins). These data indicate that the relative expression of D3Rs and D3nf isoform correlates with the functional response produced by D3R activation and this response is probably mediated by changes in the number of D3R located at the plasma membrane level.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.08/F23

Topic: C.03. Parkinson’s Disease

Title: Bridging the gap between prodromal and symptomatic phases of Parkinson's disease using translational EEG biomarkers in preclinical models

Authors: *V. DUVEAU[1], J. VOLLE[2], G. PORRAS[3], E. PIOLI[3], A. EVRARD[1], C. RUGGIERO[1], C. ROUCARD[1], E. BEZARD[4,3], Y. ROCHE[1];
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Abstract: Whereas the nature of the etiology of the process underlying clinical deterioration remains unknown, PD is characterized by the progressive loss of Substantia Nigra neurons with two main phases: a prodromal and a symptomatic phase. Despite years of research, we still lack surrogate biomarkers of disease progression. Accumulative evidences have demonstrated the presence of aberrant oscillatory synchronization of neuronal activity across the cortico-basal ganglia-thalamo-cortical circuit in both PD patients and experimental animal models in the symptomatic phase. Using state-of-the-art EEG methodologies, we here investigated the development of beta-band (~30Hz) in two rat models of PD as well as their response to the reference dopamine replacement therapy, L-DOPA, once degeneration is established. We used the unilateral 6-OHDA rat model that mimics PD late stage and the bilateral AAV-alpha-synuclein (alpha-Syn) rat model that allows investigating the progression of the degeneration over several weeks. In the late stages of the two models, a prominent beta-band (~30Hz) was observed in the motor cortex (inexistent in control rats) that was reduced after L-DOPA challenge. This treatment also induced a prominent increase in the gamma (γ)-band (80-100Hz). In the AAV-alpha-Syn rat model, we observed the progressive rise in beta-band power from the prodromal phase onwards. Since the late stage data fit with the clinical literature, we propose that the beta-band rise may represent a predictive, reliable, objective and clinically-relevant biomarker for the validation of disease modifying experimental therapeutics. In addition, our data call for a clinical validation of this biomarker.

Disclosures: **V. Duveau:** A. Employment/Salary (full or part-time):; SynapCell SAS. **J. Volle:** A. Employment/Salary (full or part-time):; SynapCell SAS. **G. Porras:** A. Employment/Salary (full or part-time):; Motac Neuroscience. **E. Pioli:** A. Employment/Salary (full or part-time):; SynapCell SAS. **A. Evrard:** A. Employment/Salary (full or part-time):; SynapCell SAS. **C. Ruggiero:** A. Employment/Salary (full or part-time):; SynapCell SAS. **C. Roucard:** A. Employment/Salary (full or part-time):; SynapCell SAS. **E. Bezard:** A. Employment/Salary (full or part-time):; Motac Neuroscience. **Y. Roche:** A. Employment/Salary (full or part-time):; SynapCell SAS.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.09/F24

Topic: C.03. Parkinson’s Disease

Support: OHSU Parkinson Center Pilot Grant program
Tartar Trust Fellowship
NIA 5R01 AG006457
I01 RX001075

Title: Freezing of gait in Parkinson's disease leads to lateralized alterations of resting state functional connectivity

Authors: *O. MIRANDA-DOMINGUEZ[1], A. RAGOTHAMAN[2], M. MANCINI[3], R. J. HERMOSILLO[1], E. J. FECZKO[4], J. G. NUTT[3], D. A. FAIR[1], F. B. HORAK[3];
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Abstract: Freezing of gait (FoG) is a brief, episodic absence or marked reduction of forward progression of the feet, despite the intention to walk. Most people with Parkinson's disease (PD) will develop this condition as the disease progresses. FoG might be triggered by different stressors, such as turning or dual-tasking, suggesting that not only motor, but higher order attention systems may be impaired in this condition. In this study, we used resting state functional MRI data to characterize differences in functional connectivity (FC) that might be specific to FoG.
We used well characterized imaging, gait, and balance data from 43 individuals, specifically: 13 participants with PD and FoG, 14 participants with PD and without FoG and 16 healthy controls. Groups were matched on age, disease severity, cognitive status, and levodopa medication. MRI data was processed using surface-based registration plus in-house denoising methods (https://github.com/DCAN-Labs). Time courses were strictly scrutinized for head movement. Surviving frames (5 minutes of data, frame displacement <= 0.3 mm) were used to characterize FC using a pre-defined set of Regions of Interest (ROIs) that also groups ROIs based on functional networks, including motor and attention systems. Differences in FC secondary to diagnosis (healthy controls, people with PD with and without FoG), ROIs, functional networks, and their interaction were identified using repeated a measures ANOVA test.
We found significant differences in FC between groups. Post hoc analysis revealed alterations at different spatial resolutions: Functional systems and ROIs. Control and PD participants exhibited significant differences in sensorimotor, subcortical, visual and higher order heteromodal systems (Dorsal Attention, Cingular-Opucular, Default). FC between the dorsal attention and visual systems indicated a trend in discriminating participants with and without FoG. Seed-based post hoc analysis revealed significant differences in FC between participants with and without FoG for several ROI pairs in the left hemisphere: the globus pallidus and two regions in the somatosensory cortex and between two ROIs belonging, one to the default mode network, and the other ROI to the insular/vestibular system. We also found significant associations between FC and objective measures of FoG for the areas identified as relevant for FOG.
These observations suggest that the interplay between motor, dorsal attention and visual systems are critical in the pathology of FOG.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

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Program #/Poster #: 382.10/F25

Topic: C.03. Parkinson's Disease

Support: NIH grant R01NS096240
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Title: Intersectional and subtractive strategies to study the origins and functions of SNc dopamine neurons

Authors: *G. CARONIA- BROWN[1], M. AZCORRA- SEDANO[2], M. M. PEREIRA LUPPI[1], J.-F. POULIN[1], F. CICCHETTI[3], D. A. DOMBECK[2], R. AWATRAMANI[1];
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Abstract: The dopaminergic (DA) neurons of the Substantia nigra *pars* compacta (SNc) are of great interest as their degeneration underpins the debilitating motor symptoms of Parkinson's disease (PD). DA neurons of the ventral SNc (vSNc) are more vulnerable than those of the dorsal SNc (dSNc) and of the Ventral Tegmental area (VTA). Recent single cell studies have furthered our understanding of DA heterogeneity and have identified multiple molecularly distinct subtypes, at least one of which is particularly vulnerable in a toxin model of PD. How these subtypes develop remains unclear. Here, using intersectional and subtractive lineage tracing techniques, we aim to determine a developmental basis for DA neuron diversity. We also use these genetic methods to unambiguously target subtypes and study their axonal projections and functions.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

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Program #/Poster #: 382.11/F26

Topic: C.03. Parkinson's Disease

Support: NIH grant P50NS098685
base grant of the Yerkes National Primate Research Center NIH/ORIP P51OD011132.

Title: GABAergic interneurons in the ventral motor and caudal intralaminar thalamic nuclei in primates: A potential source of GABAergic dysfunction in Parkinsonism?

Authors: *H. SOLOFF[1], G. M. JEYARAJ[3], A. J. SWAIN[4], S. JENKINS[2], R. M. VILLALBA[5], Y. SMITH[6];
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Abstract: Despite extensive research, our understanding of the pathophysiology of the basal ganglia-thalamocortical functional circuitry in Parkinson's Disease (PD) remains incomplete. One key feature of parkinsonism is an increased GABAergic inhibition of the ventral motor thalamic nuclei, namely the ventral anterior and ventral lateral (VA/VL) nuclear group. According to current models of the basal ganglia circuitry, this increased thalamic inhibition originates from an abnormally overactive GABAergic tone from the basal ganglia output nuclei. A less studied source of GABA in the VA/VL are the inhibitory GABAergic interneurons. Because these interneurons are absent from the rodent motor thalamus, little is known about their anatomy, function, or role in this thalamic region, and in the centromedian/parafascicular (CM/Pf) nuclear group, another main target of basal ganglia outflow in primates. Therefore, the present project aims to generate a detailed characterization of the abundance and synaptic integration of interneurons into the functional basal ganglia-thalamocortical circuitry in normal and parkinsonian states. We utilized 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce parkinsonism in adult rhesus monkeys. We performed an unbiased stereological count of interneurons and thalamocortical cells in VA/VL and CM/Pf by immunostaining with anti-GABA and anti-NeuN, respectively. Data obtained so far from 3 control and 2 parkinsonian monkeys indicate that GABAergic interneurons account for ~17% and ~8% of the total neuronal population in VA/VL and CM/Pf, respectively, without any significant difference between control and parkinsonian monkeys. Three-dimensional electron microscopic reconstruction of VA/VL and CM/Pf interneurons is in progress to determine potential changes in the synaptic microcircuitry of these interneurons in parkinsonian monkeys. Results of this study will set the stage for a deeper understanding of the functional integration of GABAergic interneurons in the physiology and pathophysiology of the basal ganglia-thalamocortical circuitry in normal and parkinsonian state.

Disclosures: H. Soloff: A. Employment/Salary (full or part-time):; Emory University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Supported by NIH grant P50NS098685 and the base grant of the Yerkes National Primate Research Center NIH/ORIP P51OD011132. **G.M. Jeyaraj:** None. **A.J. Swain:** None. **S. Jenkins:** None. **R.M. Villalba:**

None. **Y. Smith:** A. Employment/Salary (full or part-time):; Emory University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Supported by NIH grant P50NS098685 and the base grant of the Yerkes National Primate Research Center NIH/ORIP P51OD011132.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.12/F27

Topic: C.03. Parkinson's Disease

Support: K01MH103594
R21MH109775
R01NS090874
R21NS098020

Title: Using diffuse optical tomography to understand deep brain stimulation's impact on cortical networks

Authors: *A. SHERAFATI[1], A. T. EGGEBRECHT[1], T. M. BURNS-YOCUM[2], H. M. LUGAR[1], A. NARAYANAN[1], T. DOTY[1], S. EISENSTEIN[1], A. M. SVOBODA[1], M. L. SCHROEDER[1], A. Z. SNYDER[1], M. USHE[1], J. P. CULVER[1], T. HERSHEY[1]; [1]Washington Univ. In St. Louis, St. Louis, MO; [2]Indiana Univ., Bloomington, IN

Abstract: Deep brain stimulation of the subthalamic nucleus (STN DBS) in Parkinson disease (PD) can provide substantial motor benefit yet can also produce unwanted cognitive side effects. Current neuroimaging tools lack either necessary temporal resolution (PET), or optimal safety due to contraindications (fMRI) for measuring the neural mechanisms underlying the effects of STN DBS. In this study, we asked 2 questions related to this observation. First, is High-Density Diffuse Optical Tomography (HD-DOT) a feasible tool with sufficient resolution for studying PD patients and understanding the neural activity underlying benefits and side effects of the STN DBS? Second, can we measure the impact of STN DBS on functional connectivity (FC)? To answer these questions, 11 PD patients (2f; ages 55-66) with STN DBS and 11 controls (8f; ages 52-71) were scanned with a previously described HD-DOT device [1] during auditory and visual tasks, and resting state, while controls also underwent an fMRI scan with the same protocol. We validated the feasibility of HD-DOT for scanning controls and PD patients with their STN DBS ON and OFF, recapitulating fMRI results, using a previously described data analysis pipeline [2]. Auditory and visual tasks evoked anatomically appropriate patterns in both controls and PD patients. In addition, PD subjects with DBS OFF had reduced within network FC in somatomotor, visual, and auditory resting state networks and between network FC with

somatomotor and auditory, replicating findings by Gratton et al using fMRI [3]. We also found that FC between somatomotor and frontoparietal networks was greater in PD than controls. Finally, turning DBS ON appears to alter within and between network connectivity for somatomotor, auditory, and DMN (Fig 1). These preliminary results validate that HD-DOT has sufficient feasibility and resolution to accurately map task responses and FC within and between cortical networks in PD patients with STN DBS.

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[2] Sherafati A, et al. *Optics and the brain*, 2018.

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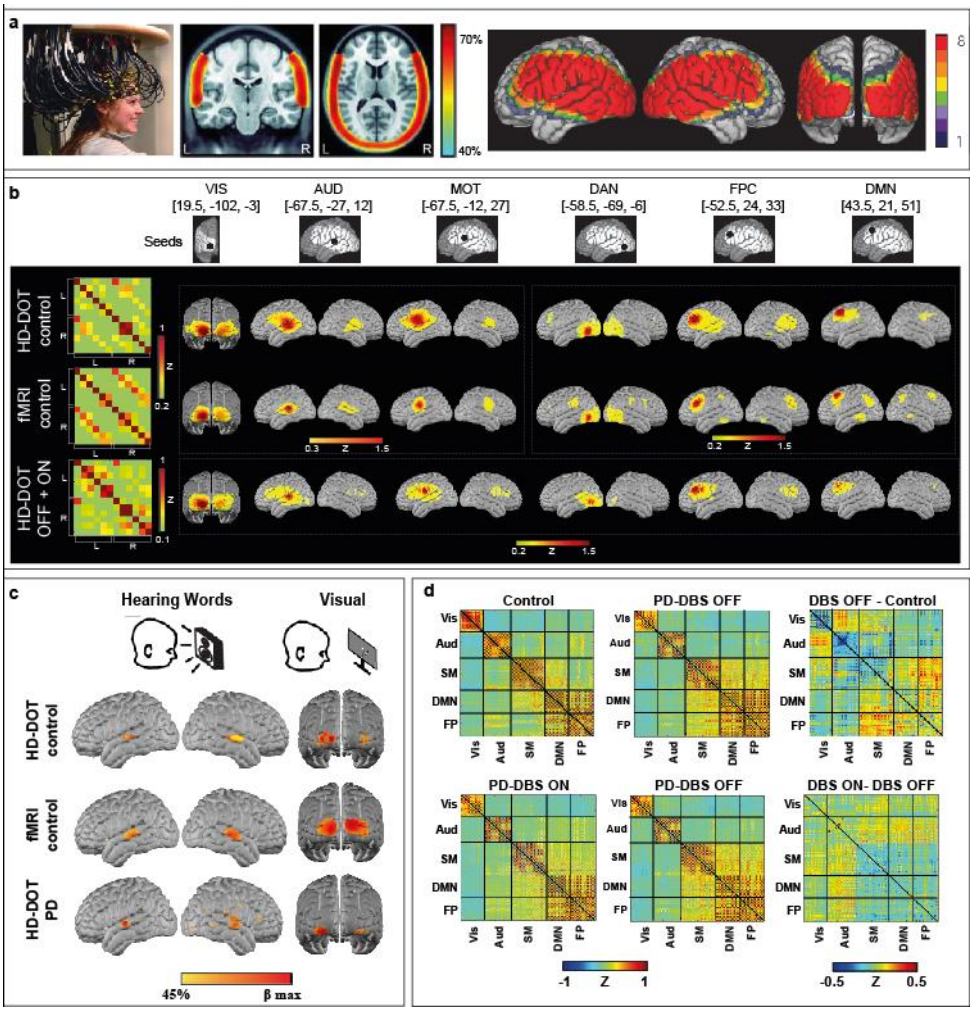

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.13/F28

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS: R01NS079518

Title: Parkinsonian affected basal ganglia output shows condition specific attenuation

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by loss of dopaminergic neurons (DANs) in the basal ganglia (BG). Motor impairment in PD is highly heterogenous and the severity and expression of impairment may depend on the context of the movement. Although the context-dependent variability of motor impairment is well documented in the clinical literature, the underlying neural mechanisms are not well understood. To investigate, we recorded neural activity from BG output nuclei of hemi-PD and control mice trained on a two-alternative forced choice task. We elicited unilateral DAN loss via 6-hydroxydopamine infusion into the left substantia nigra pars compacta and recorded from a principal output nucleus of the BG, the substantia nigra pars reticulata (SNr), ipsilateral to DAN loss. We compared behavior and neural activity between hemi-PD and control mice, and between two conditions requiring otherwise-identical orienting movements. Under the "stimulus-guided" condition, the direction of movement (left vs. right) was selected based on the identity of the stimulus. Under the "internally-specified" condition, the direction of movement was selected based on recent history of rewarded movements. Blocks of stimulus-guided and internally-specified trials were interleaved within each behavioral session. We found that DAN loss led to an ipsilateral bias on both stimulus-guided and internally-specified trials. Consistent with this bias, we found that a larger proportion of SNr neurons in hemi-PD than control mice exhibit movement-related activity promoting ipsilateral movement. In addition, in control mice, we found that SNr activity depended more strongly on movement direction (i.e., activity was more direction selective) in internally-specified than stimulus-guided trials. In hemi-PD mice, the distribution of direction preferences closely resembled that of control mice on internally-specified trials, but differed on stimulus-guided trials: specifically, SNr activity in hemi-PD mice was less direction-selective than in control mice on stimulus-guided trials. These results suggest a neural substrate for how PD may alter movements performed under some conditions more than others.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.14/F29

Topic: C.03. Parkinson's Disease

Title: How do subcortical networks shape the dynamics of pathological beta bursts? An *in-silico* dissection of Parkinsonian brain rhythms and the role of interareal phase synchronization

Authors: *T. WEST[1], S. F. FARMER[2], V. LITVAK[3], P. J. MAGILL[4], A. SHAROTT[6], H. CAGNAN[5];
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Abstract: Parkinsonism and its associated motor impairments have been attributed to an aberrant rhythmicity of large-scale neural dynamics in the basal ganglia. The biophysical mechanisms concerning the origins and control of this rhythm, occurring at beta frequencies (14-30 Hz), are currently unknown. Multiple hypotheses exist to explain the emergence of pathological oscillations as arising from alterations in the strength of connections in the cortico-basal-ganglia network. In this work we employ an inverse modelling framework in which we fit parameters to simulate data close in form to that observed empirically. Specifically, we optimize model parameters to match the spectral power and directed functional connectivity of a set of experimental recordings: local field potentials from striatum, external globus pallidus, and subthalamic nucleus; and electrocorticography from primary motor cortex in Parkinsonian rats with 6-OHDA induced dopamine depletion. After model parameters are fit to time averaged statistics of the data, we move beyond the steady state to investigate the time evolving processes that act to shape the intermittencies in rhythms- so called beta bursts -which have become of recent interest to the field.
Using a formal model comparison approach, we identify the optimal network architecture to explain the empirical data. Having established the best fitting model we then conduct a systematic *in-silico* lesion and stimulation study in order to a) determine the origin and evolution of beta rhythms in the network; b) analyse the roles of particular pathways in shaping the properties of intermittent beta activity such as burst length, duration, and phase synchronization; and c) investigate novel stimulation strategies to suppress pathological network dynamics. We find evidence for the role of the so called cortico-subthalamic "hyperdirect" pathway in modulating subcortical beta activity by supplementing antecedent bursts arising in the striatum. Overall this work provides testable predictions for experimental manipulations of pathological dynamics in the basal ganglia.

Disclosures: **T. West:** None. **S.F. Farmer:** None. **V. Litvak:** None. **P.J. Magill:** None. **A. Sharott:** None. **H. Cagnan:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.15/F30

Topic: C.03. Parkinson's Disease

Support: Doris Duke Clinical Scientist Development Award

Title: Dissociable locations of beta frequency power and coherence in patients with Parkinson's disease

Authors: ***P. M. LAURO**[1], M. PINTO[1], E. SCHAEFFER[1], S. LEE[1], W. F. ASAAD[2]; [1]Neurosci., [2]Neurosurg., Brown Univ., Providence, RI

Abstract: Exaggerated beta frequency (13 − 30 Hz) oscillations in basal ganglia nuclei such as the subthalamic nucleus (STN) are associated with Parkinson's disease (PD). Symptoms of PD are currently measured by the Unified Parkinson Disease Rating Scale (UPDRS). To understand the relationship between beta frequency oscillations, UPDRS scores, and basal ganglia anatomy, we examined microelectrode recordings (MER) from 18 patients with PD receiving deep brain stimulation (DBS) surgery. We then calculated beta frequency power at intraoperative recording sites with at least 20 seconds of data (N = 2310). As patients typically had 3 microelectrodes along the same trajectory, beta coherence was calculated between adjacent electrodes (N = 1540). Beta power and coherence from each hemisphere were then regressed with each patient's lateralized UPDRS III scores. Beta coherence was found to significantly positively correlate (r^2 = 0.15, p = 0.02) with UPDRS scores, whereas beta power showed no such relationship (r^2 = 0.04, p = 0.21). Using intraoperative CT and preoperative MR images, all MER sites were then reconstructed to patient imaging, and combined across patients to the MNI PD25 atlas. Beta power and coherence were then plotted separately. To determine where beta power or coherence were observed above chance, each voxel's average metric value was compared to a bootstrapped (10000 iterations) distribution of all metric values across voxels. Each voxel's empirical p-value was determined to be significant based on the Benjamini-Hochberg correction at a FDR of 10%. For beta power, a significant cluster of 27 $1mm^3$ voxels was found in the dorsolateral STN (MNI coordinates: x = +15.8, y = +12.0, z = -3.5). For beta coherence, four significant 7 $1mm^3$ voxel clusters were found throughout several structures superior to the STN, including the thalamus and globus pallidus pars interna (GPi). Together, these results suggest that different spectral properties of beta frequency oscillations exhibit dissociable spatial locations throughout the basal ganglia, and relate differently to PD symptoms. Future work will examine the relationship of specific PD symptoms and subtypes to spectral features and spatial locations.

Disclosures: **P.M. Lauro:** None. **M. Pinto:** None. **E. Schaeffer:** None. **S. Lee:** None. **W.F. Asaad:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.16/F31

Topic: C.03. Parkinson's Disease

Support: German Research Foundation to the GRK 1957 'Adipocyte-Brain Crosstalk'

Title: Manipulating hedonic control of eating: Effects of STN DBS in Parkinson's disease

Authors: *J. STEINHARDT[1], H. HANßEN[2], P. SCHRAMM[3], J. K. KRAUSS[4], J. VOGES[6], V. TRONNIER[5], T. F. MÜNTE[7], M. HELDMANN[7], N. BRÜGGEMANN[8];
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Abstract: Background: Body weight gain is observed in the majority of patients with Parkinson's disease (PD) who undergo deep brain stimulation (DBS) of the subthalamic nucleus (STN). Aside from changes in energy expenditure, an abnormal modulation of the mesolimbic system is accounted for changes in motivated behavior, food intake, and body weight. **Objectives:** To investigate the modulatory impact of STN DBS on the neural responses to food stimuli in PD patients.**Methods:** Fifteen medicated PD patients (mean age 63.7 years (SD 8.89)), mean disease duration 13 yrs (SD 3.49) were investigated using functional MRI (1.5T) during (i) active and (ii) switched off DBS. Food and neutral images (kitchen equipment) were presented in a slow event related design. A whole brain and a ROI-based analysis were performed. **Results:** Whole brain GLM revealed a main effect of DBS in the left lingual gyrus, right cuneus, right middle cingulate gyrus and the central operculum bilaterally (cluster level, uncorrected, $p \leq 0.001$). An interaction was found for food images during active DBS in the left lingual gyrus and the superior parietal lobe bilaterally (FWE, $p \leq 0.001$). During inactive DBS, food images were associated with an increased BOLD response in the left lingual gyrus (FWE, $p \leq 0.001$). In contrast, processing of non-food images showed an increased BOLD response in the left superior parietal lobe (FWE, $p \leq 0.001$) and during inactivated DBS in the left superior occipital gyrus (FWE, $p \leq 0.001$). ROI-based analysis revealed a decreased activation of the left amygdala (%signal change; $p=0.037$, uncorrected) and an increased activation of the right insula ($p=0.044$) during food image processing. **Discussion:** DBS may exert complementary actions on neural networks that could collectively give rise to an increased vulnerability to food cues. Firstly, DBS

globally increased attentional control and may thus bias the brain to an increased awareness of external stimuli. Secondly, reward-related circuits were activated as a function of STN DBS arguing that a co-activation of the limbic portion of the STN may influence hedonic control of food intake.

Disclosures: **J. Steinhardt:** A. Employment/Salary (full or part-time):; University of Lübeck. **H. Hanßen:** A. Employment/Salary (full or part-time):; University of Lübeck. **P. Schramm:** A. Employment/Salary (full or part-time):; University of Lübeck. **J.K. Krauss:** A. Employment/Salary (full or part-time):; University Medical Center Hannover. **J. Voges:** A. Employment/Salary (full or part-time):; University Medical Center Magdeburg. **V. Tronnier:** A. Employment/Salary (full or part-time):; University Medical Center Lübeck. **T.F. Münte:** A. Employment/Salary (full or part-time):; University of Lübeck. 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.; DFG, BMBF. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hogrefe Publishers, Thieme Publishers, Elsevier. **M. Heldmann:** A. Employment/Salary (full or part-time):; University of Lübeck. **N. Brüggemann:** A. Employment/Salary (full or part-time):; University of Lübeck. 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.; Collaborative Center for X-linked Dystonia-Parkinsonism, Else Kröner-Fresenius-Foundation, DFG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Springer. Other; UCB, Grünenthal, Tena.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.17/F32

Topic: C.03. Parkinson’s Disease

Support: Doris Duke Clinical Scientist Development Award (WFA)
Doris Duke Clinical Research Mentorship (JY & WFA)
Medtronic External Research Program (WFA)
Norman Prince Neurosciences Institute
Brown University Carney Institute for Brain Science

Title: Spatial dependence of frequency effects in deep brain stimulation

Authors: *D. E. AMAYA[1], P. M. LAURO[1], J. YU[1], D. D. LIU[1], E. L. SCHAEFFER[1], M. AHN[2], U. AKBAR[1], S. LEE[1], W. F. ASAAD[1];
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Abstract: Objective: Deep brain stimulation (DBS) has become a widely adopted treatment for Parkinson's disease (PD) and is thought to modulate the balance of activity between the direct and indirect pathways of the basal ganglia. Typically, high-frequency stimulation (~130 Hz) is delivered to a target such as the subthalamic nucleus (STN), a node in the indirect pathway of the basal ganglia. Lower frequency stimulation at the STN is generally less effective and may in fact exacerbate patient symptoms. We hypothesized that stimulating a node in the direct pathway (the Substantia Nigra pars reticulata, SNr) rather than the indirect pathway may elicit a different response to high vs. low frequency stimulation.
Methods: We recruited 6 PD patients with implanted STN DBS systems. Patients engaged in a continuous motor performance task while stimulation location and frequency were varied on different trials. Specifically, we compared stimulation at the therapeutic contact vs. the deepest implanted contact, closest to the SNr.
Results: High frequency (130 Hz) stimulation at the therapeutic contact (more dorsal) typically improved motor performance by 15% compared to lower frequency (20 Hz) stimulation at the same contact ($p<0.001$). In contrast, at the deepest contact, lower frequency stimulation often improved motor performance by 28% compared to high frequency stimulation ($p=0.066$).
Conclusions: These results suggest that lower frequency stimulation at the deepest contact (closest to the SNr) may in fact be more beneficial than high frequency stimulation at that location, consistent with the notion that driving the SNr may provide some therapeutic benefit.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.18/F33

Topic: C.03. Parkinson's Disease

Support: Canadian Institutes of Health Research (CIHR) Doctoral Award (357215) received by J. Baarbé

Queen Elizabeth II Graduate Scholarship in Science & Technology received by J. Baarbé
Dystonia Medical Research Foundation Canada Fellowship Grant received by K.J. Lizarraga
CIHR Foundation Grant (FDN 154292) received by R. Chen

Title: Phase-related high-beta oscillations in Parkinson's disease during stepping in a virtual environment

Authors: J. K. BAARBÉ, ***S. TRAN**, K. J. LIZARRAGA, J. SARAVANAMUTTU, P. SAHA, R. CHEN;
Toronto Western Hosp., Toronto, ON, Canada

Abstract: Background: Patients with Parkinson's disease (PwPD) have increased high-beta oscillations (21-35 Hz) in the sensorimotor cortex, which may be anti-kinetic and disrupt motor preparation. It is unknown how high-beta oscillations in the sensorimotor cortex change during progressive stepping in PwPD and how it is modulated by dopaminergic medications.
Hypothesis: We believe that high-beta oscillations will synchronize during progressive phases of visually-guided steps during OFF medication states in PwPD, while dopaminergic medications will desynchronize these oscillations.
Methods: We recorded whole-head EEG in 10 PwPD [2 women, median age = 65 years, median PD duration = 8 years]. PwPD were assessed on two separate days, ON-medication and OFF-medication after a 12-hour withdrawal period. PwPD were required to step on foot pedals to progress down a virtual hallway while seated. Practice trials were conducted using a metronome set at 2 Hz and subjects were asked to maintain the same pace during testing. Time-frequency data was time-warped to individual rates. High-beta power was averaged over right toe and right heel phases. The difference in high-beta power between the phases was assessed (heel-down versus toe-down) at three EEG regions: premotor/frontal (F1, Fz, F2, FC1, FCz, FC2), motor (C1, Cz, C2), and parietal (CP1, CPz, CP2, P1, Pz, P2).
Results: No difference in high-beta power was observed between toe- and heel-down phases. Stepping amplitudes remained the same between phases in both medication states. However, high-beta power between ON- and OFF-medication states was significantly different in both motor ($p = 0.04$) and parietal ($p = 0.02$) regions. As such, OFF-medications increased high-beta power compared to ON-medication states, which decreased high-beta power, during the transition from toe-to-heel phases.
Conclusion: This study demonstrates that dopaminergic medications reduce high-beta power in the motor and parietal regions during progressive phases of stepping in a virtual environment.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.19/F34

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS091236
NIH R37 NS040894
NIH R03 NS108097

Title: Frequency specific neural circuit modulation during optogenetic deep brain stimulation in subthalamic nucleus

Authors: *C. YU[1], I. R. CASSAR[2], J. SAMBANGI[3], W. M. GRILL[3];
[1]Michigan Tech. Univ., Houghton, MI; [2]Biomed. Engin., [3]Duke Univ., Durham, NC

Abstract: Deep brain stimulation (DBS) is an effective therapy for the motor symptoms of Parkinson's disease (PD). The therapeutic effects of DBS are highly dependent on stimulation frequency. High frequency DBS alleviates PD motor symptoms, while low frequency stimulation is ineffective. The subthalamic nucleus (STN) is a well-documented DBS target and has been proven clinically effective. However, the neural mechanisms underlying the therapeutic effects of STN-DBS for PD are still less understood. Here we used the unilateral 6-OHDA-lesioned rat model of PD and applied optogenetic stimulation in the STN using an ultrafast opsin (Chronos) that is able to follow high-frequency DBS. The Chronos construct was packaged into an adeno-associated virus serotype 5 (AAV5) under the control of calcium/calmodulin-dependent protein kinase II (CaMKII) promoter. We quantified the behavioral effects of different frequencies of optogenetic STN DBS on motor symptoms using pathological circling behavior and the adjusting steps test. We performed single unit recordings to quantify changes of neural circuit activity during different frequencies of optical stimulation in the STN of urethane-anesthetized rats (1.2g/kg, subcutaneous). We found that cell-specific optogenetic stimulation of STN local neurons exhibited a strong dependence on stimulation frequencies; high frequency DBS (75, 100, 130Hz) alleviated ipsilateral turning and corrected forelimb stepping bias while low frequency (5Hz and 20Hz) DBS was not effective. Different frequencies of optogenetic stimulation differentially increased or decreased the neural activity in the STN, globus pallidus externa (GPe) and substantia nigra pars reticulata (SNr). High frequency optical stimulation produced larger increases or decreases in neuronal firing rates in comparison to low frequency stimulation. As well, high frequency optogenetic DBS reduced pathological beta band oscillations in the STN and SNr, while low frequency optogenetic DBS had no effect on beta band oscillations. Our results suggest that optical stimulation at high frequencies resulted in stronger reduction of abnormal oscillatory activity in the STN-associated neural circuit and thereby was more effective

than low frequency stimulation at ameliorating pathological circling behavior and improving forelimb stepping. **Funding**: This work was partially supported by the National Institute of Neurological Disorders and Stroke under Award Numbers NIH R01 NS091236, R37 NS040894 and R03 NS108097

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.20/F35

Topic: C.03. Parkinson's Disease

Support: Spanish Goverment Grant SAF2015-67239-P
Marie Slodowksa-Curie Fellowship MSC-IF
CIBERNED

Title: Optogenetic modulation of striatal pathways elicits abnormal movements in a Parkinson's disease model

Authors: *I. CASTELA[1,2], R. CASADO[1], R. MARQUEZ[1], R. MORATALLA[3,2], J. OBESO[1,2,4], L. HERNÁNDEZ[1,2];
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Abstract: Parkinson disease (PD) is characterized by the early loss of dopaminergic neurons in the Substantia nigra *pars compacta* (SNpc) ventral tier, which leads to PD cardinal symptoms: postural instability, bradykinesia, rigidity and tremor. Currently, levodopa (L-DOPA) is the most effective therapy used, but long-term treatment is associated with the development of L-DOPA-induced dyskinesias (LIDs). We have previously reported in a rat model that simultaneous optogenetic activation of the direct and indirect striatal pathways elicited abnormal movements (optodyskinesias) that resemble those evoked by levodopa (Hernandez et al, *Mov. Dis*., 2017). Here, we present the data obtained from transgenic mice that allowed us to dissect the striatal circuitry by optically activating each pathway separately. We have found that simultaneous activation of both pathways induces abnormal movements in both control (sham) and dopamine depleted animals (different from what was obtained in rats). Moreover, when we stimulated each pathway separately, optical activation of direct pathway (D1R-expressing) neurons evoked light-induced dyskinesias, while optical activation of indirect pathway (D2R-expressing neurons) promoted freezing behavior and/or ipsilateral rotations. These experiments show that co-activation of both pathways can elicit abnormal movements, but the stimulation of direct

pathway neurons is sufficient and necessary to elicit dyskinetic movements. Finally, we show that while the inhibition of direct pathway is insufficient to block LIDs, activating the indirect pathway shows a striking blockage of this behavior, which may offer a novel way of suppressing these distressing side effects of L-DOPA therapy in PD.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.21/F36

Topic: C.03. Parkinson’s Disease

Support: NHRI-EX108-10615NC

Title: The relationship between temporal sensory discrimination and locomotor ability in Parkinson's disease

Authors: *C.-W. HUANG[1], C.-H. LIN[2], M.-T. TSENG[3];
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Abstract: Parkinson’s disease (PD) is one of the most widespread neurodegenerative diseases in the world. Patients usually experience motor difficulty such as gait freezing which limits their daily life apparently. Compelling evidence suggests early sensory declination predates motor symptoms of the disease. Since sensorimotor integration plays a key role on locomotor fluency and stability, investigation of sensory abnormality in early stage of PD provides an opportunity to elucidate deterioration of gait dysfunction. The objective of the present research is to examine the interactions of sensory and locomotor aberrance in PD. We has recruited twenty patients with PD (8 men) aged between 54 and 76 years (mean: 63.9) and twenty age- and sex-matched healthy controls (HC). Sensory and gait performances were measured in all participants. Vibrotactile stimuli were delivered from two stimulators to medial dorsum of left calf of subjects for sensory function testing. Somatosensory detection threshold (DT), temporal discrimination threshold (TDT), and spatial discrimination threshold (SDT) were evaluated. Seven-meter walking test was applied for obtaining gait velocity (GV), stride length (SL), and gait cadence (GC) which are central parameters in gait function. Our results showed that both sensory and gait performances in PD patients were impaired compared with HC. Furthermore, there was a significant task-by-group interaction, suggesting that abnormality of temporal discrimination was more severe than that of spatial discrimination in patients. Most importantly, prolonged TDT but not SDT correlated with shortened SL in patients with PD. In conclusion, these results indicate a

unique role of high-level sensory function in gait ability and suggest that impairment in the temporal processing of somatosensory stimuli might contribute to motor dysfunctions in PD.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NSF 1516288
NIH NS076524
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NIH NS101016
NIH NS104835

Title: Parkinsonian slow oscillations in the indirect pathway precede M1 oscillations and predict motor dysfunction

Authors: *T. C. WHALEN[1], A. M. WILLARD[3], J. E. RUBIN[4], A. H. GITTIS[2];
[2]Biol. Sci., [1]Carnegie Mellon Univ., Pittsburgh, PA; [3]Clarion, Clarion University, PA;
[4]Mathematics, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Oscillations in a wide range of frequency bands have been implicated in Parkinson's disease (PD), but due to low frequency noise in awake subjects, slow (0.5-3 Hz) oscillations have been understudied. We developed a novel phase metric to reliably detect single unit spike oscillations embedded in pink noise to dissect the oscillatory content of neurons in the basal ganglia of healthy and Parkinsonian (6OHDA dopamine depleted) mice. In dopamine depleted animals, a large number of neurons exhibit a 0.5-3 Hz oscillation not seen in healthy animals, but we find no oscillations in any other frequency bands Using nonparametric regression, we demonstrate that these slow oscillations are a good predictor of dopamine loss and motor dysfunction, strongly outperforming other physiological measures such as changes in firing rate, irregularity, bursting and synchrony. These slow oscillations arise immediately after loss of D2, but not D1, receptor activation, and are present throughout the indirect pathway - the substantia nigra pars reticulata (SNr), globus pallidus externa, and subthalamic nucleus. A regression analysis shows that small variations in the shape and timing of spike oscillations in the SNr predict similar changes in the motor cortex (M1) approximately 150 msec later. This suggests that slow oscillations are a major contributor to Parkinsonian dysfunction, arise in the basal

ganglia and entrain M1. Similar slow oscillations which have been observed but sparsely studied in human PD patients could be an important target for PD treatment.

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Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

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Program #/Poster #: 382.23/F38

Topic: C.03. Parkinson's Disease

Support: CIHR MOP-136778

Title: The altered dynamic amplitude of low-frequency fluctuation associated with depression and anxiety in Parkinson's disease

Authors: *J. KIM[1,2], M. VALLI[1,2], M. CRIAUD[1,2], A. MIHAESCU[1,2], S. CHO[1,2], M. CIRARDA[1,2], A. STRAFELLA[1,2,3];
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Abstract: Neuropsychiatric complications, such as anxiety and depression, are common in patients with Parkinson's disease (PD). Previous studies using the amplitude of low-frequency fluctuations (ALFF) method on resting-state functional magnetic resonance imaging (fMRI) have reported abnormal spontaneous brain activity associated with these mood disturbances in Parkinson's disease (Skidmore et al., 2013; Wang et al., 2018; Wen et al., 2013). Here, we utilized a dynamic ALFF (dALFF) method to examine the temporal variability of local brain activity in PD patients. Thirty-nine PD patients ON medication and twenty-six healthy controls (HCs) underwent 8 min 4-sec resting-state fMRI scans on a GE 3-T scanner (TR = 2s; eyes closed). Pre- and post-processing of resting-state fMRI data were performed using the CONN toolbox (motion correction, spatial normalization to MNI template, spatial smoothing with a 6 mm FWHM Gaussian kernel, nuisance signal regression via a CompCor, and band-pass filtering of 0.0227-0.1 Hz). We captured the dynamic patterns of the ALFF using a sliding windows approach implemented in the DynamicBC toolbox (Liao et al., 2014). Resting state data were segmented into a series of overlapping windows (length = 44s, overlap =90%). For each window, a whole-brain ALFF map, defined as the intensity of low-frequency oscillations, is calculated using a Fourier transformation, resulting in 72 time-varying ALFF maps. These maps were standardized by dividing the mean ALFF values. The temporal variability of the dALFF was quantified as the variance of time-varying ALFF maps. Here, we examined the group differences

between the PD and HC groups on the temporal variability map of the dALFF as well as its relationship with the degree of depression and anxiety symptoms, as measured by the Beck Depression Inventory-II and State-Trait Anxiety Inventory respectively, in the PD group (cluster-level FWE corrected $p < 0.05$). We found that PD patients compared to HC showed decreased temporal variability of the dALFF in the bilateral ventrolateral prefrontal cortex and brain stem. In addition, the depression severity in the PD group was positively correlated with the degree of the dALFF temporal variability in the right thalamus, indicating PD patients with more severe depressive symptoms exhibited more variant intrinsic brain activity in the thalamus. We also found a significant positive correlation between the degree of the anxiety symptoms and the dALFF temporal variability in the cerebellum. Our findings support the involvement of subcortical regions in mood disturbances in PD, providing further insight into behavioral complications of the disease.

Disclosures: J. Kim: None. **M. Valli:** None. **M. Criaud:** None. **A. Mihaescu:** None. **S. Cho:** None. **M. Cirarda:** None. **A. Strafella:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.24/F39

Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS108068

Title: Modulation of parkinsonism and L-DOPA-induced dyskinesia by the substantia nigra reticulata

Authors: *Y. HU, S. L. ALBERICO, T. C. MA, U. J. KANG;
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Abstract: Levodopa therapy is the most effective treatment for PD, but long-term use is often complicated by abnormal involuntary movements termed levodopa-induced dyskinesia (LID). PD and LID arise from complex compensatory changes throughout the basal ganglia, including the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr). GABAergic neurons are the main neurons in the SNr and these project to the mesencephalic locomotor region (MLR), parafascicular nucleus (PF), ventrolateral nucleus (VL), ventromedial nucleus (VM), and reticular nucleus (RT). Here, we explored the role of SNr in PD and LID by selectively regulating SNr GABAergic neurons activity in VGAT-Cre mice using chemogenetic and optogenetic techniques. Using activating (hM3d-Gq) and inhibitory (hM4d-Gi) DREADDs + clozapine-N-oxide, we bidirectionally modulated SNr GABAergic neuron activity in non-lesioned mice, dopamine-depleted mice, and mice with LID while measuring forepaw stepping,

openfield activity, and abnormal involuntary movements (AIM). In non-lesioned mice we found that increasing SNr GABAergic neuron activity with hM3d-Gq caused akinesia similar to dopamine-depleted mice (unilateral MFB 6-OHDA lesion), while the inhibition of SNr GABAergic neurons with hM4d-Gq had the opposite effect. However, in 6-OHDA-lesioned mice, both activation and inhibition of SNr GABAergic neuron activity improved PD symptoms. In mice with LID we found that increasing SNr GABAergic neuron activity significantly attenuates LID, and inhibiting SNr GABAergic neuron activity increased LID expression. To address whether specific SNr targets mediated these changes, we expressed ChR2 in SNr GABAergic neurons and stimulated their cell bodies or axonal projections to the PF, VM/VL, RT, and MLR with blue light. Optogenetic activation of SNr cell bodies improved both PD and LID symptoms. Interestingly, stimulating SNr axon terminals in PF improved PD symptoms, but did not affect LID, while stimulation SNr axon terminals in MLR significantly decreased LID without affecting PD symptoms. Because the PF is expected to be inhibited in the PD state, it was surprising to find that further inhibition could improve PD symptoms. To further address this, we expressed the activating hM3d-Gq construct in PF glutamatergic neurons in VGLUT2-CRE mice and found that increasing PF activity significantly improved LID. Our study suggests that direct modulation of basal ganglia output of the SNr GABAergic neurons improve PD symptoms and LID expression through SNr targets.

Disclosures: Y. Hu: None. **S.L. Alberico:** None. **T.C. Ma:** None. **U.J. Kang:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

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Program #/Poster #: 382.25/F40

Topic: C.03. Parkinson’s Disease

Support: NIH NINDS grant R01NS086100
NIH NINDS grant R01NS092730
Cleveland Clinic

Title: Layer-specific cortical changes in response to deep brain stimulation of the subthalamic nucleus

Authors: *D. M. TAYLOR[1,2,3], T. JOHNSON[1,2,3], S. MORALLE[1,2], S. MEADE[1,2], B. CAMPBELL[1], K. BAKER[1];
[1]Neurosciences, Cleveland Clin., Cleveland, OH; [2]Functional Electrical Stimulation Ctr. of Excellence, Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH; [3]Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus is often used clinically to treat symptoms of advanced Parkinson's disease. However, the mechanism of action of DBS is still uncertain as DBS likely alters firing behavior in multiple parts of the motor control network. DBS was traditionally thought to improve Parkinson's symptoms by orthodromic modulation of the basal ganglia-thalamo-cortical pathway. However, in recent years, antidromic activation of the cortex via 'hyperdirect pathway' neurons has been posited as a potential therapeutic mechanism of DBS. The hyperdirect pathway consists of cortical projection neurons that send axonal branches to the subthalamic nucleus. Modeling work suggests antidromic activation of these axons from the subthalamic nucleus to the cortex has the potential to have a widespread modulatory impact on the cortical network. This is because the DBS-induced action potentials not only travel antidromically to the somas in the cortex, they can also propagate throughout the extensive cortical axonal branching structure to engage synapses across a range of cortical layers and neuronal targets. In this study, we used intracortical microelectrodes to simultaneously record from different layers in the motor cortex while applying DBS to the subthalamic nucleus in animal models of Parkinson's disease. Our initial results showed slow layer-specific shifts in the baseline recorded voltages when continuous DBS is turned on/off. In addition, we also saw rapid layer-specific pulse-synced evoked response patterns reflecting the direct and synaptic effects of each individual stimulation pulse. To further tease out the therapeutic relevance of these two cortical responses occurring at different time scales, we are systematically varying DBS patterns across a range of therapeutic and non-therapeutic settings to identify response features that consistently appear across a wide range of therapeutic settings. Results from this analysis could suggest refinements in how DBS is delivered in order to maximize the therapeutic benefit while reducing side effects and power consumption.

Disclosures: D.M. Taylor: None. **T. Johnson:** None. **S. Moralle:** None. **S. Meade:** None. **B. Campbell:** None. **K. Baker:** None.

Poster

382. Circuit Mechanisms of Motor Dysfunction in Parkinson's Disease

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 382.26/F41

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS092730

Title: Potentials evoked by directional deep brain stimulation of the subthalamic nucleus region recorded using electrocorticography in the non human primate

Authors: *B. A. CAMPBELL[1], H. CHO[1], C. E. WYANT[1], A. G. MACHADO[2], K. B. BAKER[1];
[1]Neurosciences, [2]Ctr. Neurolog. Restoration, Cleveland Clin., Cleveland, OH

Abstract: Electrical stimulation in the region of the subthalamic nucleus (STN) is known to induce widespread changes in neural activity across the basal ganglia thalamocortical network. A better understanding of how deep brain stimulation (DBS) modulates cortical activity holds potential to inform its mechanistic underpinnings and help efforts to identify control signals for next-generation, adaptive DBS paradigms. In the current study, the effects of STN DBS on cortical LFP activity was examined in two, naïve non-human primates instrumented with scaled, six contact directional DBS lead (Abbott Medical) in the area of the STN combined with bilateral, twelve-channel subdural, ECoG paddle electrodes spanning anterior parietal to prefrontal cortical regions. The relative effect of annular versus directional and biphasic versus monophasic (anodal versus cathodal) stimulation paradigms as well as changes in pulse width (20 - 200 usec) and frequency (3.1 - 185.1 Hz) on evoked cortical LFPs was evaluated in the awake animal. Annular stimulation below the threshold for motor activation (~ lateral capsule spread) was associated with multi-phasic cortical potentials marked by early, high-frequency components that included peaks as early as 0.5 ms post-stimulation, maximal across motor and premotor cortices, followed by sequentially broader peaks that persisted for as long as 100 ms post-stimulation. Marked differences in the cortical potentials were observed based upon stimulus polarity, with pseudo-monopolar cathodal stimulation yielding larger evoked responses than anodal stimulation conditions. During biphasic, symmetric stimulation, the order of the anode/cathode pulses failed to have a significant impact on component latencies or amplitudes. Responses were further sensitive to directionality, with differences in response morphology observed across the three, 120-degree directions afforded by the lead. Finally, the short-latency (<10 ms) components did not appear to be significantly influenced by pulse frequency up to 185.1 Hz. These data begin to characterize the effects of STN DBS stimulation parameters on LFP activity across cortical motor and premotor regions and provide the foundation for further comparisons to be made across the parkinsonian state, including untreated and treated conditions.

Disclosures: B.A. Campbell: None. **H. Cho:** None. **C.E. Wyant:** None. **A.G. Machado:** None. **K.B. Baker:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.01/F42

Topic: C.03. Parkinson’s Disease

Support: Parkinson Canada

Title: Exercise interacts with dopamine to improve motor learning in Parkinson's disease

Authors: *J.-F. NEPVEU[1], M. M. BECK[2], D. MEDEIROS[3], L. RODRIGUES[3], B. DE LAS HERAS[3], M. WEISS[3], C. CENTENO[3], J. SALIB[5], L. BHERER[6], A. DAGHER[3], J. DOYON[7], J.

LUNDBYE-JENSEN[8], M. ROIG[4];
[1]Memory and Motor Rehabil. Lab. (MEMORY-LAB), Laval, QC, Canada; [2]Univ. of Copenhagen, Copenhagen, Denmark; [4]Physical and Occup. Therapy, [3]McGill Univ., Montreal, QC, Canada; [5]Univ. de Bordeaux, Bordeaux, France; [6]Univ. de Montréal, Montréal, QC, Canada; [7]McConnell Brain Imaging Ctr., Montreal, QC, Canada; [8]Dept. of Neurosci. and Pharmacology, Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Parkinson's Disease (PD) leads primarily to motor impairments such as tremor, rigidity, bradykinesia and postural instability. The pathogenesis of these impairments is related to dopaminergic neurotransmission in the basal ganglia. Despite having shown an enormous potential to help maintain motor function in PD, little is known about how cardiovascular exercise interacts with dopamine (DOPA). Understanding this interaction is crucial because the rapid loss of dopaminergic neurons is the pathophysiological signature of PD and impairs their capacity to acquire and retain motor skills. We investigated the interactive effects of cardiovascular exercise and DOPA on motor learning in people with PD. Twenty-five individuals (52-75 years old) diagnosed with moderate PD reported to the laboratory at 8 a.m. in a fasting state after an overnight withdrawal of levodopa (L-DOPA) and were randomly divided into four groups: DOPA+EXE, DOPA+CON, NDOPA+EXE, NDOPA+CON. Before practicing a visuomotor task the DOPA groups received their normal dose of L-DOPA while the NDOPA groups received a placebo administered in a double-blinded fashion. Twenty minutes after practicing 5 blocks of a visuomotor task, the EXE groups performed 15 mins of high-intensity interval training cycling while the participants of the CON groups underwent 15 mins of rest. Participants performed one block of the motor task 48 hours after motor practice to assess skill retention. A linear mixed model with group and block of motor practice as fixed interaction terms and subject as random intercepts was used to assess differences among groups in motor skill acquisition and retention. The linear mixed model revealed a significant group x block interaction ($F = 3.36$; $p < 0.001$). Planned comparisons revealed no differences among groups at baseline (p-value range: 0.46-0.92) or when comparing the changes from baseline to the last block of motor practice (i.e. skill acquisition) (p-value range: 0.19-0.88). In contrast, motor skill retention (estimate ± standard error) was significantly greater in DOPA+EXE than NDOPA+CON (5.51 ±1.67, z=3.92, p = 0.001) and in DOPA+EXE compared to DOPA+CON (3.45 ± 1.74, z=1.99, p = 0.05). These preliminary findings indicate that a single bout of intense cardiovascular exercise can improve skill retention in PD but only when dopamine precursors are co-administered. Conclusively, exercise appears to have a synergistic relationship with DOPA to optimize motor learning. Timely combining the effects of L-DOPA medication with sessions of exercise and motor learning could maximize the effects of cardiovascular exercise on motor function in people with PD.

Disclosures: **J. Nepveu:** None. **M.M. Beck:** None. **D. Medeiros:** None. **L. Rodrigues:** None. **B. de Las Heras:** None. **M. Weiss:** None. **C. Centeno:** None. **J. Salib:** None. **L. Bherer:** None. **A. Dagher:** None. **J. Doyon:** None. **J. Lundbye-Jensen:** None. **M. Roig:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.02/F43

Topic: C.03. Parkinson's Disease

Support: Kent State University's Brain Health Research Institute (BHRI) Pilot Program

Title: Optimizing dance interventions to improve motor function in people with Parkinson's disease and older adult

Authors: *P. GATES[1], J. H. KIM[1], R. MELCZAK[1], J. MEGGITT[1], F. DISCENZO[2], A. L. RIDGEL[1];
[1]Kent State Univ., Kent, OH; [2]Rockwell Automation (retired), Cleveland, OH

Abstract: Dance comprises a broad range of techniques and styles which have been utilized in classes specifically designed for individuals with Parkinson's disease (PD) and healthy older adults. Previous studies have shown that a series of dance sessions can improve balance, posture and mobility for people diagnosed with PD and healthy older adults. However, these studies have not analyzed the linkage between repetitive movement types and persistent changes in motor skills. In order to begin understanding the causative factors of repetitive movement types that result in improved motor skill performance, the functional relationship between movement features and observed performance improvements needs to be examined. PURPOSE: To identify dance movement patterns resulting in the greatest improvement in tests of gait, balance and upper extremity function using partnered and non-partnered dance to music in PD and healthy older adults. We hypothesize that scripted variation in movement will promote improvements in motor performance. METHODS: Participants with and without PD in structured group dance classes were recruited for this study. Performance measures of upper and lower extremity were collected before and after each dance class. Motion capture, video and live observations were used to examine movement patterns. RESULTS: Individuals with PD had slower baseline performance in the 9 hole peg test (9HPT) than healthy older adults in both left ($p=0.026$, 33.5s vs 24.9s) and right hand ($p=0.008$, 31.2s vs 26.5s). There was also a significant improvement in the 9HPT for the left hand after the dance classes in the individuals with PD ($p=0.035$, 3.44s). Participants with PD saw no differences in hip range of motion or maximum movement angles between the different dance types, however those without PD did see such differences ($p< 0.01$ for left and right hip flexion, and left and right hip rotation). Factors that led to observed improvements in mobility and movement execution included: repetition of foundational weight shifts in a separate exercise, engagement of the spine and arms in counterbalancing movement in the legs, incorporating flexion at the knee into the dance stride, and partnering with a moderately-skilled dancer. Increased amplitude and ease of stride and greater lift in the feet in

locomotion were also documented. CONCLUSION: These preliminary results suggest that repetitive shifts in balance and movement during dance with music can lead to upper extremity motor performance and increased amplitude of movement in the lower extremity in individuals with PD. The differences in ROM and maximum angles of motion suggest possible future directions for dance instruction.

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Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.03/F44

Topic: C.03. Parkinson's Disease

Support: NIH Grant UH3 NS103468
NIH Grant R01 NS040894
NIH Grant R37 NS040894

Title: Deep brain stimulation of the subthalamic nucleus may not modulate beta burst activity in persons with Parkinson's disease

Authors: *S. L. SCHMIDT, J. J. PETERS, D. A. TURNER, W. M. GRILL;
Duke Univ., Durham, NC

Abstract: Oscillations in the local field potential (LFP) represent synchronous neural activity and have traditionally been viewed as relatively stable over the time scale of seconds. However, recent focus has shifted to short, high-amplitude LFP events known as bursts. The presence of bursts was correlated to increased performance on working memory tasks and decreased performance on motor and sensory perception tasks. Further, bursts of beta frequency oscillations within the motor circuit appear to be highly correlated with the motor symptoms of PD. Effective treatment of motor symptoms of PD by DBS or administration of levodopa appears to decrease the duration of beta bursts, and adaptive DBS controllers based on the amplitude of the beta signal have shown promising results. However, it remains unclear what the appropriate timescale is for burst analysis. We examined the effect of traditional signal processing methods including filtering, thresholding and minimum burst duration on burst analysis. We used methods drawn from publications on burst dynamics and compared the results to ground truth using an on/off burst oscillator. The number and duration of bursts were highly dependent on the amplitude threshold and smoothing applied by the filter. Specifically, the selection of the threshold determined the amount of time spent in bursts, and the mean burst duration was highly constrained by the smoothing caused by the filter. We then quantified

subthalamic LFPs collected from 18 participants (15 male) with PD during lead placement or battery replacement surgery. All clinical studies were approved by the IRB of Duke University Health System and participants provided written informed consent. We found that using thresholds determined by matching raw amplitudes greatly changed the fraction of time spent in bursts and therefore constrained burst amplitude, duration and rate, thereby leading to biased results. We then analyzed the performance of burst classification with two support vector machines (SVM) using continuous and cycle-by-cycle amplitude data. The resulting burst dynamics were not constrained by a predefined fraction of time spent in bursts. When applied to the participant data, the SVMs produced a range of time spent in bursts (0.5- 47%) across participants. However, there was no significant effect of DBS on time in bursts, burst rate, or duration. Unbiased identification of burst features may lead to identification of biomarkers generally and, in the case of PD, inform adaptive DBS strategies to improve symptom control and reduce side-effects.

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Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.04/F45

Topic: C.03. Parkinson’s Disease

Title: Comparative analysis of structural deficit between olfaction and gustation in terms of diagnostic accuracy and consistency in Parkinson’s disease

Authors: *N. S. OSAKWE, H. CHATTERJEE, G. ELUMALAI, N. SEWRAM;
Texila American Univ., East Bank Demerara, Guyana

Abstract: Smell and taste deficit can be diagnostically challenging to the otolaryngologist as patients with Parkinson’s disease are usually unable to differentiate between deficits of smell and taste. Standardized questionnaire and procedures, good medical history taking and some diagnostic tools like CT scans, MRI etc. have been employed to differentiate and diagnose smell from taste disorder in previous research but no structural analysis have been used for this purpose. Hence, this study demonstrates a structural analysis between the deficit of olfaction and gustation in patients with Parkinson’s disease. With the Institutional Review Board approval, we used 80 Magnetic resonance imaging (sMRI) scans obtained from Parkinson’s Progression Markers Imitative. The total of 40 subjects (20 male and 20 female) with Parkinson’s disease and 40 subjects (20 male and 20 female) as the control group; with the age range from 30- 80 years were used. The main eligibility criteria for diagnostic accuracy and consistency from scans

obtained includes a diagnosis of Parkinson disease within 2 years at Screening with confirmation from imaging core that screening dopamine transporter SPECT scan is consistent with dopamine transporter deficit (or for sites where DaTSCAN^{TM} is not available, that VMAT-2 PET scan is consistent with VMAT deficit) and patients not on PD medication within at least 6 months. Exclusion: Received any drugs that might interfere with dopamine transporter SPECT imaging within 6 months of Screening. The Inclusive criteria for the control group of both sex is same age range with the subjects with PD at Screening. Exclusion includes: Current or active clinically significant neurological disorder, first degree relative with idiopathic PD, received any drugs that might interfere with dopamine transporter SPECT imaging within 6 months of Screening. The datasets from the subjects with PD and the control group are processed and fiber analysis are performed with Diffusion Imaging fiber tractography tracing fiber tracts extending from the VPM nucleus and the gustation system and from the primary cortex to the temporoparietal junction for the olfaction system. Data was statistically analyzed using structural equation modeling (SEM), mean and standard deviation (SD) .There is a significant p-value of .0008 between the both groups of Gustation system and shows a significant p-value of .00001 between both groups of olfaction system. These findings serve as a source for therapeutic intervention for Diagnostic Accuracy and Consistency of Patients with PD; Future research could focus on the functional implications of degeneration in PD, using techniques such as fMRI.

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Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.05/F46

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation
department grant from Rush Neurological Sciences
Parkinson’s disease Foundation

Title: Long-term post-mortem studies following neurturin gene therapy in advanced Parkinson’s disease

Authors: *Y. CHU[1], B. T. RAYMOND[3], W. C. OLANOW[4], J. H. KORDOWER[2];
[2]Dept Neurol Sci., [1]Rush Univ. Med. Ctr., Chicago, IL; [3]RTBioconsultants Inc, San Diego, CA; [4]Mount Sinai Sch. of Med., New York, NY

Abstract: We performed postmortem studies on 2 patients with advanced Parkinson’s disease 8 and 10 years following AAV2-neurturin (CERE120) gene therapy. CERE120 was delivered to

the putamen bilaterally in one case (10 years), and to the putamen plus the substantia nigra pars compacta (SNc) bilaterally in the second (8 years). In both patients there was persistent, albeit limited, neurturin expression in the putamen covering approximately 3 & 12% of the dorsal putamen. In the putamen dense staining of tyrosine hydroxylase (TH)-positive fibers were observed in areas that contained detectable neurturin expression. In the SNc, neurturin expression was detected in 9.8% & 18.95% and 22.02% & 39% of remaining melanin-containing neurons in the patient with putamenal and combined putamenal and SNc gene delivery, respectively. Melanized neurons displayed intense TH and RET proto-oncogene expression in SNc neurons in the patient where CERE120 was directly delivered to the nigra. There was no evidence of increased inflammation or activated microglia in areas of CERE120 delivery. There was also no difference in the degree of Lewy pathology in comparison to control PD patients. Our observations demonstrate long-term transgene expression with limited axonal sprouting and TH upregulation, though the TH upregulations was greater following CERE120 gene delivery to the putamen plus nigra, compared to putamen alone. However, neither dosing approach showed clinical benefit. Therefore, if these cases are representative of other patients their respective cohorts, the dopaminergic changes observed were not likely sufficient to provide significant anti-parkinsonian benefits. This study provides the longest term evidence of persistent transgene expression following gene delivery to the CNS and the first human results when targeting the terminal fields in the putamen as well as in the cells of origin.

Disclosures: Y. Chu: None. **B.T. Raymond:** None. **W.C. Olanow:** None. **J.H. Kordower:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.06/G1

Topic: C.03. Parkinson’s Disease

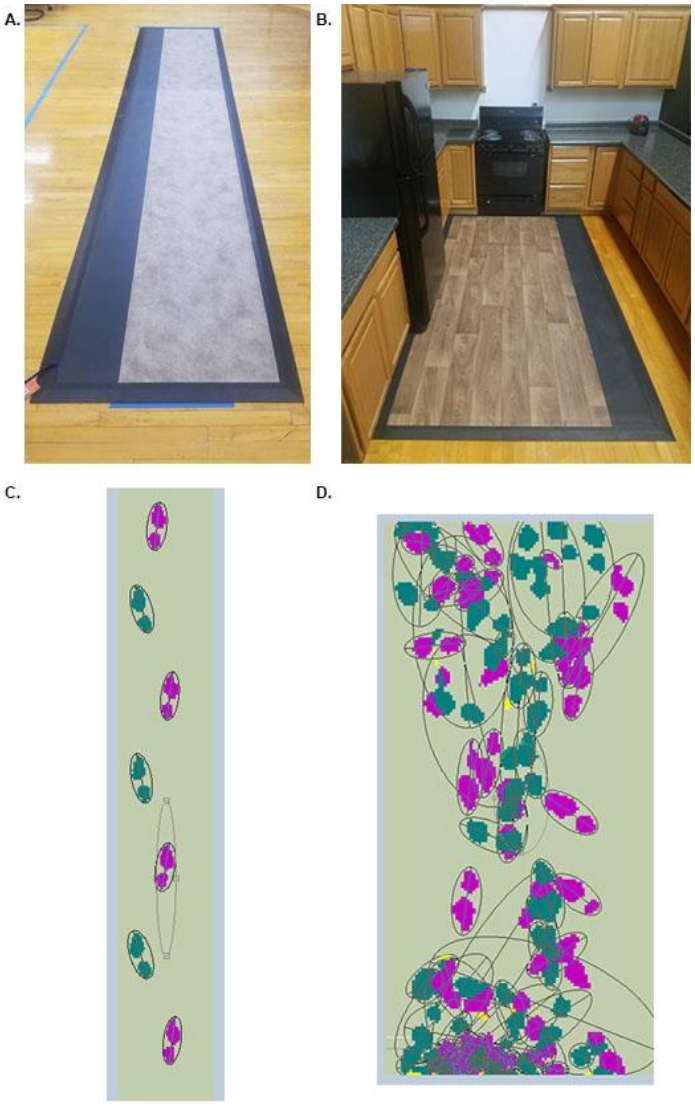
Support: UL1TR002373
KL2TR002374
Conway Fellowship

Title: Do spatiotemporal measures of functional gait predict straight-line gait in individuals with Parkinson's disease?

Authors: *K. L. DOYLE, M. TOEPFER, K. A. PICKETT;
Kinesiology, Univ. of Wisconsin -- Madison, Madison, WI

Abstract: Introduction. As Parkinson disease (PD) progresses, postural instability leads to loss of independent function and inability to perform gait-dependent activities of daily living (ADLs)

and instrumental activities of daily living (IADLs). Spatiotemporal measures of straight-line gait, collected on an instrumented walkway are commonly used to measure gait function in both clinical and research settings. We examined standardized spatiotemporal measures of straight-line gait compared to functional gait performed during an instrumented kitchen task in individuals with PD. **Methods.** Individuals who met the inclusion and exclusion criteria for a larger randomized control intervention study and completed the baseline assessment were included in this cross-sectional analysis. All individuals were previously diagnosed with "definite PD". Each participant performed straight-line normal forward and dual task walking on a Protokinetics Zeno® walkway, followed by non-linear walking on a similar Zeno© walkway designed for placement in a research kitchen (Fig1, A & B). The non-linear task involved performance of an IADL task (cooking) as outlined in a standardized assessment battery (Performance Assessment of Self-Care Skills). Means were compared between gait tasks and a linear regression model was used to analyze correlations between spatiotemporal parameters (cadence, velocity, stride length, base of support, and percent of cycle in double support). **Results.** Preliminary findings for this exploratory study are presented here. Overall, stride width and length were smaller during the functional task while time spent in double support was longer and mean velocity was slower. **Discussion.** To better understand disease progression and treatment efficacy on community-dwelling individuals with PD, it is important to implement the use of ecologically valid and novel measures that capture performance of "real world" functional tasks. Moreover, portable gait mats may enable researchers and clinicians to assess patients in-home and identify individualized treatment options.



Disclosures: **K.L. Doyle:** None. **M. Toepfer:** None. **K.A. Pickett:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.07/G2

Topic: C.03. Parkinson’s Disease

Support: ISF Grant 18-04

Title: Enhancement of hand functions in individuals with Parkinson’s disease: Impact of virtual reality training

Authors: *Z. ELIAS[1], B. BALAKRISHNAN[1], M. GANESAN[2];
[1]Univ. of Dubuque, Dubuque, IA; [2]Clarke Univ., Dubuque, IA

Abstract: Background: Individuals with Parkinson's disease (PD) have impaired hand function due to tremors, rigidity, and bradykinesia. Purpose: The purpose of this project is to test the feasibility, and efficacy of virtual reality training of hand functions in PD. Design: A pre-post quasi-experimental study design. Methods: In this pilot study, 3 individuals with idiopathic PD diagnosed by a neurologist were recruited from the community. Hand functions were assessed using the hand components of the motor score section of the Unified Parkinson's Disease rating scale (UPDRS) at baseline and after the 4 weeks of training. Intervention: The intervention includes virtual hand exercise using a RAPAEL Smart Glove™ with a visual display that focuses on hand functions including pronation, supination, radial deviation, ulnar deviation, flexion and extension of the wrist. In addition, the training also focuses on timing and coordination of movement. Both training and assessment were carried out during the "ON" period of medication. Results: All the three patients showed improvement of the hand functions reflected through a decrease in the UPDRS motor score hand components. The improvement obtained in each patient was more than the minimal clinical important difference (MCID) score of 4.63 in UPDRS motor score. The decrease in score was found in both the more affected side and the less affected side. Conclusion: The training result favors safety, feasibility and improvement of hand functions in individuals with PD.

Disclosures: Z. Elias: None. **B. Balakrishnan:** None. **M. Ganesan:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.08/G3

Topic: C.03. Parkinson's Disease

Title: Plasticity of frequency dependent tremor control in Parkinson disease (PD) subthalamic nucleus (STN) deep brain stimulation (DBS) implanted individuals

Authors: *E. L. HARGREAVES[1], D. P. SCHNEIDER[2], R. DIPAOLA[2], J. CHEN[2], S. F. DANISH[1], D. L. CAPUTO[2];
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Abstract: Plasticity of tremor control in DBS implanted individuals targeting the STN for PD is demonstrated. Specifically, over time the necessary frequency lessens. The DBS records of 85 sequential cases implanted at our institution from January 2013 to August 2016 were examined. From these records we drew 20 tremor dominant PD STN cases, 8 nontremor dominant PD STN cases, and 9 Essential Tremor (ET) VIM DBS cases. The value of the DBS frequency parameter

at the end of the initial contact screen was contrasted to the same parameter immediately following an initial device exchange. Frequency is minimized throughout programming to assist gait and reduce battery depletion, while maintaining adequate symptom control. Tremor dominant PD individuals had a mean UPDRS III “Off” tremor subscore at the contact screen of 5.61 (sem=1.05) and a total UPDRS III “Off” score of 33.58 (sem=2.11). The final frequency parameter value for the Tremor group at the end of the contact screen was an average of 182.22Hz (sem=5.56 range 150Hz-200Hz). By the programming session following battery exchanges, an average of 3.64 years later, the frequency had been reduced to an average of 131.39Hz (sem=7.59 range 75Hz-170Hz). At this time the Tremor group had a mean UPDRS III “On” tremor subscore of 0.39 (sem=0.153) and a total UPDRS III “On” score of 11.61 (sem=1.84). The NoTremor group exhibited a similar reduction in frequency from the contact screen to the device exchange some 4.35 years later (119.37Hz sem=4.99 - 76.25Hz sem=4.92). The accompanying UPDRS III “Off” tremor subscore was 0.50 (sem=0.40) and total score was 27.6 (sem=6.29), at the device exchange the “On” tremor subscore was 0.37 (sem=0.28) and the total “On” score was 17.64 (sem=7.33). In contrast the ET group exhibited no difference in frequency from the contact screen to the device exchange across some 4.16 years (177.27Hz sem=2.04 - 185.91Hz sem=7.96).As such, both the Tremor and NoTremor PD groups exhibited similarly significant reductions in the necessary frequency value over time, which did not hold true for the ET group. However, both initial and endpoint frequencies were different in the PD subgroups. The higher frequency necessary to maintain tremor may also have contributed to a shorter device duration. Similar comparison cannot be made to the ET group as different individuals deactivated the device diurnally. Identifying the necessary frequency for the Tremor and ET groups is straight forward, whereas it is not so straight forward for the NoTremor group. Thus, it is definitive to say that the frequency parameter and its control over tremor in the Tremor group exhibits plasticity, which cannot be said for the NoTremor PD and ET groups.

Disclosures: E.L. Hargreaves: None. **D.P. Schneider:** None. **R. DiPaola:** None. **J. Chen:** None. **S.F. Danish:** None. **D.L. Caputo:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.09/G4

Topic: C.03. Parkinson’s Disease

Support: NINDS U01NS080818
NINDS U01NS080840

Title: Pharmacokinetic variability of isradipine in participants with Parkinson’s disease from the phase 3 STEADY-PD clinical trial

Authors: C. S. VENUTO[1], *D. SURMEIER[4], A. WATTS[2], K. BIGLAN[5], R. HAUSER[6], S. HENDERSON[1], K. HODGEMAN[1], R. HOLLOWAY[3], E. KAYSON-RUBIN[1], D. KINEL[7], A. LANG[8], C. LUNGU[9], J. LOWELL[3], C. G. TAROLLI[3], S. SHARMA[1], D. OAKES[2], I. SHOULSON[3], **T. SIMUNI**[10];
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Abstract: Background: Isradipine is a dihydropyridine calcium channel inhibitor that has demonstrated concentration-dependent protective effects in animal models of Parkinson's disease (PD). Moreover, systemically administered isradipine engages calcium channels in brain dopaminergic neurons in mouse models, lowering mitochondrial oxidant stress and mitophagy. In addition, epidemiological studies showed association of dihydropyridine use with reduced risk of PD. Hence, a phase 3, randomized, double-blinded, placebo-controlled trial tested whether 10 mg of isradipine administered twice daily - the highest dose tolerated by PD patients in a phase 2 study - slowed the progression of PD. The study collected plasma pharmacokinetic samples to conduct posthoc analysis of correlation of PK with the clinical outcomes and as indirect proxy of subject compliance. **Methods:** Sparse samples for quantification of plasma isradipine concentrations were collected 3 and 6 months post-baseline visit. The samples were collected during the following time windows relative to last dose: ~2 - 3 hours (i.e., maximum concentration [C_{max}]), ~4 - 8 hours, and ~12 hours (i.e., trough [C_{min}]) post-dose. Quantitation of isradipine was performed using a validated liquid chromatography with tandem mass spectrometry assay. The assay measurement range for isradipine was from 0.100 to 100 ng/mL. **Results:** The study recruited 336 participants of whom 170 received isradipine. Of these participants, 166 had PK samples for analysis. Six samples from four different participants were below the assay limit of quantitation suggesting adherence issues, rapid metabolism of isradipine, or other pharmacokinetic factors. In the remaining participants, the median (minimum, maximum) C_{max} and C_{min} isradipine concentrations were 3.1 (0.4, 17.4) ng/mL and 0.6 (0.1, 4.1) ng/mL, respectively. **Conclusions:** There was substantial variability in the plasma concentrations of isradipine in our clinical trial participants. Additionally, the observed C_{max} value was lower than expected compared to previously reported values for isradipine in non-PD populations. Population pharmacokinetic modeling of these data is underway to identify individual characteristics associated with the inter-individual pharmacokinetic variability of isradipine. Correlations between isradipine exposure, PD sub-type, age and PD progression also will be determined.

Disclosures: C.S. Venuto: None. **D. Surmeier:** None. **A. Watts:** None. **K. Biglan:** A. Employment/Salary (full or part-time):; Full, Eli Lilly and Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly and Company. **R. Hauser:** None. **S. Henderson:** None. **K. Hodgeman:** None. **R. Holloway:** None. **E. Kayson-Rubin:** None. **D. Kinel:** None. **A. Lang:**

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Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.10/G5

Topic: C.03. Parkinson's Disease

Support: NAD RESEARCH, INC.

Title: Intravenous administration of nicotinamide adenine dinucleotide alleviates tremors associated with Parkinson's disease: A case report

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Abstract: Introduction: A number of pharmacological agents currently exist for treating symptoms associated with Parkinson's disease (PD), however these drugs are not optimal because they typically target dopamine reuptake in depleted dopaminergic systems. Nicotinamide Adenine Dinucleotide (NAD+) is present in all cells and serves a vital role in cell function; in fact, disruption or depletion of NAD+ is associated with a number of disease states, including PD. Clinicians in Springfield, LA developed an intravenous administration protocol of NAD+ for a patient experiencing symptoms associated with PD. Subsequent observation of a rapid and sustained treatment effect on alleviation of PD symptoms ***provide rationale for further describing and quantifying the positive effects of IV NAD+ on tremors associated with PD.*** **Method:** Client, male age 59 was diagnosed with PD four years prior and reported taking traditional PD medications with no alleviation of symptoms. Client received IV NAD+ (two days of 1,500mg) followed by (four days of 750mg) for a total of six days. Tremors were recorded in the client's dominant right hand using an accelerometer and gyroscope built into the client's smart phone. Researchers recorded measurements (Hz) for baseline (day 0; 44.5Hz average at 34hr), day 4 and day 6 (13 measurements total) on three axes; vertical, lateral and anterior-posterior. Following day 6, client received sublingual NAD+ tablets (300mg 2Xday) and recorded mean tremors for 14 days. **Findings:** 1) Overall, the vertical axis tremors decreased by 75.9%, the lateral axis by 83.0% and the anterior-posterior axis by 9.1%. 2) Mean tremor on day six was 20.6Hz, resulting in a decline from baseline by 54.7%. 3) Client self-report of tremors continued to decline two weeks post IV NAD+ (average 12Hz) by using maintenance supplements and application of a relaxation technique. **Conclusion:** These data show the longevity and endurance of the initial IV NAD+ followed by sublingual tablets (300mg 2x/day)

in maintaining decline and alleviation of PD symptoms. Additionally, these results replicate previous findings and establish a protocol for empirically measuring the effects of IV NAD+ on PD symptoms (specifically tremors), and longevity of treatment results as compared to traditional pharmacotherapies.

Disclosures: L.E. Rutherford: None. **S.L. Broom:** F. Consulting Fees (e.g., advisory boards); NAD Research, Inc. **T. Olds:** A. Employment/Salary (full or part-time):; Springfield Wellness Center. **R.F. Mestayer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Royalty received from Archway Apothecary for NAD products. **P.N. Mestayer:** A. Employment/Salary (full or part-time):; Director/Owner Springfield Wellness Center. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Royalty received from Archway Apothecary for NAD products.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.11/G6

Topic: C.03. Parkinson’s Disease

Support: R01 NR014852

Title: Analyzing the differences in neural activation between conventional and interleaving deep brain stimulation of the subthalamic nucleus for dyskinesia suppression

Authors: *G. DUFFLEY[1], C. AQUINO[2], C. R. BUTSON[3];
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Abstract: Introduction: Dyskinesias are a common side effect of dopaminergic therapy, a common treatment for the symptoms of Parkinson’s disease (PD). Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has been shown to reduce PD symptoms and thereby reduce the need for dopaminergic medications, but it is not thought to have a direct effect on dyskinesias. Interleaving stimulation (ILS), which is a stimulation modality where two distinct stimulation waveforms are delivered to the patient out of phase with each other, has shown potential to improve dyskinesias. The objective of this study was to evaluate how neural activation differs between conventional and ILS in order to improve dyskinesias following STN DBS.
Methods: We identified 23 PD patients who had received ILS of the STN. Of those patients, 11 received bilateral ILS and 12 received unilateral ILS, for a total of 34 DBS leads to analyze. We computed the volume of tissue activated (VTA) for each patient’s best conventional setting and

ILS setting. For each ILS setting, we computed two separate volumes one, where activation occurred at the frequency of the either of the independent waveforms and one where activation occurred at the total frequency of the waveforms combined. We generated heatmaps of activation resulting from each type of VTA across patients.
Results: The heatmaps revealed that both ILS and conventional DBS most commonly activated the lateral portion of STN, but ILS additionally activated the region of zona incerta (ZI), located dorsal-lateral to the STN. Out of the 34 DBS leads, 28 interleaving stimulation settings generated a volume of high frequency activation. The most common location of the high frequency activation was on the border of dorsal STN and ventral ZI.
Conclusions: ILS causes two primary changes as compared to conventional DBS; there is a small region of high frequency activation on the border of STN and ZI, and the center of ZI is activated. Future work will analyze if stimulation of ZI activates pallidothalamic fibers in H field of Forel, which is a hypothesized mechanism of ILS dyskinesia suppression.

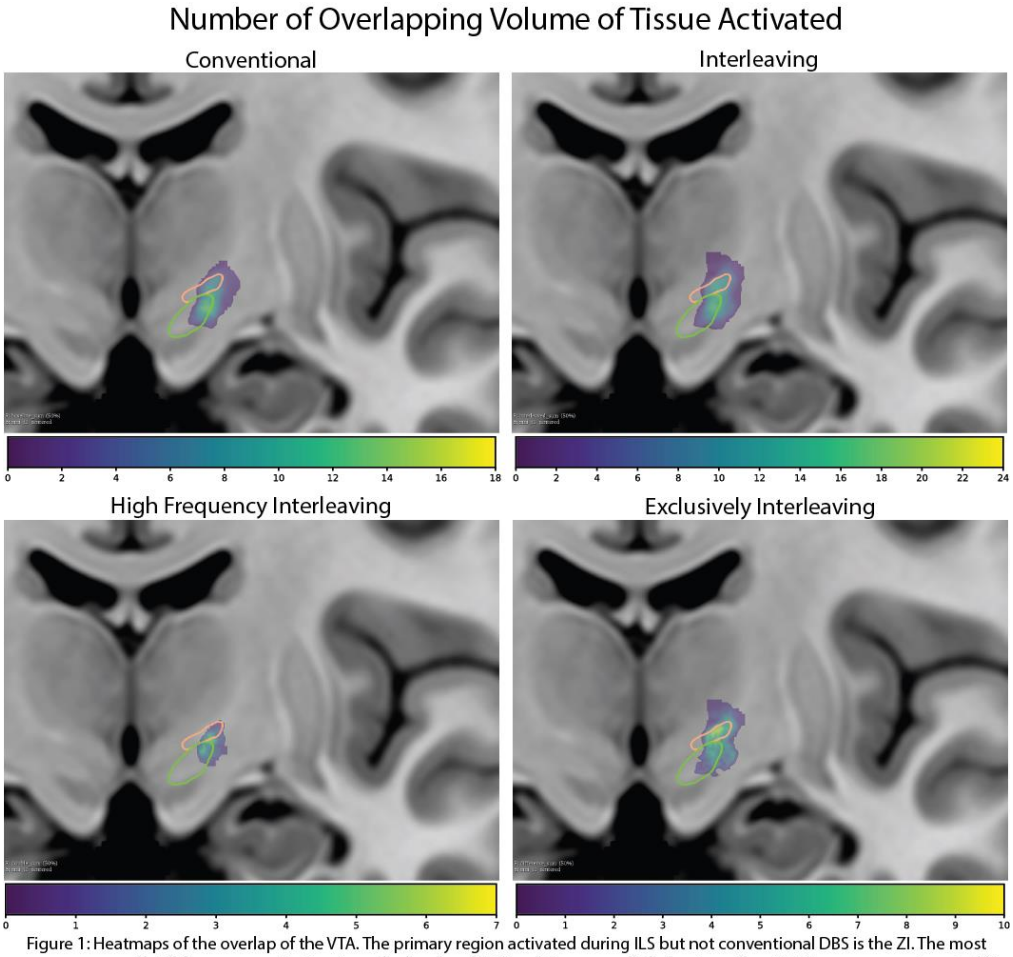

Figure 1: Heatmaps of the overlap of the VTA. The primary region activated during ILS but not conventional DBS is the ZI. The most commona area of high frequency activation is on the border of STN and ZI. green: subthalamic nucleus (STN), orange: zona incerta (ZI).

Disclosures: **G. Duffley:** None. **C. Aquino:** None. **C.R. Butson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IntelectMedical. F. Consulting Fees (e.g., advisory boards); NeuroPace, Advanced Bionics, Boston Scientific, IntelectMedical, Abbott, Functional Neuromodulation.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.12/G7

Topic: C.03. Parkinson's Disease

Support: NIH BRAIN Initiative Grant 1 UH3 NS100553-01
The Michael J. Fox Foundation for Parkinson's Research (MJFF) Grant

Title: Biomarkers to guide directional DBS for Parkinson's disease: 2 year interim analyses from a randomized, double-blind crossover trial

Authors: *H. C. WALKER[1], C. GONZALEZ[1], Z. IRWIN[1], C. HURT[2], D. KUHMAN[2], A. NAKHMANI[3], R. MARTIN[4], S. BLACK[5], M. WADE[1], M. AWAD[3], R. VADEN[1], N. BENTLEY[5], B. GUTHRIE[5];
[1]Neurol., [2]Physical Therapy, [3]Electrical Engin., [4]Neuropsychology, [5]Neurosurg., UAB, Birmingham, AL

Abstract: Introduction
Deep brain stimulation (DBS) is effective for motor symptoms of Parkinson's disease (PD), but improvement varies significantly across trials, within individuals, and over time. Directional DBS electrodes allow greater precision and flexibility, but at the cost of markedly increased complexity during postoperative programming. Our overall goals are to contrast the clinical effects of directional and circular DBS and to identify electrophysiology biomarkers that rapidly and objectively identify effective, well-tolerated stimulation contacts.
Methods
We are conducting a randomized, double-blind crossover study of directional versus circular unilateral subthalamic DBS for PD. Our primary clinical outcomes include within-participant contrasts in the UPDRS part 3 motor score "off" meds and novel patient preference measures incorporating the NIH PROMIS battery at 2 and 4 months post-op versus pre-op baseline. During surgery, we record stimulus-evoked potentials with scalp EEG and an ECOG strip over ipsilateral primary sensorimotor cortex as well as subcortical local field potentials from the directional DBS lead both at rest and during simple arm, mouth, and leg movements. We are building predictive models to examine whether stimulus evoked activity or subcortical local field potentials (LFPs) better guide rapid, effective activation of directional DBS leads. We have implanted 12 of 30 participants (10 male, 2 female) as of year 2 of 5. Primary clinical outcomes are blinded until study conclusion.
Results
Both directional and circular DBS significantly improved motor outcomes at 2 and 4 months versus baseline (54.0±16.9 to 30.4±12.6 and 33.1±14.3, 43.7% and 38.6% improvement,

respectively, p<0.001, n=7, linear mixed effects model). Within individuals, directional DBS technology differentially modulates motor improvement, side effect thresholds, stimulus-evoked activity, and subcortical LFPs. One patient experienced an isolated seizure the day after surgery without neuroimaging abnormalities, and there have been no other SAEs.

Discussion

Directional DBS therapy is safe and effective for motor symptoms. Our interim analyses show significant within-participant contrasts in motor outcomes and both stimulus evoked activity and LFPs, in the context of directional DBS therapy. These contrasts show considerable promise as potential biomarkers to guide new approaches to DBS therapy with next-generation directional and closed-loop DBS systems.

Disclosures: **H.C. Walker:** A. Employment/Salary (full or part-time):; UAB. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institutes of Health, MJFF. **C. Gonzalez:** None. **Z. Irwin:** None. **C. Hurt:** None. **D. Kuhman:** None. **A. Nakhmani:** None. **R. Martin:** None. **S. Black:** None. **M. Wade:** None. **M. Awad:** None. **R. Vaden:** None. **N. Bentley:** None. **B. Guthrie:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.13/G8

Topic: C.03. Parkinson’s Disease

Support: The Michael J Fox Foundation for Parkinson's Research
The Cures Parkinson's Trust

Title: Analyses of pharmacokinetics, CSF exposures and CSF dopamine and its metabolite levels in NILO-PD, a phase 2A study of nilotinib in moderate to advanced Parkinson's disease

Authors: *K. M. MERCHANT[1], T. SIMUNI[2], B. FISKE[3], C. COFFEY[4], H. MATTHEWS[5], R. WYSE[5], P. BRUNDIN[6], D. K. SIMON[7], M. SCHWARZSCHILD[8], D. WEINER[9], J. ADAMS[10], L. TRUSSI[10], L. BAKER[10], M. KOSTRZEBSKI[11], T. WARD[2], G. GARY RAFALOFF[12], C. VENUTO[10];
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Abstract: Nilotinib is a drug approved for treatment of chronic myeloid leukemia, but not for PD. Several studies in cells and animal models show that nilotinib can reduce alpha-synuclein pathology and thereby has the potential to slow PD progression; this hypothesis gained supported from a preliminary, open-label study in moderate to advanced PD patients (Pagan et al., 2016) raised the additional possibility of a symptomatic effect. A recent study conducted by Pagan et al. (2019) showed that cerebrospinal fluid (CSF) concentrations of nilotinib are approximately 0.5 to 1% of those in the plasma and are associated with alterations in dopamine metabolite levels. However, the pharmacokinetic (PK) profile CSF exposures and biomarker changes after a steady-state level of nilotinib, have not been systematically studied. We initiated a Phase 2a randomized, double-blind, placebo-controlled, parallel group study termed NILO-PD. The primary outcome of NILO-PD is safety and tolerability. Secondary/exploratory outcomes include nilotinib serum PK, CSF exposures together with a battery of serum and CSF biomarkers, whichs include levels of CSF dopamine and its metabolites in CSF. The study has enrolled 76 participants with moderate to advanced PD randomized 1:1:1 to a once daily dose of placebo:nilotinib (150 mg):nilotinib (300 mg) for 6 months. Recruitment started in November 2017 and was completed in December 2018. Baseline characteristics of study participants are summarized in the table below.

Characteristics	**Mean (SD)**
Age (years)	64.6 (7.5)
Sex (% female)	31.6
Age at diagnosis (years)	54.7 (8.0)
Disease duration (years)	9.9 (4.7)
MDS-UPDRS Off score	66.4 (19.5)
MDS-UPDRS On score	48.3 (16.1)
MoCA score	27.1 (2.2)

The study is scheduled to complete data collection in September 2019. Here we report the protocol-specified interim analyses at baseline and 3 months for serum PK, CSF exposures and CSF dopamine metabolite levels as well as correlations between exposures and dopamine metabolite changes. The primary outcome results are expected by December 2019.

Disclosures: **K.M. Merchant:** A. Employment/Salary (full or part-time):; Self-employed at TransThera Consulting Co.. 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.; Advisor and grant recepient to the Michael J Fox Foundation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CSO at Vincere Biosciences, ELi Lilly stock holder as a retiree. F. Consulting Fees (e.g., advisory boards); Lysosomal Therapeutics, Sinopia Biosciences, Origami Therapeutics, angel investors. Other; Member of NCATS and CAN Review Board, Member of Wellcome Trust Interview Panel. **T. Simuni:** 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.; NINDS, Michael J Fox Foundation, Parkinson Foundation, Biogen, Roche, Neuroderm, Sanofi, Sun Pharma, Northwestern Foudnation. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); GE Medical, TEVA, Lundbeck. F. Consulting Fees (e.g., advisory boards); Acadia, Abbvie, Allergan, Anavex, Accorda, Denali, GE Medical, Neuroderm, Neurocrine, Sanofi, Sunovion, TEVA, Takeda, Voyager, US World Meds, Parkinson Foundation, Michael J Fox Foundation. **B. Fiske:** None. **C. Coffey:** None. **H. Matthews:** None. **R. Wyse:** None. **P. Brundin:** 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.; Renovo, Roche, Teva/Lundbeck. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acousort AB. F. Consulting Fees (e.g., advisory boards); Renovo Neural, Inc., Roche, Teva Inc, Lundbeck A/S, AbbVie, Neuroderm, Fujifilm-Cellular Dynamics, IOS Press Partners, LifeSci Capital, LLC, Axial Biotherapeutics, CuraSen. **D.K. Simon:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; BioElectron Technology, Inc; , Lysosomal Therapeutics, Inc. , Mission Therapeutics, Voyager Therapeutics, Weston Brain Institute, National Parkinson Foundation, NIH (NINDS). F. Consulting Fees (e.g., advisory boards); Biogen, Halloran Consulting Group/ Cerevance Therapeutics, Michal J. Fox Foundation. **M. Schwarzschild:** None. **D. Weiner:** None. **J. Adams:** None. **L. Trussi:** None. **L. Baker:** None. **M. Kostrzebski:** None. **T. Ward:** None. **G. Gary Rafaloff:** None. **C. Venuto:** F. Consulting Fees (e.g., advisory boards); Michael J Fox Foundation.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.14/G9

Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS092950

Title: Anti-Parkinsonian medication improves impaired eye-hand coordination in participants with Parkinson's disease

Authors: *M. J. MUNOZ[1], R. ARORA[2], E. GEORGE[1], G. PAL[3], L. A. VERHAGEN METMAN[3], L. C. GOELZ[1,4], D. M. CORCOS[1], F. J. DAVID[1];
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Abstract: Eye-hand coordination has been infrequently studied in Parkinson's disease (PD) despite being an important skill of everyday life. In addition, the effect of medication on eye-hand coordination remains unclear. Studying the effect of medication on eye-hand coordination can provide insight into the role of the basal ganglia in the control of eye-hand coordination. Thus, we addressed the following two questions: 1) what is the effect of anti-Parkinsonian medication on eye-hand coordination while performing a visually guided reaching task, and 2) does anti-Parkinsonian medication improve performance to the levels of age and sex matched healthy control participants? Twenty-one participants with PD (19 male) and 8 healthy controls (7 male) completed a visually guided reaching task. Participants were asked to look and reach as accurately as possible to a target LED that appeared in their periphery immediately after fixating on a central fixation LED. Visual stimuli were presented in total darkness. The PD participants performed this task over two days; one day was on medication while the other day was off medication. The order of on and off medication was randomized. Eye-hand coordination was operationally defined as the latency between the onset of eye movement and the onset of hand movement, i.e., eye-hand latency. The outcome measures were eye-hand latency, pointing latency (the time taken to begin movement), pointing peak velocity normalized to amplitude, and pointing error magnitude normalized to peak velocity. All statistical analyses were performed using SAS Proc Mixed. The fixed effect was medication status (on vs off). Subjects were considered random effects. Covariates were off medication Movement Disorders Society-Unified Parkinson's disease Rating Scale Part III score, Montreal Cognitive Assessment total score, age, and sex. In addition, independent t-tests were performed to compare outcomes between PD on medication and healthy control participants. Relative to off medication, anti-Parkinsonian medication 1) reduced eye-hand latency, 2) had no effect on pointing latency, 3) increased pointing velocity, and 4) reduced pointing error. Medication improved eye-hand latency to healthy control participant levels, but deficits in pointing latency, peak velocity, and error magnitude remained. Our findings showed that medication improved eye-hand coordination and suggest a basal ganglia role in the circuitry controlling visually guided limb movements.

Disclosures: M.J. Munoz: None. **R. Arora:** None. **E. George:** None. **G. Pal:** None. **L.A. Verhagen Metman:** None. **L.C. Goelz:** None. **D.M. Corcos:** None. **F.J. David:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.15/G10

Topic: C.03. Parkinson's Disease

Title: Effects of cycling cadence variability on motor function and cortical inhibition in Parkinson's disease

Authors: *A. L. RIDGEL[1,2], A. J. GARBIN[3], Y.-C. CHUNG[3], J. AXELROD[3], S. YASSA[3], B. E. FISHER[3];
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Abstract: Prior research has demonstrated that forced cycling improves motor symptoms commonly experienced by individuals with PD. Further, the degree of improvement is predicted by the variability in one's cadence. However, the mechanisms behind these changes are poorly understood. The purpose of this study was to assess the effect of cadence variability (degree of complexity) on motor function and cortical inhibitory mechanisms during forced and voluntary cycling in adults with Parkinson's disease (PD).We hypothesized that an increase in cortical inhibition, a cortical mechanism that is reduced in individuals with PD, may be responsible for the motor improvements secondary to forced cycling. Individuals with mild to moderate PD completed three 30-minute cycling sessions consisting of high cadence variability forced cycling (HCV), low cadence variability forced cycling (LCV), and voluntary cycling (VC) while off medication. During the two forced cycling conditions, cadence was set to 80 rpm, while participants were encouraged to maintain this same cadence during voluntary cycling. Motor function was assessed using the Unified Parkinson's Disease Rating Scale III and cortical inhibitory mechanisms were assessed using a transcranial magnetic stimulation paradigm of short interval cortical inhibition (SICI) before and after each cycling session. Variability in cadence and power during each session was calculated using entropy analysis. Mean cadence was greater during HCV and LCV relative to VC (80.3 & 81.4 vs. 75.5 rpm). Cadence variability was higher during HCV (1.4) compared to LCV (1.1), while power and power variability were higher during LCV (4.6 & 0.32) compared to HCV (2.9 & 0.14). We observed the greatest increase in cortical inhibition as indicated by reduced SICI following HCV (Pre 0.724, Post 0.527) while the greatest improvement in UPDRS was seen following LCV (5.5). While our inhibitory and behavioral changes following high and low cadence variability forced cycling at first seem counter-intuitive, we postulate they may be secondary to different mechanisms. Specifically, increased modulation in kinematic output and somatosensory input during the high cadence variability condition may be responsible for increased inhibition. The increased power and power variability in the low cadence variability condition is indicative of greater kinetic variability and motor output, potentially resulting in the observed greater impact on motor behavior. Further, these effects are less apparent in the voluntary cycling condition due to their significantly lower cadence.

Disclosures: **A.L. Ridgel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Inventor. **A.J. Garbin:** None. **Y. Chung:** None. **J. Axelrod:** None. **S. Yassa:** None. **B.E. Fisher:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.16/G11

Topic: C.03. Parkinson's Disease

Title: A smartphone-based motor function battery to assess Parkinson's disease patients in the community setting

Authors: E. E. ECK, ***B. L. TRACY**;
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Abstract: Parkinson's disease produces slow movement, motor variability, postural instability, and impaired gait. It is often difficult for patients in studies to access laboratories with sophisticated measurement tools. It can be of interest to capture symptom severity and the extent of functional disability in remote clinical settings or in studies at exercise facilities. Smartphones contain quality movement sensors, are portable, user friendly, lightweight, and feasible when remote community-based testing is desired. Here we describe a smartphone-based motor function battery to measure PD symptoms before and after a rehabilitative exercise training session (boxing). Eight smartphone-instrumented (iPod Touch) functional tasks were devised to assess key features of PD-related function. Raw movement data was acquired on the device in the boxing gym and transferred wirelessly to the lab for subsequent analysis. Postural Stability: device attached to the lateral hip, the SD of acceleration for the X and Z axes (A/P and M/L) was measured with eyes closed and open. Walking (10m gait test): Devices strapped above the ankle. Heel strike timestamps were detected from acceleration spikes from both feet separately; gait speed, step/stride length, and variability of step/stride times were calculated. 5x Sit-To-Stand: Device attached to thigh. The speed of the rising phase of each repetition, body mass, and rise distance was be used to calculate chair rise power. Upper and lower body movement speed: Device attached to the distal forearm or distal shank. The peak acceleration of the initial phase of an abbreviated horizontal arm punch (jab) or isolated knee extension kick was measured. Functional Reach: Device attached to lateral trunk. Flexing at the hip, the standing subject reached out as far as possible with horizontal arms and without losing balance. The greatest trunk tilt value and the stability at the maximum position indicates the ability to maintain balance under challenge. Rapid tapping (20s): Seated at a table, the subject tapped the device as rapidly as possible for 20s. The mean rate and variability of tapping rate was calculated using the timestamps of acceleration spikes. Tremor: Devices attached to the distal forearm of both arms separately. Arms were held steady for 30s. The SD of acceleration in three axes was taken as a measure of tremor amplitude. Smart devices may allow efficient, low cost field assessment and increase access to testing for community-based intervention studies when lab visits are not possible.

Disclosures: E.E. Eck: None. **B.L. Tracy:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.17/G12

Topic: C.03. Parkinson's Disease

Support: VA Merit Grant

Title: Niacin supplement for Parkinson's disease

Authors: *B. GIRI[1], M. SEAMON[2], B. BABAN[2], R. CHONG[2], J. C. MORGAN[3], S. PUROHIT[2], C. WAKADE[4];
[1]Physical Therapy, Augusta Univ., AUGUSTA, GA; [2]Augusta Univ., Augusta, GA; [3]Dept Neurobiol, Med. Coll GA, Augusta, GA; [4]Res. and Develop., CNVAMC, Augusta, GA

Abstract: We propose over-the-counter niacin supplementation to improve inflammation in Parkinson's disease (PD). α-synuclein is known to form aggregates in brains of PD patients, contributing to neuroinflammation. We have reported up-regulation of niacin receptor (GPR109A) in PD patients and niacin intervention has been beneficial in reducing inflammation. In this exploratory study, we examined GPR109A expression, cytokine (pro and anti-inflammatory) profile, and α-synuclein load in the plasma samples of early- and late-stage patients (based on H & Y) at baseline and after 6 months of niacin supplementation. In this double-blind within-subjects study, informed consent was obtained on PD subjects under protocols approved by the Institutional Review Board. Behavioral, cognitive assessments, and blood biochemistry testing were carried out at baseline and after 6 months of niacin supplement. Exosomes were separated through size exclusion chromatography from plasma samples. Exosomes were identified by electron microscopy as well as by exosome markers. Western blot densitometry analyses were performed on high exosome fractions to determine α-synuclein oligomers to exosome marker ratio. Levels of niacin metabolites, GPR109A, and cytokines were determined in the blood. Our study showed that α-synuclein oligomers to exosome marker ratio in PD patients was higher at baseline compared to that after 6 months of niacin supplement. Baseline GPR109A expressions were significantly lower in late stage patients compared to early stage at baseline and after niacin treatment. Niacin supplements reduced GPR109A, increased niacin metabolite levels and decreased α-synuclein oligomers level in exosomes. Niacin may be beneficial in PD by its anti-inflammatory effects through GPR109A mechanisms, as well as modulating α-synuclein load.

Disclosures: B. Giri: None. **M. Seamon:** None. **B. Baban:** None. **R. Chong:** None. **J.C. Morgan:** None. **S. Purohit:** None. **C. Wakade:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.18/G13

Topic: C.03. Parkinson's Disease

Title: Electrophysiological responses to deep brain stimulation in Parkinson's disease

Authors: A. C. GUEST[1], D. GRAHAM[2], K. J. O'NEILL, III[2], Z. MIRZADEH[3], F. PONCE[3], *B. GREGER[2];
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Abstract: Deep brain stimulation (DBS) is a technique used to alleviate motor symptoms of advanced Parkinson's disease (PD). DBS electrodes are surgically placed into deep brain structures, e.g. subthalamic nucleus (STN), and when current is passed to the electrodes, the motor symptoms of PD are relieved. Although DBS is a well-established treatment for PD, its mechanism is poorly understood. In this study, patients with PD underwent neurosurgery for DBS electrode placement. Intraoperatively, we recorded action potentials (APs) from isolated neurons and local field potentials (LFPs) from a microelectrode placed in STN. We simultaneously recorded LFPs from an epicortical micro-electrocorticography (micro-ECoG) grid placed over premotor cortex. The micro-ECoG grid consisted of 16 microelectrodes with an inter-electrode spacing of 1 mm. DBS was performed while recording from these two devices. The microelectrodes were placed either ipsilateral or contralateral to the DBS electrodes. LFPs recorded on the micro-ECoG grid placed over premotor cortex that were aligned on APs from neurons in the STN and averaged showed increased amplitude. This increase in LFP amplitude was only present on a subset of micro-ECoG electrodes that varied for each STN neuron. These results demonstrate functional connectivity between single neurons in the STN and patterns of small regions of premotor cortex. We also examined the electrophysiological responses to DBS on the microelectrode placed in STN using subtherapeutic, therapeutic, and supratherapeutic stimulation frequencies (30, 70, 140, and 250 Hz). AP aligned and averaged LFPs in the ipsilateral STN showed an increase in amplitude in the theta band during 140 Hz DBS, a therapeutic frequency. There was a moderate increase in amplitude in the theta band with 250 Hz DBS, but there were little or no changes in LFP amplitude at frequencies of 30 Hz, 70 Hz, or for recordings on the contralateral side. Our results provide insight into the effects of DBS on neural tissue and the functional connectivity between the STN and premotor cortex. Future studies will be performed with patients undergoing awake neurosurgical procedures allowing us to simultaneously record the electrophysiology measures described above and the parameters of the patient's tremor during DBS. By linking the dynamics in electrophysiological recordings from micro- and meso-scale neural circuits with the reduction of tremor during DBS we aim to

advance the understanding of the mechanism of DBS, and provide insight into the pathophysiology of PD.

Disclosures: **A.C. Guest:** None. **D. Graham:** None. **K.J. O'Neill:** None. **Z. Mirzadeh:** None. **F. Ponce:** None. **B. Greger:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.19/G14

Topic: C.03. Parkinson's Disease

Support: Arizona State University - Mayo Clinic Seed Grant

Title: Real-time feedback during treadmill training for individuals with Parkinson's disease

Authors: *N. KRISHNAMURTHI[1], D. BASKARAN[2], S. PARIKH[2], V. VENUGOPAL[2], N. MUTHUKRISHNAN[2], E. DRIVER-DUNCKLEY[4], P. MAHANT[5], M. C. OSPINA[6], **J. J. ABBAS**[3];
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Abstract: Common motor impairments in Parkinson's disease (PD) include reduced step length, shuffling gait, and postural instability; these can be aggravated by the presence of stooped posture. The severity of these impairments worsen over time and affect quality of life. Neurorehabilitation therapies may help to sustain, or possibly improve, motor function and the ability to perform daily activities. The application of visual and auditory cues is one such neurorehabilitative therapy which has been shown to provide some benefits in gait in PD. However, due to impaired proprioception, people with PD are not aware if they are following the cues successfully. This limitation may be overcome by providing real-time feedback of performance, which can encourage modulation of movement patterns to improve gait and posture. In earlier work, we demonstrated that, in a single session, people with PD could successfully follow treadmill-based real-time feedback (RTF) of step length and back angle to improve gait and posture during and immediately after walking with feedback. To investigate whether the observed immediate benefits can be sustained and transferred to overground walking without feedback, we are conducting a long-term (3 sessions/week for 6 consecutive weeks) real-time feedback training (RTFT). Thus far, five participants (age 69 +/- 5 years) with mild to moderate PD (Hoehn and Yahr stage III or below) received RTFT that involves providing real-time feedback of step length or back angle by displaying it (pictorially) on a monitor placed in

front of the participant, at eye-level. The target step length was set between 110-120% of the step length obtained during a baseline non-feedback walking trial, and the target back angle was set at the maximum upright posture exhibited during a quiet standing task. Evaluation sessions conducted post-training (after 6-week training) and at follow-up (after 12 weeks) indicate increases in step length, gait speed, and cadence, and decreases in step time and double support in four of five participants, and these benefits are sustained at follow-up. These results provide preliminary evidence that RTFT can be an effective rehabilitative modality in improving gait and posture in people with PD.

Disclosures: **N. Krishnamurthi:** None. **D. Baskaran:** None. **S. Parikh:** None. **V. Venugopal:** None. **N. Muthukrishnan:** None. **E. Driver-Dunckley:** None. **P. Mahant:** None. **M.C. Ospina:** None. **J.J. Abbas:** None.

Poster

383. Parkinson's Disease: Clinical Trials

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 383.20/G15

Topic: C.03. Parkinson’s Disease

Support: National Institute of Nursing Research at National Institutes of Health Grant 1R21NR017484-01A1

Title: Gait and balance monitoring using wearable technology for real-time feedback in Parkinson’s disease

Authors: ***N. MUTHUKRISHNAN**[1], J. ABBAS[1], P. TURAGA[2], T. INGALLS[2], N. KRISHNAMURTHI[3];
[1]Sch. of Biol. and Hlth. Systems Engin., [2]Sch. of Arts Media and Engin., [3]The Edson Col. of Nursing and Hlth. Innovation, Arizona State Univ., Tempe, AZ

Abstract: Several recent studies have employed real-time feedback (RTF) of gait parameters to improve walking patterns in Parkinson’s disease (PD). In earlier work, we investigated the effects of RTF of step length and back angle during treadmill walking and observed that people with PD could follow the feedback and utilize it to modulate movements favorably in a manner that transferred, at least acutely, to overground walking. Our study, and most others with RTF in the literature, utilized training while walking on a treadmill. However, people in the moderate to advanced stages of PD experience balance impairments and therefore may find it difficult to train on a treadmill and may not be willing to go through a specialized program involving RTF. Recent advances in wearable sensors can be leveraged to develop a wearable real-time feedback (WRTF) system that can evaluate movements and provide feedback during daily activities that involve overground walking. The work presented here addresses the challenge of obtaining

accurate gait and balance measures from wearable sensors in real-time. We have developed algorithms to detect gait events and calculate parameters such as step-length and trunk uprightness using data from wearable sensors (inertial measurement units) that were placed on the feet, sternum and lumbar region of the back. The algorithms perform quaternion-based correction of the sensor‘s orientation using a computationally simple complementary filter for position and posture calculations. Data collected during 30-meter walking trials from healthy individuals and people with PD were used to calculate gait and posture measures. Preliminary results demonstrate accurate and reliable gait detection and subsequent calculation of step/stride length, swing time, and back angle. The algorithms do not incur substantial computational demands and therefore are suitable for real-time applications. In an ongoing study, we are testing the validity of the measures from the wearable system against gold-standard measurements. In subsequent work, the WRTF system will be evaluated as a potential rehabilitation tool for use in a home/community setting by investigating the impacts of using the system on walking patterns and posture in people with PD.

Disclosures: N. Muthukrishnan: None. **J. Abbas:** None. **P. Turaga:** None. **T. Ingalls:** None. **N. Krishnamurthi:** None.

Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 384.01/G16

Topic: C.03. Parkinson’s Disease

Title: Relations among mood, cognition, severity of symptoms and autonomic functions in Parkinson's disease patients

Authors: *D. P. LOPES[1], F. B. RODRIGUES[1], E. P. PEDROSA[1], C. E. PINTO[1], C. S. G. CAMPBELL[2];
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Abstract: Introduction: Reduced production of dopamine in Parkinson’s Disease (PD) substantially affects motor skills and other primary body functions, including mood and cognition. At the same time, losses occur in the autonomic nervous system, which can be estimated by heart rate variability (HRV). **Objectives:** To assess HRV and its relationship with symptoms of anxiety, depression, cognition and severity of the disease. **Methods**: Twenty-six patients were evaluated according to scales of anxiety and depression (HADS-A and HADS-D), Mini-Mental Parkinson (MMP) cognition scale, and disease severity scales (UPDRS - motor examination and Hoehn & Yahr scale). HRV was measured with the patients standing and sitting. **Results**: We observed high levels of anxiety (30.7%), depression (34.6%) and impaired

cognition (64%). Parasympathetic activity, inferred by the RMSSD values, was associated with the intensity of depressive symptoms ($r = -0.382$; $p \leq 0.05$), while the LF/TP values, which reflect both sympathetic and parasympathetic activity, were associated with the score obtained in the MMP ($r = 0.503$; $p \leq 0.05$). Severity and overall time of the disease were also associated with autonomic function. **Conclusions**: It is suggested that HRV works as an integrative measure and peripheral marker of the body's adaptability and self-regulatory capability, as well as a practical and sensible resource regarding the disease's progression.

Disclosures: D.P. Lopes: None. **F.B. Rodrigues:** None. **E.P. Pedrosa:** None. **C.E. Pinto:** None. **C.S.G. Campbell:** None.

Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 384.02/G17

Topic: C.03. Parkinson's Disease

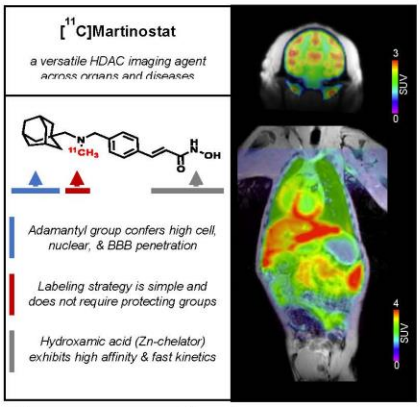
Support: NIH Grant R21NS109833
NIH Grant R33AA025192

Title: The investigation of epigenetic mechanisms *in vivo* in Parkinson's disease patient brains quantified by non-invasive PET imaging using a PET/MR

Authors: *S. FIEDLER[1], L. WERNECK[1], B. NTAGANDA[1], S. N. GOMPERTS[2], C. WANG[1]; [1]Massachusetts Gen. Hosp., Boston, MA; [2]Massachusetts Gen. Hosp., Charlestown, MA

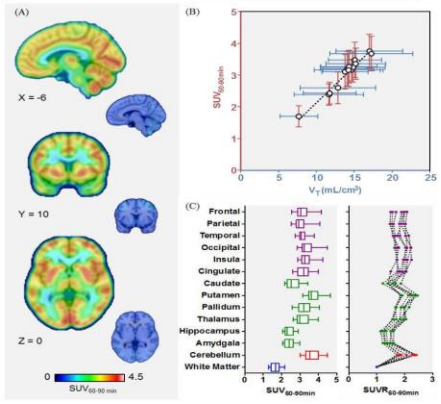
Abstract: The purpose of this study is to evaluate altered regional histone deacetylase (HDAC) expression in Parkinson's Disease (PD) brains using [^{11}C]Martinostat as a positron emission tomography (PET) radiotracer. The hypothesis tested is that HDAC enzyme expression is altered in PD brains. A combined PET/magnetic resonance imaging (MRI) system will be taken advantage of to collect simultaneous images. Research from diverse disciplines supports the hypothesis that HDAC dysfunction can lead or contribute to brain diseases, including PD, but limited investigation of the underlying processes and mechanisms relevant to PD. To overcome this limitation, the first HDAC radiotracer was developed (Figure 1). Our laboratory has now successfully imaged a growing cohort of healthy adults (18-65 years; >50) (Figure 2), and PD patients. In our first publication, we performed PET imaging on eight healthy volunteers. Standard uptake value-Revised (SUVR)$_{60\text{-}90min}$ showed that the regional distribution patterns test-retest reliability of [^{11}C]Martinostat binding are remarkably consistent in all subjects and volume of distribution (V_T) is well correlated with SUVR. These results support the use of SUVR$_{60\text{-}90min}$ as an appropriate surrogate outcome measurement for V_T (Figure 3). Our PET HDAC imaging in PD is the first time ever to visualize epigenetic expression in the brain of these patient

populations. In our preliminary study, HDAC binding in PD subjects (a 74-year-old cognitively normal and a 71-year-old man with left-predominant PD-Mild cognitive impairment (MCI)) differed from that of a 65-year-old normal control in two respects. First, binding in the putamen was markedly asymmetric in PD, with elevated binding greatest in the hemi-putamen underlying the greater motor impairment. Second, occipital cortical binding was increased markedly and selectively in the PD-MCI patient with visual hallucinations. These early imaging data represent a major step forward in understanding epigenetic mechanisms in vivo and are already providing insights into regional HDAC expression.



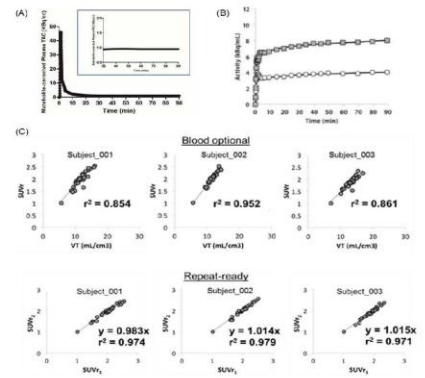
The Design of [^{11}C]Martinostat

Figure 1. [^{11}C]Martinostat: a translational PET imaging probe. We have developed a potent HDAC imaging agent, termed [^{11}C]Martinostat, incorporating three key structural features to create a versatile and translational probe for visualizing HDAC expression in vivo. Intravenous injection of trace amounts of [^{11}C]Martinostat (nanogram scale) in baboon and imaging by PET/MR demonstrates quantifiable uptake in the brain and in diverse peripheral organs. This illustrates the potential for [^{11}C]Martinostat as a broadly-applicable tool in evaluating HDAC density in humans.



Human Brain PET/MR Imaging with [^{11}C]Martinostat

Figure 2. Group [^{11}C]Martinostat $SUV_{60\text{-}90min}$ images show small intersubject variation of localized, regional [^{11}C]Martinostat binding. (A) Mean images (upper left) and standard deviation images (inset on the lower right) of $SUV_{60\text{-}90min}$ from healthy volunteers (n=8; 4 males, 4 females, mean age ± standard deviation: 28.6 ± 7.6 years). (B) Correlation of regional distribution volume (V_T) values, derived from a two-tissue compartmental model using metabolite-corrected arterial plasma as input function, and $SUV_{60\text{-}90min}$ values (n=6). The V_T values and $SUV_{60\text{-}90min}$ were significantly correlated (r^2=0.96; correlation analysis, p<0.0001). (C) *(Left)* Regional $SUV_{60\text{-}90min}$ of cortical (purple), subcortical (green), cerebellar (red), and white matter (blue) volumes of interest. *(Right)* Regional $SUV_{60\text{-}90min}$ values were normalized to the subject's white matter $SUV_{60\text{-}90min}$ value ($SUVR_{60\text{-}90min}$). Each dashed line represents $SUVR_{60\text{-}90min}$ values from a single subject (n=8).



Test-Retest and Blood Analysis for Human Imaging

Figure 3. Human data: (A) Metabolite-corrected time activity curves (TAC) from human plasma (inset: 30-90 min TAC); (B) TACs and compartmental model fitting (two-tissue compartmental model) results for superior frontal cortex and white matter. TACs of the superior frontal cortex (square) and white matter (circle) regions from all subjects are shown for the 90-minute scan duration. (C) (Top) Quantitation of [^{11}C]Martinostat distribution in human brain PET scans. SUVr, standardized uptake with normalization to white matter, is purely image-based however aligns incredibly well with distribution volumes (VT) obtained using plasma-input function from sampled arterial blood. (Bottom) High reliability in PET test-retest with [^{11}C]Martinostat. Excellent correlations of HDAC density measures in whole brain from same day test-retest demonstrate stability of repeated HDAC measures.

Disclosures: S. Fiedler: None. **L. Werneck:** None. **B. Ntaganda:** None. **S.N. Gomperts:** None. **C. Wang:** None.

Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 384.03/G18

Topic: C.03. Parkinson's Disease

Support: K23 NS097625-01A1

Title: Cognitive outcomes of STN-DBS in GBA-PD

Authors: *G. PAL[1], E. HILL[1], T. FOLTYNIE[2], V. LYTHE[2], R. ALCALAY[3], P. GARCIA[3], K. MARDER[3], R. SAUNDERS-PULLMAN[4], S. BRESSMAN[4], J. AASLY[5], B. OUYANG[1], Y. LIU[1], S. ANDERSON[1], B. BERNARD[1], L. VERHAGEN[1];
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Abstract: Objective: To determine the rate of cognitive decline in glucocerebrosidase (GBA) mutation carriers with subthalamic nucleus deep brain stimulation (STN-DBS).
Background: About 12-17% of Parkinson's disease (PD) subjects undergoing DBS carry mutations in the GBA gene. The rate of cognitive decline in GBA mutation carriers after DBS has never been examined in a large cohort.
Methods: PD subjects with bilateral STN-DBS and GBA genotyping were identified from Rush University Medical Center, University College London Institute of Neurology, Norwegian University of Science and Technology, Icahn School of Medicine at Mount Sinai, Columbia University, and Parkinson's Progression Markers Initiative (PPMI). Data collected included age, age of onset, sex, years before DBS, years since DBS, family history, UPDRS, MMSE, MoCA, and Mattis Dementia Rating Scale (MDRS). MMSE and MoCA scores were converted to MDRS scores(1). Subjects were required to have a baseline MDRS score >=130 to be included in the analysis. Subjects were fully sequenced for GBA mutations and categorized into 4 groups: risk variants, mild, severe, or non-mutation carriers. Linear mixed modeling was used to compare rate of change in MDRS scores over time comparing the four groups, adjusting for age, sex, years before DBS, and years since DBS.
Results: Data were available for 30 GBA mutation carriers and 44 non-mutation carriers. GBA mutation carriers included 11 subjects with risk variants, 15 subjects with mild mutations, and 4 subjects with severe mutations. There was no significant difference in age, sex, age of onset, years before DBS, years since DBS, baseline MDRS scores, family history, or baseline UDPRS-III (OFF/ON) scores between GBA mutation carriers and non-mutation carriers. MDRS scores worsened over time in all 4 groups (-0.84 points per year, p = 0.0007). MDRS scores decreased faster in mild GBA mutation carriers (-1.68 points per year p = 0.0004) and severe GBA

mutation carriers (-1.8 points per year, p = 0.01) compared with non-mutation carriers. Median follow-up time was 5.5 years and was not significantly different between the groups (p = 0.68). By the time of meeting, additional subject data will be available and the analyses will be updated accordingly.
Conclusions: PD subjects with mild or severe GBA mutations with STN-DBS have faster cognitive decline compared with non-mutation carriers. This is the largest cohort to date to examine cognitive decline in GBA mutation carriers after STN-DBS. Additional studies are needed to compare cognitive decline in GBA mutation carriers with and without STN-DBS.

Disclosures: G. Pal: A. Employment/Salary (full or part-time):; Rush University Medical Center, NINDS. 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.; MOVES-PD, NILO-PD. **E. Hill:** None. **T. Foltynie:** None. **V. Lythe:** None. **R. Alcalay:** None. **P. Garcia:** None. **K. Marder:** None. **R. Saunders-Pullman:** None. **S. Bressman:** None. **J. Aasly:** None. **B. Ouyang:** None. **Y. Liu:** None. **S. Anderson:** None. **B. Bernard:** None. **L. Verhagen:** None.

Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 384.04/G19

Topic: C.03. Parkinson’s Disease

Support: NIH Grant U01 NS102038

Title: Neurite orientation dispersion and density imaging (NODDI) and free-water imaging in Parkinsonism

Authors: *T. MITCHELL[1], E. HERSCHEL[5], D. B. ARCHER[1], W. T. CHU[2], S. A. COOMBES[1], S. LAI[3], B. J. WILKES[1], N. R. MCFARLAND[4], M. S. OKUN[4], M. L. BLACK[1], T. SIMUNI[6], C. COMELLA[8], T. XIE[9], T. B. PARRISH[7], H. LI[10], A. KURANI[7], D. M. CORCOS[5], D. E. VAILLANCOURT[1];
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Abstract: Neurite orientation dispersion and density imaging (NODDI) uses a three-compartment model to probe brain tissue microstructure, whereas free-water (FW) imaging models two-compartments. It is unknown if NODDI detects more disease-specific effects related to neurodegeneration in Parkinson's disease (PD) and atypical Parkinsonism. We acquired multi- and single-shell diffusion imaging at 3 Tesla across two sites. NODDI (using multi-shell; isotropic volume [Viso]; intracellular volume [Vic]; orientation dispersion [ODI]) and FW imaging (using single-shell; FW; free-water corrected fractional anisotropy [FAt]) were compared in 44 PD, 21 multiple system atrophy Parkinsonian variant (MSAp), 26 progressive supranuclear palsy (PSP), and 24 healthy control subjects in the basal ganglia, midbrain/thalamus, cerebellum, and corpus callosum. There was elevated Viso in posterior substantia nigra across Parkinsonisms, and Viso, Vic, and ODI were altered in MSAp and PSP in the striatum, globus pallidus, midbrain, thalamus, cerebellum, and corpus callosum relative to controls. The mean effect size across regions for Viso was 0.163, ODI 0.131, Vic 0.122, FW 0.359, and FAt 0.125, with extracellular compartments having the greatest effect size. A key question addressed was if these techniques discriminate PD and atypical Parkinsonism, using regions previously identified in a different cohort (putamen, superior cerebellar peduncle, vermis). Both NODDI (AUC: 0.946) and FW imaging (AUC: 0.932) had high accuracy, with no significant difference between models. This study provides new evidence that NODDI and FW imaging offer similar discriminability between PD and atypical Parkinsonism, and FW had higher effect sizes for detecting Parkinsonism within key regions across the basal ganglia and cerebellum.

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

Location: Hall A

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Program #/Poster #: 384.05/G20

Topic: C.03. Parkinson's Disease

Support: NIA K01AG051777
AARG-17-529121

Title: Associations between cortical thickness and white matter connectivity

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Abstract: The regionally-specific relationship between longitudinal changes in white matter (WM) microstructure and cortical thinning across adult lifespan was studied in a few previous studies to evaluate the causal relationship between changes in white matter integrity and cortical degeneration. In the current cross-sectional study, we further investigated the white-to-grey matter relationship in a group of Parkinson's patients and healthy controls in order to explore the associations in the context of pathological degeneration. Instead of the often-used fractional anisotropy to characterize white matter integrity, we examined white matter connectivity between cortical region pairs in order to understand how variability in cortical thickness relates to the variability in the connectivity between the region pairs. Nine Parkinson's patients (3F/6M; age:58±9.4 years) and 13 healthy controls (10F/3M; age: 65±6.6 years) received both T1-weighted structural imaging and multi-shell diffusion weighted imaging (DWI) using a 3T GE scanner. T1 images were parcellated with the DKT atlas using FreeSurfer v6.0 and the DWI data were pre-processed with the MRtrix3 software. After pre-processing, the multi-shell multi-tissue constrained spherical deconvolution model was estimated for the DWI data, then ten million streamlines were estimated by a probabilistic fiber tracking algorithm among all pairings of the parcellated regions. Connectivity between region pairs was quantified with the intensity of structural connectivity. To relate cortical thickness with the connectivity between region pairs, linear regression model was tested with age, gender and mean cortical thickness of each region pair as the explanatory variables, while connectivity was the outcome variable. Using the False Discovery Rate to correct for multiple comparisons, we found that the mean cortical thickness of (1) the left lateral occipital and the middle temporal area significantly predicted the WM connectivity at $P < 0.0001$, and (2) the right posterior cingulate and the precuneus predicted the associated WM connectivity with $P < 0.0001$. The findings demonstrate the interconnectedness between grey and white matter health and warrants further exploration in longitudinal data.

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

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Topic: C.03. Parkinson's Disease

Support: Deutsche Forschungsgemeinschaft (DFG) SFB1286/B09

Title: Cytoskeletal alterations contributing to synapto-axonal dysfunction in Parkinson's disease

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Abstract: Parkinson´s disease (PD) is a movement disorder with a strong degeneration of dopaminergic neurons. Motor symptoms arise once ~30% of these neurons are lost. However, more than 50% of tyrosine hydroxylase positive striatal dopaminergic terminals have degenerated by this point. Synapto-axonal degeneration preceding the loss of the somata in a dying back manner is hence an early pathological step in PD. The aim of this project is to establish the alterations of the synapto-axonal proteins levels in PD as a quantitative description of pathological changes in the synapse, and to identify novel potentially therapeutically relevant molecular targets.

To this end, we analysed the proteome of human post mortem hippocampal tissue of 16 PD patients and 14 age-matched controls (AMC). Via Selected Window Acquisition of All Theoretical Precursors (SWATH) mass spectrometry, 2089 proteins were quantified across all samples. A linear model was fitted to the data with moderated t-statistics via empirical Bayes, followed by Benjamini-Hochberg correction for multiple-testing. 55 proteins were found to be differentially expressed. Amongst these we identified proteins involved in the synapse organization: synapsin 1, a neural phosphoprotein coating synaptic vesicles that regulates neurotransmitter release; neuromodulin, a major component of growth cones and involved in axonal and dendritic filopodia induction; and myristoylated alanine-rich C-kinase substrate, the most prominent cellular substrate for protein kinase C, which binds to proteins like calmodulin, actin and synapsin.

To correlate human post-mortem data with longitudinal disease development, we analysed three time points in the hippocampus of male prnp.aSyn.A53T mice. Animals were analysed at 100, 250, and 400 days of age and compared to age-matched wild-type litter mates (n=5, each). 1734 unique proteins were identified, from which 2, 14, and 12 differentially expressed proteins were identified in 100, 250, and 400 days old mice, respectively. Amongst these, synapsin 1 was identified in both mouse (at all 3 time points) and human samples.

Our results demonstrate the feasibility of comparative proteomics from human post-mortem tissue and mouse animal models. Furthermore, they support the idea of synapto-axonal alterations contributing to PD pathogenesis and identify molecular correlates that could represent therapeutic targets.

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

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Program #/Poster #: 384.07/G22

Topic: C.03. Parkinson's Disease

Support: The Michael J. Fox Foundation for Parkinson's Research Grant 2018A009209

Title: Fostering inclusivity in research engagement for underrepresented populations in Parkinson's disease: The FIRE-UP PD study

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Abstract: Greater diversity in clinical trial enrollment improves study generalizability and therapy efficacy. However, underrepresented groups (URGs) in Parkinson's disease (PD) research remains rare, even in online PD research platforms such as Fox Insight (FI). We define URGs as women, racial and ethnic minorities, individuals of low socioeconomic status (SES; below the US median household income of $57,000), or rural residents. This study addresses barriers to research for URGs in PD research as well as the awareness of PD clinical research opportunities for underrepresented participants. This pilot study focuses on enrollment to FI via a small exploratory intervention. We hypothesized that interventions at sites focused on engaging URGs would enroll a greater proportion of URGs than at passive control sites serving similar populations. We implemented a stratified randomization design for eight sites within the Parkinson Study Group (PSG) network, in which pairs of sites were assigned to either intervention or control conditions. Sites were paired based on ability to address specific recruitment barriers outlined by Picillo and colleagues (2015). Each intervention site additionally identified a URG population and a 6-month intervention to address the URG barrier. Sites in Chicagoland, Boston, Denver, and Miami were randomly assigned to the intervention condition. Sites targeted low SES, racial and ethnic minorities; Black/African Americans; and two sites targeted Hispanic/Latino URG populations, respectively. Sites in Chicago; the rural Midwest; King County, WA; and San Francisco served as controls. Control sites maintained current recruitment infrastructure and workflow modified through the display of FI materials and a dedicated tablet device for surveys and FI registration. All sites report the Trust in Medical Researchers Scale (TIMRS), attitudes towards genetic testing, surveys regarding awareness, engagement, and interest in PD research, as well as URG accrual to FI, prior to and following the intervention period. Intervention and follow-up continue through December 2019. We report change in site-level TIMRS and accrual to PD research studies, as well as digital media metrics to determine engagement with PD research.

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 384.08/G23

Topic: C.03. Parkinson’s Disease

Support: 2016048SF/YX02（1）

Title: Application of tract-based spatial statistic analysis of Parkinson's disease

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Abstract: Parkinson's disease (PD) is a common nervous system degenerative disease in the aged, which mainly manifested as resting tremor, bradykinesia, rigidity and postural balance disorder. we used tract-based spatial statistics (TBSS) to analyze the microstructure of white matter in PD patients with different courses and explore whether TBSS method may be used as an imaging biomarker of PD diagnosis and monitoring.30 Parkinson's disease (PD patients in our hospital were collected. The whole brain T1 structure image and diffusion tensor imaging (DTI) function image were obtained . A data processing software PANDA is used to preprocess DTI data. We compared brain white matter by TBSS. The fractional anisotropy(FA) value of the brain region was obtained by PANDA software. The fiber bundle of FA decreased in early PD group,including the body of corpus callosum, splenium of corpus callosum, middle cerebellar peduncle,left posterior thalamic radiation, genu of corpus callosum, right posterior thalamic radiation,right posterior corona radiata, left anterior corona radiata, right superior corona radiata, right external capsule, right anterior limb of internal capsule, left anterior limb of internal capsule,and right superior longitudinal fasciculus as well($P<0.05$, FWE correction, voxel>500).The fiber bundle of FA decreased in advanced PD group, including genu of corpu callosum, body of corpus callosum, right anterior coron radiata, left anterior coron .radiata,right posterior thalamic radiation, right superior coron radiata, right posterior corona radiata, right anterior limb of internal capsule,right external capsule, left superior corona radiata,left anterior limb of internal capsule, and left posterior thalamic radiation as well($P<0.05$, FWE correction, voxel>500). Extensive white matter fiber bundles were damaged in PD patients,and the damage of the white matter fiber bundles was more severe in advanced PD patients. The FA value of the damaged white matter fiber bundle in PD patients was negatively correlated with the H-Y classification. The FA value of impaired white matter fiber bundle in PD patients was not correlated with the score of UPDRS- I and UPDRS- III scale.

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

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Program #/Poster #: 384.09/G24

Topic: C.03. Parkinson's Disease

Support: NSF Graduate Research Fellowship (KAM)
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Rackham Merit Fellowship (KAM)

Title: Atlas-independent, individualized tissue activation modeling to map the optimal location of subthalamic deep brain stimulation for Parkinson's disease

Authors: *K. A. MALAGA[1], J. T. COSTELLO[2], K. L. CHOU[3,4], P. G. PATIL[1,3,4];
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Abstract: Deep brain stimulation of the subthalamic nucleus (STN DBS) is an established treatment for the motor symptoms of Parkinson disease (PD). Motor outcomes after STN DBS can vary considerably across patients and depend strongly on the location of stimulation relative to the surgical target. The objective of this retrospective study was to map the optimal location of STN DBS for PD using an atlas-independent, individualized tissue activation modeling approach and to assess the relationship between the therapeutic volume of tissue activated (VTA) and motor improvement. The stimulation-induced electric field in the brains of 40 PD patients treated with STN DBS was modeled using finite element analysis. Neurostimulation models were tailored to each patient, incorporating individual STN anatomy, anisotropic brain conductivity, electrode position and orientation, and stimulation settings. A voxel-based analysis of the VTAs was used to map the optimal location of stimulation. The amount of stimulation in specific regions relative to the STN was measured and compared between patients with optimal and sub-optimal stimulation, as determined by their motor outcomes (MDS-Unified Parkinson's disease Rating Scale III) and VTA. The relationship between VTA location and motor improvement was assessed using linear regression. Variability across patients in terms of STN anatomy, active contact position, and VTA location was also evaluated. Individualized VTA modeling mapped the optimal location of stimulation to a region medial, posterior, and dorsal to the STN centroid. This region was external to the STN and corresponds to the zona incerta (ZI). VTA location and active contact position differed significantly between patients with optimal and sub-optimal stimulation in the dorsal-ventral and anterior-posterior directions. There were significant linear relationships between the amount of dorsal and posterior stimulation, as measured by the VTA, and motor improvement. These relationships were more robust than those between active contact position and motor improvement. There was high variability in STN anatomy, active contact position, and VTA location across patients. Accurate predictions of the spread of stimulation in the brain are important for optimizing STN DBS for PD. High variability across patients in terms of neuroanatomy and stimulation location highlights the need for individualized modeling approaches. This study mapped the optimal location of stimulation to a region external to the STN, which provides further evidence for ZI as an optimal stimulation target for PD.

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

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Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation -- Computational Grant 14848
R56 AG 058854 (Thompson)

Title: Polygenic risk for Parkinson's disease in unaffected individuals associates with microstructure of disease-related white matter regions

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Abstract: Parkinson's disease (PD) affects up to 10 million people worldwide; still, little is known about its cause or progression. Early structural brain changes in PD may occur in the white matter (WM; Worker et al., 2014), which can be studied using diffusion tensor imaging (DTI) derived fractional anisotropy (FA). Identifying genetic risk factors underlying WM abnormalities may help in the detection of PD in presymptomatic stages, and allow for targeted treatments, yet the effect of the genetic risk for PD on WM is unknown. Here we calculated a PD polygenic risk score (PRS) for 8,632 subjects from the UK Biobank (4,518F/4,114M; mean age: 62.5 yrs, range 45-79 yrs) by summing risk variants, weighted by association statistics from Nalls et al. (2014), using a $p<10\text{-}5$ threshold. FA estimates were extracted and averaged bilaterally for 27 regions of interest (ROIs) per subject. For each ROI, we used a linear regression to test the association between PRS and FA, covarying for age, sex, and ancestry using the first four components from multi-dimensional scaling analysis. The anterior limb of the internal capsule (ALIC) showed a significant positive association after multiple comparisons correction using the false discovery rate method; higher genetic risk was associated with higher FA ($p=0.002$). A follow-up study of separate left and right ALIC showed significant positive associations ($p=0.001$ and $p=0.006$, respectively). To assess disease relevance of the ALIC, we then compared ALIC FA of 142 PD patients to 63 healthy controls (HC) from the Parkinson's Progression Markers Initiative. PD patients showed significantly higher FA in the bilateral ALIC compared to HC ($p=0.015$). Higher FA in PD compared to HC was also seen in left and right ALIC separately ($p=0.017$, and $p=0.025$ respectively). The association of higher genetic risk for PD with higher FA in the ALIC follows the pattern of effects seen in patients vs HC suggesting the disease-related microstructure differences may be partially driven by genetic risk. We note that the ALIC contributes to thalamic connections; larger thalamic volumes observed in PD patients vs HC (Van der Werf, 2019) may be partially driven by genetic risk and WM

differences. Our study suggests genetics as a promising target for future research on PD-related brain changes.
This research has been conducted using the UK Biobank Resource under Application Number '11559'. Data used in the preparation of this article were obtained from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/data). For up-to-date information on the study, visit www.ppmi-info.org

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

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Topic: C.03. Parkinson's Disease

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Dystonia Medical Research Foundation
NINDS, NIH Intramural Program

Title: Distribution of tremorogenic activity and tremor in the upper limbs of persons with essential tremor

Authors: *S. K. CHARLES[1], D. STANDRING[2], A. PIGG[2], J. THOMPSON-WESTRA[3], K. MENTE[4], C. MAURER[4], D. HAUBENBERGER[3], M. HALLETT[4];
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Abstract: Although Essential Tremor is one of the most common movement disorders, we do not currently know where to intervene (which muscles or joints) to suppress tremor in an optimal manner. To start addressing this challenge, we characterized the distribution of tremorogenic muscle activity and tremor throughout the upper limb. We recorded surface electromyography (sEMG) in the 15 major superficial muscles of the upper limb and motion capture data from the torso to the hand in 25 subjects with ET while they performed both postural and kinetic tasks. The amount of tremorogenic activity in each muscle was computed as the power in the normalized, high-pass filtered, and rectified sEMG, integrated over the tremor band (4-12 Hz). To compute the amount of tremor in each of the 7 main degrees of freedom (DOF) comprising the shoulder, elbow, forearm, and wrist, we used inverse kinematics algorithms to transform motion capture data into angular displacement in each DOF, differentiated to obtain angular displacement in each DOF, and calculated the tremor-band power in the angular acceleration.

Averaged across all subjects, we found that the anterior deltoid had the most tremorogenic activity overall and much more tremorogenic activity than neighboring proximal muscles. Wrist extensors also had high amounts of tremorogenic activity compared to other distal muscles. The triceps longus had the least amount of tremorogenic activity. Tremor was found to be most pronounced in wrist flexion-extension, then forearm pronation-supination, and then shoulder internal-external rotation. Shoulder abduction-adduction exhibited the least amount of tremor. Importantly, the distribution of both tremorogenic activity and tremor were quite stereotyped, being similar across repetitions, postures, tasks, and subjects. For example, the mean correlation coefficients characterizing the distribution of kinetic tremor across repetitions, postures, tasks, and subjects were 0.94, 0.86, 0.52, and 0.72, respectively. We are currently working on elucidating the relationship between tremorogenic muscle activity and tremor to determine which muscles have the largest effect on tremor and should therefore be targeted to suppress tremor in an optimal manner.

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 384.12/G27

Topic: C.03. Parkinson’s Disease

Support: NIH Academy Enrichment Program

Title: Exploring phosphorous metabolites in Parkinson's disease at 7Tesla

Authors: *K. THOMAS[1], P. BEDARD[1], D. EHRLICH[2], M. HALLETT[1], S. G. HOROVITZ[1]; [1]Med. Neurol. Branch, Human Motor Control Section, [2]Med. Neurol. Branch, Parkinson's Dis. Clin., NIH - NINDS, Bethesda, MD

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder in which neurons in the substantia nigra (SN) of the basal ganglia die, leading to its hallmark motor symptoms. Because impaired mitochondrial function may contribute to cell death in PD, we are motivated to investigate signs of mitochondrial dysfunction in the SN. To do so we explored phosphorous metabolites in PD patients and healthy volunteers (HV) using magnetic resonance spectroscopy imaging (MRSI).

28 datasets (14 PD, 14 HV) collected in a 7T siemens scanner using a $^{31}P/^{1}H$ birdcage coil are included in this study. Data consisted of an MPRAGE and a whole brain 3D ^{31}P-MRSI with voxel size of 17.28 cc (FOV=200 x 180 x 180 mm^3, matrix=12 x 12 x 12). MPRAGE data were segmented in Freesurfer. Spectroscopy data were processed in JMRUI to fit eight phosphorous metabolites indicative of energy and phospholipid metabolism. This data was registered to the MPRAGE with in-house programs.

Four regions of interest (ROIs) were selected bilaterally: left SN, right SN, left putamen (PUT) and right PUT. Two metabolites of interest were selected for this analysis: glycerophosphoethanolamine (GPE) to assess membrane integrity and phosphocreatine (PCr) to assess cell energy. We compare groups with t-tests assuming unequal variance between the HV and PD patients for each ROI separately.

Our results showed that PD patients have significantly ($p = .0419$) larger GPE/PCR in the right SN compared to HV. To determine whether an increase in GPE or decrease in PCr the reason for this difference, we also did t-tests of the ratios GPE over total (sum of all eight metabolites we calculated), as well as PCr over total. We found in the right SN that there was a trend towards significance between the groups in the ratio GPE/total ($p = .081$) compared to the ratio PCr/total ($p = .3479$). None of the ratios assessed were found to be associated with the subjects' age or intracranial volume, and the tissue composition of the voxel selected that encompassed the SN was similar for both groups. These ratios in the left and right PUT showed no significant difference between groups.

We were able to detect changes in the area where the disorder is believed to start, the SN. Our results would be compatible with an increase in membrane breakdown in PD patients when compared to HV. Our results suggest that the increased degradation seen in the SN of PD patients is not detectable in the PUT. Further exploration of other metabolites and brain areas will provide a better understanding of the energetics and membrane integrity in PD.

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

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Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation

Title: Transcriptomic analysis of whole blood samples from the Parkinson's progression markers initiative for biomarker discovery

Authors: *E. HUTCHINS[1], D. CRAIG[3], I. VIOLICH[3], E. ALSOP[1], S. HUTTEN[4], B. CASEY[4], A. REIMER[4], S. LEVY[5], M. R. COOKSON[6], R. GIBBS[6], C. BLAUWENDRAAT[6], K. R. VAN KEUREN-JENSEN[2];
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Abstract: Transcriptomic analysis of whole blood samples has the potential to uncover biological pathways disrupted by disease processes. Detectable changes in the transcriptome of a readily accessible biofluid, such as blood, have the potential to be valuable biomarkers. The goal of this project was to develop and share a comprehensive RNA resource from whole blood samples taken from normal healthy control subjects, patients diagnosed with Parkinson's disease (PD), and subjects at-risk for PD, for the purposes of biomarker discovery and to provide a broader understanding of RNA changes associated with PD. Approximately 4700 whole blood samples from 1610 subjects were obtained from the Michael J. Fox Foundation's Parkinson's Progression Markers Initiative cohort, including longitudinal samples at baseline, 6 months, 1 year, 2 year, and 3 years. High quality RNA samples were globin and ribodepleted, and strand-specific libraries were created for sequencing. Targeted sequencing depth was 100 million read pairs per sample, yielding a highly diverse transcriptome for downstream analyses. We identified a large number of coding and non-coding RNA transcripts. We will present data comparing the transcriptomic landscape of PD and normal controls, as well as transcriptomic changes associated with disease progression. These data include not only protein-coding RNA, but several RNA biotypes, including lncRNA and circRNA, and their potential deregulation in association with PD. We will also provide details on how to access the data and how to access a web portal built specifically to visualize and query the RNASeq data.

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Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 384.14/G29

Topic: C.03. Parkinson’s Disease

Support: BERGEN RESEARCH FOUNDATION

Title: H3K27 acetylation landscape dysregulation in patients with Parkinson's disease

Authors: *J. SUNDARESAN, G. T. TRAN, L. TOKER, G. S. NIDO, C. DÖLLE, C. TZOULIS;
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Abstract: Parkinson's disease (PD), is the most common movement disorder and second most common neurodegenerative disease. It has a lifetime risk of 1-2% with a steady increase of prevalence with age, affecting 1-2 per 1000 of the population at a given time. Risk factors for the disease range from lifestyle and environment to genetics with no particular factor taking precedence over others. Our previous work suggested alteration in some of the histone acetylation profile, in particular H3K27, between PD and age matched control brains. Here, we compare genome-wide enrichment of H3K27ac in the pre-frontal cortex (BA9) of PD individuals against age matched cognitively normal controls. The dataset includes 17 PD and 11 control brains for whom H3K27ac profile was determined by CHIP sequencing. We find a distinct difference in H3K27ac binding pattern between the two groups. Furthermore, we elucidate the genomic locations of significant H3K27ac changes with expression quantitative trait loci. Our results establish the basis for an epigenetic link to PD pathology.

Disclosures: J. Sundaresan: None. **G.T. Tran:** None. **L. Toker:** None. **G.S. Nido:** None. **C. Dölle:** None. **C. Tzoulis:** None.

Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 384.15/G30

Topic: C.03. Parkinson’s Disease

Title: Cortical and subcortical microstructural alterations detected by diffusion tensor imaging in early-stage Parkinson's disease

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Abstract: Previous diffusion tensor imaging (DTI) studies of Parkinson's disease (PD) have shown alterations in microstructure integrity, particularly in frontal white matter and subcortical grey matter. The progression of these microstructure degradations in PD is not fully understood. Our objective is to examine changes in microstructure integrity in early-stage PD in relation to cognitive and motor decline using a prospective approach.
Using data from the Parkinson's Progression Markers Initiative (PPMI), we examined individuals in early-stage PD who had diffusion images at both 12-month (N = 60, aged 39-76, 20 F) and 24-month (N = 44, aged 39-77, 12 F) timepoints. We conducted a paired t-test across the two timepoints, and multiple regression analyses at each timepoint to test for alterations in whole brain voxel-wise fractional anisotropy (FA) and mean diffusivity (MD) in associations with cognitive and motor symptoms.
In accordance with previous findings, advanced age was associated with higher MD and lower FA in widespread brain regions at both timepoints. This age-related pattern of microstructural degradation seemed to relate to cognitive impairment across subjects in the 12-mo timepoint, but less so in the 24-mo timepoint. Significantly higher MD in the right medial frontal white matter was observed in the 24-mo relative to 12-mo timepoint. At both timepoints, cognitive and motor impairment across subjects were associated with higher MD and lower FA in frontal white matter and various cortical and subcortical regions. Intriguingly, severity of rigid and akinetic symptoms was associated with higher FA and lower MD in the pons, thalamus, and cerebellum in the 12-month timepoint. A similar pattern of FA and MD effects was found with rigid/akinetic symptoms across subjects at 24 months. These findings suggest that impairment in cognitive and motor abilities in early-stage PD may relate to degradation in frontal white matter, and that alterations of microstructure integrity in subcortical regions may uniquely relate to more severe rigid and akinetic symptoms in early-stage PD. Tracking such microstructural changes may have implications in treatment management.

Disclosures: M. Amandola: None. **H. Leung:** None.

Poster

384. Parkinson's Disease Human Studies: Genetics and Diagnostics

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 384.16/G31

Topic: C.03. Parkinson's Disease

Title: Subcortical brain volumes as potential markers of Parkinson's disease progression

Authors: *R. M. WALES, M. WONG, M. KARIBIAN, H.-C. LEUNG;
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Abstract: Several recent volumetric longitudinal studies have proposed the volumes of subcortical structures as potential biomarkers of early Parkinson's disease (PD), but the relationship between these brain volumes and cognitive and/or motor outcomes remains unclear. In this study, we examined hippocampus, caudate, and putamen volumes in relation to cognitive functions (executive function, memory, and visuospatial domains) and motor symptom severity (UPDRS III total, akinetic/rigidity, and tremor scores). Imaging (T1 MRI), clinical, and behavioral data from early stage PD patients and healthy controls were obtained from the Parkinson's Progression Markers Initiative database. FreeSurfer was used to extract subcortical volumes from each individual at each time point (baseline, 12-, 24-, and 48-mo follow-ups). Changes in cognitive and motor assessments were measured by calculating the slope between time points. Multiple regressions, accounting for age, gender, years of education, and study site, were used to examine the relationships between volumetric, cognitive, and motor measures.Comparing subcortical volumetric rates of change between PD and healthy controls revealed significantly more hippocampal atrophy in PD patients over a one year period than healthy controls. This effect remained even after controlling for intracranial volume. A multiple regression analysis of the relationship between volumetric, cognitive, and motor measures revealed that, at baseline, smaller putamen volumes were associated with more severe rigid/akinetic symptoms across subjects. Intriguingly, more severe rigid/akinetic symptoms at baseline seemed to predict putamen atrophy over four years. Some of these associations may also be driven by a combination of age, gender, and cognitive dysfunctions. These data suggest that hippocampal atrophy is evident in early PD, and that the baseline motor symptom profile (e.g., rigid/akinetic) may predict subcortical atrophy during early stage of the disease

Disclosures: R.M. Wales: None. **H. Leung:** None. **M. Wong:** None. **M. Karibian:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.01/G32

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant P30 AG059299
NIH Grant P50 AG005131

Title: Sensory weighting for postural control and fall-risk changes with progression of Huntington's disease

Authors: *M. B. MAY[1], J. COREY-BLOOM[5], P. E. GILBERT[2], D. J. GOBLE[3], H. S. BAWEJA[4];
[2]Dept Psychol, [3]Exercise and Nutritional Sci., [4]Doctor of Physical Therapy Program, [1]San Diego State Univ., San Diego, CA; [5]Dept. of Neurol., Univ. of California San Diego, San Diego, CA

Abstract: Huntington's disease (HD) is a genetic neurodegenerative movement disorder caused by more than 36 repetitions of the CAG nucleotide sequence. HD is typically diagnosed between the ages of 30-50 years. Prior to diagnosis the gene carriers are considered *pre-manifest*. Chorea, characterized by involuntary hyperkinetic motor movements, is a hallmark symptom of manifest HD. Dynamic posturography is an effective test to distinguish the movement dysfunction between pre-manifest and manifest HD. Furthermore, postural sway measurements during sensory information manipulations are of great value in understanding disease progression. However, the role of sensory information in postural control with disease manifestation remains unclear. Therefore, the purpose of this study was to understand the effect of sensory information processing on postural control in Huntington's disease. 82 subjects participated in the study: 26 gene-negative (ARN), 20 gene-positive (ARP), 26 HD, and 10 healthy controls (HC). All subjects performed a static balance assessment using a portable force plate (Balance Tracking Systems, San Diego, CA). Testing consisted of 1 familiarization and 3 experimental trials of quiet standing with feet shoulder width apart and hands on the hips. This was performed twice to examine sensory weighting: once with eyes open (EO) and once with eyes closed (EC). Each trial lasted 10 seconds during which the total center of pressure (COP) sway, COP antero-posterior sway, COP medio-lateral sway excursions, velocities and 95% CIs of the COP area were quantified. All subjects' exhibited greater postural sway with eyes closed versus open in all directions. However, HD patients demonstrated the most significant decline in balance with their eyes closed when compared with pre-manifest (HD>ARP>ARN) and healthy control subjects. Previous studies from our group suggest that this decline in postural control with the absence of vision is
indicative of a higher risk for falls. Our findings also indicate that manifest HD patients are at a higher risk for falls and are highly dependent on vision for postural control when compared with pre-manifest and healthy older adults.

Disclosures: M.B. May: None. **J. Corey-Bloom:** A. Employment/Salary (full or part-time):; University of California San Diego. 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.; UCSD Huntington's Disease Society of America Center of Excellence, UCSD Shiley-Marcos Alzheimer's Disease Research Center. **P.E. Gilbert:** A. Employment/Salary (full or part-time):; San Diego State University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH Grant P30 AG059299. **D.J. Goble:** A. Employment/Salary (full or part-time):; San Diego State University. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity Stake in

Balance Tracking Systems, Inc., Pending Patent (OMB 0651-0032). **H.S. Baweja:** A. Employment/Salary (full or part-time):; San Diego State University.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.02/G33

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: De Luca Foundation
NINDS T32 NS082168

Title: Wearable-sensor driven responsive deep brain stimulation for the improved treatment of essential tremor

Authors: *S. CERNERA[1], J. ALCANTARA[1], E. OPRI[2], J. CAGLE[1], M. S. OKUN[1], A. GUNDUZ[2];
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Abstract: **Introduction:** Essential tremor (ET) is one of the most common adult onset tremor disorders that is clinically associated with chronic, disabling action tremors within the 4-12 Hz range. For patients unresponsive to medication, deep brain stimulation (DBS) is an effective therapeutic option. Despite significant improvements with DBS, its paradigm is continuous. Additionally, the current methodology is an inefficient solution for patients with tremor, which can dynamically change throughout the day. Since ET symptoms are paroxysmal, DBS does not have to be continuously delivered. We hypothesize that wearable sensors, specifically those that measure the electromyogram (EMG), are capable of detecting correlates of tremor or movement that will provide the control signal for responsive DBS (R-DBS) in a targeted and personalized manner. **Rationale:** R-DBS could reduce adverse effects of continuous DBS, deliver personalized stimulation, and decelerate battery depletion of the implantable neurostimulator (INS), while delivering an equally effective treatment. **Methods:** EMG data were recorded using Trigno Acquisition Unit (Delsys, Inc, Natick, MA) sensors from three ET subjects. Sensors were bilaterally placed on seven upper limb muscles. Data were recorded while the subject was performing several tasks in both DBS-on and -off states. From EMG signals, we extracted correlates that accurately delineated both tremor from voluntary movement and movement from rest conditions through power analysis. Correlates that separated movement from rest conditions were used in the R-DBS algorithm. Feasibility of R-DBS using wearable sensors was evaluated using Nexus-D, which is a Medtronic investigational system able to titrate stimulation parameters by communicating with the subject's INS. For R-DBS, two separate paradigms were tested. The first focused on a linear detector using a calibrated threshold, while the second was a multi-sensor paradigm using a support vector machine. The feature used in both paradigms was

the power within the subject's tremor frequency band (±2 Hz). **Results:** Across subjects, DBS decreased tremor power by -66.44±-28.10% within the EMG. Additionally, R-DBS decreased the average voltage by 1.51±0.63V across trials. The average sensitivity of movement detection during R-DBS was 78.7±7.85%; however, DBS was always active during movement since stimulation amplitude was continuously titrated. **Conclusions:** From these results, we have proven the feasibility of an R-DBS paradigm based solely on external wearable sensors. Furthermore, R-DBS was more efficient than and just as efficacious as continuous DBS.

Disclosures: S. Cernera: None. **J. Alcantara:** None. **E. Opri:** None. **J. Cagle:** None. **M.S. Okun:** None. **A. Gunduz:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.03/G34

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: European Union's Horizon 2020 research and innovation programme (Project EXTEND - Bidirectional Hyper-Connected Neural System) under grant agreement No 779982

Title: Reduction of tremor in essential tremor patients using electrical stimulation of afferent pathways

Authors: A. PASCUAL VALDUNCIEL[1], *F. O. BARROSO[1], M. GONZALEZ SÁNCHEZ[2], J. PEREZ SÁNCHEZ[2], S. MUCELI[3], M. K. JUNG[3], F. GRANDAS[2], D. FARINA[3], J. L. PONS[1];
[1]Spanish Natl. Res. Council (CSIC), Madrid, Spain; [2]Hosp. Gen. Universitario Gregorio Marañón, Madrid, Spain; [3]Imperial Col. London, London, United Kingdom

Abstract: Essential Tremor (ET) is one of main disorders leading to pathological tremor, which is the most prevalent movement disorder in the world and may hamper the execution of activities of daily living. Tremor management approaches such as Deep brain stimulation or medication are not effective in about 25% of the patients. Thus, other solutions should be considered, especially those that present less risks and are less invasive. Functional electrical stimulation has been tested as an alternative to suppress tremor. However, the development of fatigue, the discomfort and interference with voluntary movements are drawbacks that may impede the adaptation of similar solutions in long-term rehabilitation of tremor patients.
Recently, stimulation of muscle afferents has been proposed as a novel approach to reduce pathological tremor: the stimulation of Ia afferents may inhibit antagonist α-motoneurons (a mechanism known as reciprocal inhibition). Specifically, for the case of pathological tremor, the

reciprocal inhibition may be modulated via stimulation of Ia afferents of the muscle without tremor in order to suppress tremor in the antagonist muscle. To evaluate this application, we used surface stimulation of wrist flexors and extensors of ET patients using low-level intensity of stimulation and tested two different strategies: 1) out-of-phase stimulation of the antagonist of the muscle presenting the tremorogenic burst of activity (based on EMG activity); and 2) simultaneous stimulation of the two muscles. Periods of stimulation and without stimulation were alternated every 30s. IMUS were placed over the hand and forearm to quantify the tremor reduction. Preliminary results indicated that the out-of-phase strategy achieved better tremor suppression results than continuous stimulation. This reduction of tremor was even higher when stimulation was applied over the nerves innervating each muscle and not over the muscle belly directly.
Electrical stimulation of afferent pathways poses as a promising approach for reducing tremor in ET patients. We are currently evaluating if intramuscular stimulation can improve tremor reduction when compared to superficial stimulation of the nerves. Our long-term goal is to use fully deployable technologies to maximize tremor suppression.

Disclosures: A. Pascual Valdunciel: None. **F.O. Barroso:** None. **M. Gonzalez Sánchez:** None. **J. Perez Sánchez:** None. **S. Muceli:** None. **M.K. Jung:** None. **F. Grandas:** None. **D. Farina:** None. **J.L. Pons:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.04/G35

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Cohn Rush University Research Fellowship
Rush Translational Sciences Consortium Award
2018 Rush University Dean’s Fellowship
Samantha’s Search for the Cure Foundation
Hope for Hayley Foundation

Title: Efficacy of long term intrathecal 2-hydroxypropyl-ß-cyclodextrin treatment on balance and gait deficits in Niemann-Pick type C1

Authors: *J. A. O'KEEFE[1], J. M. JOYCE[2], K. E. WROBEL[3], N. PURCELL[2], B. OUYANG[4], Y. LIU[5], E. BERRY-KRAVIS[6];
[1]Cell & Mol. Med., Rush Univ. Med. Ctr., Chicago, IL; [2]Cell & Mol. Med., [3]Rush Med. Col., [4]Neurolog. Sci., [6]Pediatrics and Neurolog. Sci., [5]Rush Univ., Chicago, IL

Abstract: Niemann-Pick Type C (NP-C) is an autosomal recessive neurodegenerative disorder characterized by lysosomal accumulation of cholesterol in brain and peripheral tissues. NPC is caused by mutations in NPC1 or NPC2, proteins that normally transport cholesterol. There is great phenotypic variability but cerebellar ataxia, apraxia, dystonia and cognitive decline are seen in many patients. 2-Hydroxypropyl-ß-cyclodextrin (HP-ß-CD) extends life and slows disease in NPC animal models. We examined the efficacy of HP-ß-CD in slowing neurological deterioration in patients with NPC via balance and gait testing. Six patients were treated with HP-ß-CD intrathecally in an Expanded Access Protocol (IND119856) at Rush University Medical Center (RUMC) for 15-60 months. Six additional patients were enrolled in a RCT (Vtesse, VTS301) at the RUMC site. Balance and gait outcome measures were tracked longitudinally with computerized dynamic posturography (CDP) and an inertial sensor system, to determine potential modification of disease course and longitudinal change over time. The primary balance outcome measure was the composite postural sway score on CDP. Gait variables were secondary outcome measures. Longitudinal change of each measure was analyzed using linear mixed models. Global function was assessed by the NPC-Neurological Severity Scale (NPC-NSS). CDP composite scores improved significantly over time ($p=0.0006$). Double support and swing times and cadence were stable ($p=0.24$-0.45). Stride length and velocity and gait variability worsened significantly ($p<0.0001$) and turn duration significantly increased ($p=0.002$). These findings of improved balance support the concurrent lack of worsening or mild improvement on the NPC-NSS, which deviates from the expected disease trajectory based on the natural history of NPC. Cadence, time in double support and swing phase showed overall stabilization likely due to stabilization of ataxia, while stride length and velocity, gait variability and turns worsened, likely due to worsening dystonia in 5/12 patients. Future studies are needed to explore the effect of dystonia on worsening gait measures in NPC. CDP balance measures and inertial sensor based gait and turn outcomes are feasible to determine efficacy of pharmaceutical interventions in neurological populations. Ongoing tracking with these sensitive measures will quantify the chronic impact of HP-ß-CD on balance and gait function in NPC, help differentiate good responders from poor responders and may distinguish improvements in balance/ataxia from worsening dystonia, a symptom which might be based on differences in regional brain penetration of the drug.

Disclosures: **J.A. O'Keefe:** 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.; Sub-investigator, A Phase 2b/3 RCT of VTS270 in NPC1. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Hope for Hayley Foundation, Samantha's Search for the Cure Foundation. **J.M. Joyce:** None. **K.E. Wrobel:** None. **N. Purcell:** None. **B. Ouyang:** None. **Y. Liu:** None. **E. Berry-Kravis:** 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.; Site PI: Phase 2b/3 RCT of VTS270 in NPC1. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Hope for Hayley and Samantha's Search for the Cure Foundations. F. Consulting Fees (e.g., advisory boards); Vtesse, Sucampo and Mallinckrodt to consult on trial design/perform the VTS301 trial.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.05/G36

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Neuroprotective effects of melatonin pretreatment on a Parkinson's disease experimental model induced by manganese mixture inhalation

Authors: M. RODRÍGUEZ-ALCÁNTARA, O. MEJÍA-GARCÍA, A. GUTIÉRREZ-VALDEZ, E. MONTIEL-FLORES, J. ESPINOSA-VILLANUEVA, J. ORDOÑEZ-LIBRADO, P. ALEY-MEDINA, *M. AVILA-COSTA;
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Abstract: Parkinson disease (PD) is a neurodegenerative process characterized for the depletion of substantia nigra compacta dopaminergic cells and striatal dopamine innervation, an as a consequence the patients show motor coordination and cognitive impairments. We developed a useful PD experimental model, where the animals inhale the mixture of MnCl2and Mn(OAc)3. This model promote the auto-oxidation of the dopamine, inducing bilateral and progressive degeneration of the SNc dopaminergic neurons in mice, bilateral tremor and rigidity. On the other hand, Melatonin (Mel) has antioxidant properties, increasing the mitochondrial complexes I and IV activity, furthermore it has been suggested that this action contribute to the protective effect in neurodegenerative diseases. Therefore in this study we aim to determine if Mel pre-treatment has a neuroprotective effect against the alterations produced by the inhalation of the mixture of MnCl2and Mn(OAc)3 one hour two times a week for five months. Before Mn exposure, animals were trained to perform motor tests and were evaluated each week after the exposure. The experimental groups were: control group, Mn mixture without Mel pretreatment exposed mice and Mel pretreated/Mn exposed group, the treatment was given orally for a month (10mg/kg). After five months of Mn inhalation we found that Mel-pretreatment, partially reduced the motor and cytological alterations observed in the exposed group. Therefore, Mel pretreatment is a promising procedure to delay the disease progression.

Disclosures: M. Rodríguez-Alcántara: None. **O. Mejía-García:** None. **A. Gutiérrez-Valdez:** None. **E. Montiel-Flores:** None. **J. Espinosa-Villanueva:** None. **J. Ordoñez-Librado:** None. **P. Aley-Medina:** None. **M. Avila-Costa:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.06/G37

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Postural control deficits with progressive supranuclear palsy differ from idiopathic Parkinson's disease

Authors: *K. E. DILLON[1], C. J. MCELROY[2], I. LITVAN[2], J. FILOTEO[2], D. J. GLOBE[3], H. S. BAWEJA[1];
[1]San Diego State Univ., San Diego, CA; [2]Univ. of California at San Diego, La Jolla, CA; [3]Oakland Univ., Rochester, MI

Abstract: Progressive Supranuclear Palsy (PSP) is an atypical degenerative Parkinsonian disorder affecting the basal ganglia and brainstem. Cardinal signs include muscle weakness, oculomotor impairments, difficulty speaking, trouble swallowing, imbalance and lack of coordination. Postural control assessments using force platforms can provide an objective window into a disease mostly studied subjectively. These measurements can also be used to distinguish postural sway signatures unique to PSP from other Parkinsonian disorders. Therefore, the purpose of this study was to determine postural control deficits accompanying PSP and idiopathic Parkinson's disease (PD). 6 PSP (61-80 years), 12 PD (66-84 years) and 12 older adults (OA; 60-85 years) volunteered to participate in the study. All subjects performed a static balance assessment using a portable force plate. All testing consisted of 3 trials each of quiet unperturbed standing with eyes closed and eyes open; feet shoulder width apart and hands on the hips. Each trial lasted 20 seconds during which the total center of pressure (COP), COP antero-posterior (AP), and COP medio-lateral (ML) sway displacements were calculated. A principle component analysis was used to calculate the 95 and 99% confidence intervals (CI) of the area within which the COP would lie. Furthermore, changes in low-frequency postural oscillations were quantified by examining the absolute wavelet power in 3 frequency bins from 0-4 Hz. We found that low frequency oscillation in postural sway varied differentially with removal of vision (eyes closed v. eyes opened) and disease (PSP vs. PD). Furthermore, these postural oscillations increase with increasing fall risk. This effect is most pronounced in the PSP>PD>OA. Furthermore, PSP and PD patients at high fall risk are highly dependent on vision for postural control when compared to healthy controls and patients at low risk for falls. Most importantly, our findings suggest that non-linear measures of postural sway are more sensitive to changes in sensory weighting and neuropathologies than standard linear measures of postural sway assessment.

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Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.07/G38

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Brain Initiative UH3NS095553

Title: Adaptive cortico-thalamic closed-loop deep brain stimulation for an enhanced treatment of essential tremor

Authors: *E. OPRI[1], S. CERNERA[1], R. MOLINA[2], M. S. OKUN[3], K. D. FOOTE[4], A. GUNDUZ[1];
[1]Biomed. Engin., [2]Electrical Engin., [3]Neurol., [4]Neurosurg., Univ. of Florida, Gainesville, FL

Abstract: Essential tremor (ET) is defined as a rhythmical, involuntary oscillatory movement of the limbs and is one of the most common movement disorders. Intention tremor occurs mostly in the upper limbs (with slow oscillations between ~4-12 Hz) during the initiation and execution of goal-directed reaching motions, while it is absent at rest. It has currently been suggested that a synchronous pathological oscillation in a network that includes the premotor (PM) and primary motor (M1) cortices, the ventral intermediate nucleus (Vim), and the cerebellum is suppressed through deep brain stimulation (DBS) by jamming the “tremor cells” in the thalamus. Three patients affected by ET were chronically implanted with both cortical (M1) and thalamic (VIM) leads, connected to a Medtronic Activa PC+S neurostimulator. Together with inertial and EMG collected data, it was possible to explore biomarkers related to movement intention/execution, and to tremor. The Activa PC+S neurostimulator allows us to use thalamocortical neuromarkers to modulate the stimulation parameters in real time, enabling a truly responsively delivered DBS. This closed-loop system was tested in real-case scenarios, such as reaching for a cup to drink from it. Hence, we show the feasibility and implementation of a closed-loop system using cortical neuromarkers evoked during different behavioral tasks, such as moving a hand or reaching a cup, to enable the control of stimulation activation and deactivation. We show that responsive DBS efficiently suppresses tremor modulating the amplitude of the stimulation based on the patient state (i.e., rest: no stimulation, movement: stimulation). Our results suggest that a reliable control of responsive DBS is possible with the use of a single or a combination of targeted brain areas. In addition, standard DBS and closed-loop DBS was shown to have similar performances in tremor suppression. Hence, this enhanced DBS therapy solution has the potential to decrease the possible patient’s side effects, such as balance and speech impairment,

and slow down battery depletion, by being inactive during non-tremor statuses, while delivering an equally effective but more efficient treatment.

Disclosures: E. Opri: None. **S. Cernera:** None. **R. Molina:** None. **M.S. Okun:** None. **K.D. Foote:** None. **A. Gunduz:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.08/G39

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Stimulation rebound in deep brain stimulation for essential tremor

Authors: *B. I. FERLEGER[1], S. S. COOPER[2], A. B. BROWN[2], A. L. KO[3], H. J. CHIZECK[1], J. A. HERRON[3];
[1]Electrical and Computer Engin., [2]Neurosci., [3]Dept. of Neurolog. Surgery, Univ. of Washington, Seattle, WA

Abstract: Deep brain stimulation (DBS) is an established treatment for Parkinson's disease (PD), essential tremor (ET), dystonia, and several other movement and behavioral disorders [Baizabal-Carvallo 2014, Rodriguez-Oroz 2005, Krauss 2004]. Adaptive DBS (aDBS) seeks to mitigate battery drain and side effects of DBS by using feedback from biomarkers to modulate stimulation parameters, generally with a binary, all-or-nothing stimulation control system [Herron 2016, Opri 2016, Houston 2019]. A recent study identified and quantified a "rebound effect" in patients receiving DBS treatment for ET [Paschen 2019]. This effect manifests when sudden DBS deactivation results in a temporary increase in the severity of motor symptoms before settling to a steady state. In three trials across two patients receiving DBS for ET, videos of patients completing the drawing task of the Fahn-Tolosa-Marin (FTM) tremor rating scale were taken at 8-10 minute intervals. Inertial measurement unit (IMU) gyroscope data and recordings from one electrode implanted over the hand portion of the motor cortex were logged in one of these trials. One to four ratings were taken before DBS deactivation to account for motor task learning, with four after to evaluate rebound. Videos were blinded and shuffled for review by two neurologists. Rebound effect was present in all trials by clinical ratings and IMU data from the applicable trial. Mean clinical time-to-peak was T_p=10 min (SD=5.7), far longer than the near instantaneous peak reported previously [Paschen 2019]. Mean time-to-settle, defined as rating r_i for which $|r_{i-1}-r_i|$<0.5 points, was T_s=34 min (SD=1.7). Mean difference between peak (r_p) and steady-state (r_s) rating was r_p-r_s=0.83 (SD=0.24). IMU data yielded T_{gp1}=4 min, with 41% difference between peak and steady state 4-8 Hz band-power [Neumann 2017]. This diverges from the clinical assessment of T_{p1}=13 min. Cortical recordings show 12-30 Hz band power 27% lower at T_{gp1} than at T_{s1} during identical motor tasks. This additional beta

desynchronization may result from the increased effort required to complete tasks in the presence of tremor or correlate with tremor severity itself [Kondylis 2016, Weiss 2015]. Rebound effect has potential implications for the mechanism of DBS treatment in ET, indicative of an acute accommodation in response to DBS and a period of post-DBS washout comparable to the ramp-up washout seen in PD [Cooper 2013]. These findings imply inadvertent complications may stem from binary aDBS, supporting the case for nuanced, non-binary aDBS control systems [Little 2013, Priori 2013]. Rebound effect itself may serve as a testbed for future work in tremor estimation from cortical data.

Disclosures: B.I. Ferleger: None. **S.S. Cooper:** None. **A.B. Brown:** None. **A.L. Ko:** None. **H.J. Chizeck:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic. **J.A. Herron:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.09/G40

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: the Ministry of Science and ICT, NRF-2018R1C1B6008884
the Ministry of Science and ICT, NRF-2018M3A9E8066249

Title: Emotional symptoms in CNS demyelinating diseases: Comparison between multiple sclerosis and neuromyelitis optica spectrum disorders

Authors: *L. CHOI[1], G. KIM[1], S.-M. KIM[1,2], S. LEE[1], H.-W. KIM[1], H. KIM[1], S. KIM[1], Y.-M. LIM[1], K.-K. KIM[1], E.-J. LEE[1,2];
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Abstract: Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) are autoimmune demyelinating diseases of the CNS, having distinct immunologic features. The target antigen of autoimmunity in NMOSD is the water channel aquaporin-4 on astrocytes. Meanwhile, primary targets of MS are myelin and oligodendrocytes. As demyelinating diseases progress, emotional symptoms can develop. Here we compared the prevalence and clinical features of anxiety and depressive symptoms between MS and NMOSD. A total of 98 patients (72 with relapsing remitting MS, 26 with NMOSD) were evaluated for emotional symptoms with Hospital anxiety and depression scale (HADS). The HADS consists of two divisions, HADS-anxiety and HADS-depression; each score ranges from 0 to 21 (≥10 suggests significant anxiety or depression). As for baseline characteristics, female proportion was higher in NMOSD patients, while age was comparable. Clinically. although annualized relapse rates were

significantly higher in the NMOSD group (MS vs. NMOSD: 0.3 [0.1−0.5] vs. 0.6 [0.3−1.0], p=0.005), current functional status was similar (Expanded Disability Status Scale [EDSS], 2.5 [1.0−4.0] vs. 3.5 [1.0−3.5], p=0.550). As for emotional symptoms, both HADS-anxiety (6 [3−9] vs. 7.5 [5−9], p=0.403) and HADS-depression (6 [3−8] vs. 7 [5−8], p=0.600) were comparable between the disease groups; the proportion of patients with significant anxiety (38.9% vs. 50.0%, p=0.325) and depression (34.7% vs. 42.3%, p=0.492) were also comparable. As for disease phenotypes in the MS group, the most common first-attack phenotype in patients with significant anxiety was brain lesion (32.1%), while the phenotype in those with significant depression was myelitis (40.0%). In contrast, in NMOSD patients with significant emotional symptoms, the most common first-attack phenotype was optic neuritis (46.2% for anxiety, 45.5% for depression). In both disease groups, a low EDSS level was the only factor significantly associated with anxiety and depression. These findings suggest that anxiety and depression are fairly common in both MS and NMOSD patients, and their presence are associated with poor functional status. In addition, disease phenotypes related with emotional symptoms might be distinct between MS and NMOSD.

Disclosures: **L. Choi:** None. **G. Kim:** None. **S. Kim:** None. **S. Lee:** None. **H. Kim:** None. **H. Kim:** None. **S. Kim:** None. **Y. Lim:** None. **K. Kim:** None. **E. Lee:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.10/G41

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: RES/0165/7628/099

Title: A novel non-human primate model of essential tremor

Authors: *M. APANAVICIUTE[1], L. SAIEVA[1], M. BAKER[1], S. BAKER[2];
[1]Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [2]Inst. of Neurosci., Inst. of Neurosci., Newcastle upon Tyne, United Kingdom

Abstract: Essential tremor (ET) is the most common movement disorder, affecting ~1% of people and characterized by a 4-12Hz postural/kinetic tremor affecting the hands, which progresses over years to involve head, arms, face and voice. Histological examination of autopsy tissue from some ET patients has demonstrated pathological changes in locus coeruleus (LC) and cerebellar cortex (Louis et al., 2007), suggesting a role for noradrenergic LC projections to cerebellum (LC-NA) in the pathogenesis of ET.
Two female rhesus macaques were trained to hold the right hand outstretched and maintain this posture for 3.5 seconds for food reward. The animals were then implanted surgically

(subcutaneous EMG electrodes in shoulder, arm and hand muscles; head piece with EMG output connectors; chamber with microelectrode access to cerebellum/brainstem). LC neurons were first stimulated using an excitatory DREADD (for technical details see Vazey & Aston-Jones, 2014) to increase LC firing rate (confirmed by single unit recordings from LC) and thus increase NA activity in cerebellar cortex. LC-NA terminals in the hand/arm area of cerebellar cortex were then lesioned by injecting 6-hydroxydpamine (6OHDA). The effects on kinetic/postural upper limb tremor were quantified by spectral analysis of EMG.
Upper limb tremor was not observed following LC overactivation. However, 1 day after 6OHDA injections a postural 4Hz tremor indistinguishable from ET emerged in both distal and proximal muscles. Tremor was attenuated significantly ($p<0.05$) by the following medications commonly used to alleviate ET (% reduction): propranolol (~40%); primidone (~40%); and carbamazepine (~30%).
Loss of LC-NA terminals in cerebellar cortex is thus sufficient to produce upper limb tremor resembling ET in non-human primates.

Acknowledgements
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Reference
Louis et al. (2007) *Brain* 130(12):3297-3307
Vazey & Aston-Jones (2014) *Proc Natl Acad Sci U S A.* 111(10):3859-3864

Disclosures: M. Apanaviciute: 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.; Neurexpert. **L. Saieva:** None. **M. Baker:** None. **S. Baker:** 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.; Neurexpert.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.11/G42

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: The efficacy of phase-specific stimulation essential tremor during daily activities

Authors: *C. REIS[1], N. HALIASOS[3], P. BROWN[2], M. WOOLRICH[4], H. CAGNAN[2]; [1]Nuffield Dept. of Clin. Neurosci., [2]Nuffield Dept. of Clin. Neurosciences, Brain Network Dynamics Unit, Oxford, United Kingdom; [3]Barking, Havering and Redbridge Univ. Hosp. NHS

Trust, Romford, United Kingdom; [4]Oxford Ctr. for Human Brain Activity, Oxford, United Kingdom

Abstract: Essential tremor is one of the most common movement disorders. Although its cause remains unknown, several lines of evidence have shown that specific neurons in the ventral thalamus fire synchronously with peripheral tremor. Such synchrony is effectively disrupted with Deep Brain Stimulation (DBS), which delivers a continuous high frequency (130-180Hz) electric current to the target nucleus – e.g. ventral intermediate thalamus (ViM), posterior subthalamic area or zona incerta, achieving symptom suppression of up to 80%. Yet, around 50% of DBS implanted patients report side-effects, suggesting that stimulation may also disrupt physiological neural activity. A novel stimulation strategy is currently being developed with the aim of targeting only the tremor related neural activity termed phase-specific DBS. Hitherto, Phase-specific DBS has been delivered to the ViM, phase locked to patient's tremor as the patient was holding a static posture. This achieved tremor reduction by 85% whilst using less than half of the energy of conventional DBS. However, as tremor severity is variable across daily activities, i.e. during different movement states, it becomes crucial to investigate whether this strategy can provide sustained tremor suppression while the patient is moving. Here we aim to evaluate the feasibility of phasic DBS during movement by testing alternating stimulation strategies (sham, continuous and phasic-specific) and alternating movement states (rest, holding a posture and moving freely). Moreover, using Hidden Markov Modelling, which allows for a thorough description of the evolution of observed states dependent on hidden factors, we will model the variability of tremor amplitude during different stimulation and movement states. From such, we expect to provide a patient-specific description of the internally (movement) and externally (stimulation) generated perturbations that lead to the transition between different tremor severities.
Overall, the importance of this work is twofold: 1) to determine the applicability of phase-specific DBS as a novel clinical approach and 2) to better understand the dynamic relationship between central and peripheral tremor activity.

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Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.12/G43

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NSF Graduate Research Fellowship Program (1747505)
NIH P41 Center for Integrative Biomedical Computing (CIBC) (GM103545)

Tourette Association of America International TS Registry Grant

Title: Structural connectivity predicts clinical outcomes of deep brain stimulation for Tourette syndrome

Authors: *K. A. JOHNSON[1,2], G. DUFFLEY[1,2], D. SERVELLO[5], A. BONA[5], M. PORTA[6], J. L. OSTREM[7], E. BARDINET[8], M.-L. WELTER[8,9], A. M. LOZANO[10], J. BALDERMANN[11], J. KUHN[11], D. HUYS[11], T. FOLTYNIE[12], M. HARIZ[12], E. M. JOYCE[12], L. ZRINZO[12], Z. KEFALOPOULOU[12], F.-G. MENG[13], Z. LING[14], A. Y. J. M. SMEETS[15], L. ACKERMANS[15], V. VISSER-VANDEWALLE[16], A. Y. MOGILNER[17], M. H. POURFAR[17], L. ALMEIDA[18], A. GUNDUZ[19,18], W. HU[18], K. D. FOOTE[20], M. S. OKUN[18], C. R. BUTSON[3,1,4];
[1]Dept. of Biomed. Engin., [2]Scientific Computing and Imaging Inst., [3]Scientific Computing & Imaging Inst., [4]Departments of Neurology, Neurosurgery, and Psychiatry, Univ. of Utah, Salt Lake City, UT; [5]Neurosurgical Dept., IRCCS Inst. Ortopedico Galeazzi, Milan, Italy; [6]IRCCS Inst. Ortopedico Galeazzi, Tourette's Syndrome and Movement Disorders Center, Italy; [7]Dept. of Neurol., Univ. of California San Francisco, San Francisco, CA; [8]Inst. du Cerveau et de la Moelle Epiniere, Paris, France; [9]Sorbonne Universités, Univ. of Pierre and Marie Curie Univ. of Paris, The French Natl. Inst. of Hlth. and Med. Res. U 1127, The Natl. Ctr. for Scientific Res. 7225, Paris, France; [10]Dept Neurosurg., Toronto Western Hosp. Rm 4-431 West, Toronto, ON, Canada; [11]Dept. of Psychiatry and Psychotherapy, Univ. of Cologne, Cologne, Germany; [12]Unit of Functional Neurosurgery, Sobell Dept. of Motor Neurosci., Univ. Col. London Inst. of Neurol., London, United Kingdom; [13]Beijing Neurosurgical Inst., Capital Med. Univ., Beijing, China; [14]Dept. of Neurosurg., PLA Gen. Hosp., Beijing, China; [15]Dept. of Neurosurg., Maastricht Univ. Med. Ctr., Maastricht, Netherlands; [16]Dept. of Stereotaxy and Functional Neurosurg., Univ. Hosp. Cologne, Cologne, Germany; [17]Ctr. for Neuromodulation, Departments of Neurol. and Neurosurg., New York Univ. Langone Med. Center,, New York City, NY; [18]Fixel Inst. for Neurolog. Diseases, Dept. of Neurol., [19]J Crayton Pruitt Family Dept. of Biomed. Engin., [20]Fixel Inst. for Neurolog. Diseases, Dept. of Neurol. and Neurosurg., Univ. of Florida, Gainesville, FL

Abstract: Deep brain stimulation (DBS) can be an effective therapy for select cases of Tourette syndrome (TS), but clinical outcomes remain variable across patients. Our recent study showed that the anatomical location of stimulation does not predict outcomes, but it is unknown if there are specific pathways that are associated with improvement in TS symptoms. Our objective was to identify the pathways that predict clinical outcomes of DBS for TS in a large multisite cohort. Retrospective imaging and Yale Global Tic Severity Scale (YGTSS) scores of 34 TS patients receiving bilateral globus pallidus internus (GPi) DBS were collected in a collaboration with the International TS DBS Database and Registry and the International Neuromodulation Registry. Volumes of tissue activated (VTA) were generated using stimulation parameters for each patient. The VTA were used to generate probabilistic tractography in diffusion-weighted imaging of 40 Human Connectome Project subjects. A map of average connectivity weighted by percent improvement in YGTSS scores was computed across patients to identify the pathways associated with the highest improvement in TS symptoms. The pathways were used to perform a leave-one-out regression analysis of connectivity versus percent improvement in YGTSS scores. The

pathway associated with the greatest improvement in TS symptoms projected through thalamus, anterior cingulate cortex (ACC), and regions in prefrontal cortex (PFC). Average connectivity along this pathway was significantly correlated with percent improvement in YGTSS scores ($R = 0.46$, $p = 0.007$), and the correlation remained significant across leave-one-out iterations ($p < 0.01$). Average connectivity along the thalamus-ACC-PFC pathway reliably predicted response across TS patients receiving GPi DBS. Interestingly, regions within thalamus have been targeted with DBS for TS, and there may be a common pathway that should be modulated to improve TS symptoms. Future work will compare connectivity maps across GPi and CM thalamus to converge on networks to target with DBS to improve outcomes of DBS for TS.

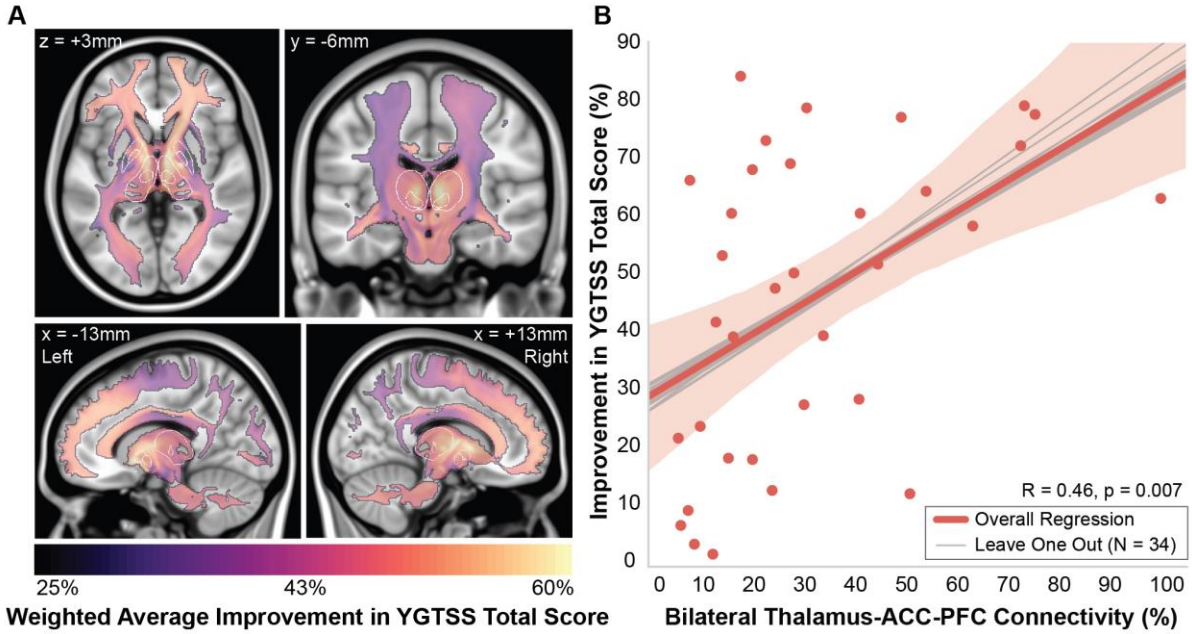

Figure 1. (A) The pathway associated with the the highest percent improvement (≥ 53%) included thalamus, anterior cingulate cortex (ACC), and regions in prefrontal cortex (PFC). **(B)** Average connectivity along the thalamus-ACC-PFC pathway was correlated with percent improvement in YGTSS total score. The correlation remained significant in a leave-one-out analysis, and the regression lines were relatively stable (as shown with the gray lines).

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even if those funds come to an institution.; Medtronic GmbH, German Research Foundation (KU2665/1-2) and the Marga and Walter Boll Foundation. **D. Huys:** None. **T. Foltynie:** None. **M. Hariz:** None. **E.M. Joyce:** None. **L. Zrinzo:** None. **Z. Kefalopoulou:** None. **F. Meng:** None. **Z. Ling:** None. **A.Y.J.M. Smeets:** None. **L. Ackermans:** None. **V. Visser-Vandewalle:** None. **A.Y. Mogilner:** None. **M.H. Pourfar:** None. **L. Almeida:** None. **A. Gunduz:** None. **W. Hu:** None. **K.D. Foote:** None. **M.S. Okun:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH, NPF, Michael J. Fox Foundation, Parkinson Alliance, Smallwood Foundation, Bachmann-Strauss Foundation, Tourette Syndrome Association, UF Foundation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Demos, Manson, Amazon, Smashwords, Books4Patients, Cambridge. F. Consulting Fees (e.g., advisory boards); National Parkinson Foundation. Other; Associate editor for New England Journal of Medicine Journal Watch Neurology, CME and educational activities on movement disorders (in the last 36) months sponsored by PeerView, Prime, QuantiaMD, WebMD, Medicus, MedNet, Henry Stewart, and by Vanderbilt University. **C.R. Butson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IntelectMedical. F. Consulting Fees (e.g., advisory boards); NeuroPace, IntelectMedical, Advanced Bionics, Abbott, Boston Scientific, Functional Neuromodulation.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.13/G44

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Regulation of orchestrated glial cell activation and neurodegeneration by anti-semaphorin 4D antibody pepinemab (VX15/2503) as a potential therapeutic strategy for Huntington’s and Alzheimer’s disease

Authors: *E. EVANS[1], T. FISHER[1], J. LEONARD[1], A. READER[1], V. MISHRA[1], C. MALLOW[1], A. HOWELL[1], E. SMITH[1], A. FEIGIN[2], M. ZAUDERER[1];
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Abstract: SEMA4D signaling through its receptors (PLXNB1, PLXNB2) triggers activation of inflammatory glial cells, inhibits migration and differentiation of glial progenitor cells, and disrupts endothelial tight junctions that are required for the integrity of the BBB. Blockade of SEMA4D in neurogenerative and neuroinflammatory disease is investigated in preclinical mechanistic studies and the first clinical trial of anti-SEMA4D antibody, pepinemab

(VX15/2503), to evaluate therapeutic potential in a randomized, double-blind, placebo-controlled phase 2 study of patients with late prodromal and early manifest Huntington's disease (HD). Mechanistic studies include histopathological observations of marked differences in expression, distribution, and colocalization of SEMA4D with neuronal and glial markers in transgenic Q175 HD mice compared to wild type controls. Preclinical studies suggest that SEMA4D plays an important role in inflammatory activation of astrocytes, in which state they downregulate glucose transporter, reducing their normal function in brain energy metabolism. Several clinical studies have demonstrated that loss of FDG-PET signal, a measure of glucose uptake, correlates with cognitive decline in Alzheimer's disease (AD) patients. Antibody neutralization of SEMA4D ameliorates neurodegenerative processes in preclinical transgenic YAC128 HD and CVN AD mouse models. SIGNAL is the first trial of anti-SEMA4D antibody pepinemab administered to HD subjects evaluating safety, tolerability and efficacy. Study endpoints include clinical features of HD and FDG-PET and volumetric MRI imaging in prospectively defined brain regions of interest (ROI), along with cognitive, motor, and patient-reported outcomes. In SIGNAL Cohort A (n=36), no concerning safety signals were identified following up to 12 monthly IV administrations; blinded safety data from cohort B suggest a similar safety profile. Pepinemab treatment trended toward stabilization of disease-related reduction in MRI volume and was favored over placebo in 24/31 ROI. FDG-PET also favored pepinemab in all ROI, with median increase in FDG uptake from baseline of 8.6% (range: 0.5-20.4%) compared to placebo achieving significance ($p<0.05$) in a majority of frontal and parietal brain ROI (11/14). Analysis of cohort A guided the design of Cohort B, enrolling 265 HD subjects for 17 to 35 months of treatment. Enrollment in cohort B was completed Dec 31, 2018 and clinical evaluation will continue through June 2020. These findings have shown pepinemab to be well tolerated in subjects with HD and warrants further investigation in neurodegenerative diseases including HD and AD.

Disclosures: **E. Evans:** A. Employment/Salary (full or part-time):; Vaccinex. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vaccinex. **T. Fisher:** A. Employment/Salary (full or part-time):; Vaccinex. **J. Leonard:** A. Employment/Salary (full or part-time):; Vaccinex. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vaccinex. **A. Reader:** A. Employment/Salary (full or part-time):; Vaccinex. **V. Mishra:** A. Employment/Salary (full or part-time):; Vaccinex. **C. Mallow:** A. Employment/Salary (full or part-time):; Vaccinex. **A. Howell:** A. Employment/Salary (full or part-time):; Vaccinex. **E. Smith:** A. Employment/Salary (full or part-time):; Vaccinex. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vaccinex. **A. Feigin:** 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.; Vaccinex, Teva. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Voyager Therapeutics. F. Consulting Fees (e.g., advisory boards); Oxford Medica. **M. Zauderer:** A. Employment/Salary (full or part-time):; Vaccinex. E. Ownership Interest (stock, stock options,

royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); vaccinex.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.14/H1

Topic: C.04. Movement Disorders other than Parkinson"s Disease

Support: NIH 1R21DC011841
NIH 1 R01 DC016315-01A1

Title: Abnormal electrocortical responses during vocalization as a neural correlate of the dystonic voice symptoms in spasmodic dysphonia

Authors: *J. KONCZAK[1], S. KHOSRAVANI[1], A. MAHNAN[1], Y. ZHANG[2], P. J. WATSON[2];
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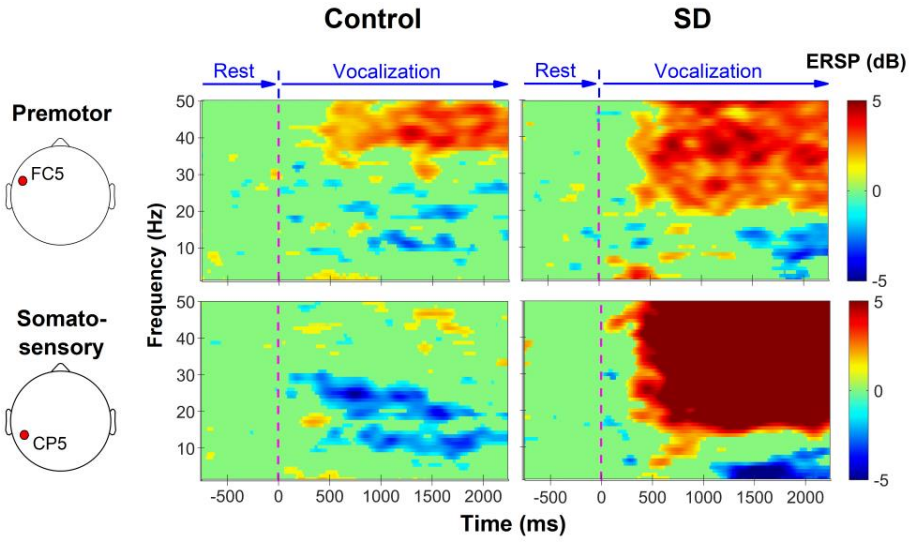
Abstract: Spasmodic dysphonia (SD) is a voice disorder that results in choked or strained speech. It is considered to be a form of focal dystonia. Onset occurs spontaneously during mid life and becomes chronic thereafter. There is no effective cure for SD. The neural features of abnormal cortical processing associated with SD are largely unknown. To obtain a better understanding of the underlying cortical neural mechanism of the disease, we analyzed electroencephalographic (EEG) signals of 10 SD individuals and 10 healthy age and gender matched volunteers. The participants produced 50 vowel /a/ vocalization epochs for duration of 2500ms. During early vocalization (500-1000ms) the SD group showed significantly larger alpha band spectral power over the left motor cortex. During late vocalization (1000-2500ms) SD patients showed a significantly larger gamma band coherence between left somatosensory and premotor cortical areas (see Fig. 1). The results indicate two atypical patterns of cortical activity in people with spasmodic dysphonia during voice production: 1) a reduced movement-related desynchronization of motor cortical networks, 2) an excessively large synchronization between left somatosensory and premotor cortical areas. These findings document that the pathophysiology of SD is characterized by an abnormally high synchronous activity within and across cortical neural networks involved in voice production that is mainly lateralized in the left hemisphere. Fig. 1 Spectrograms of somatosensory (CP5) and premotor (FC5) cortical electrodes in one healthy and one SD participant during vocalization. Green indicates no significant change in relation to rest. Blue color depicts the suppression of oscillatory activities in relation to the baseline, and the red color shows the excitation of oscillations in relation to the baseline level. Vocalization in the healthy participant is associated with the suppression of lower frequency

oscillations and gamma band excitation. The SD patient shows a smaller degree of suppression in the majority of frequency bands.



Disclosures: **J. Konczak:** None. **S. Khosravani:** None. **A. Mahnan:** None. **Y. Zhang:** None. **P.J. Watson:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.15/H2

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH 1 R01 DC016315-01A1

Title: Effects of long term laryngeal vibrotactile stimulation in people with spasmodic dysphonia

Authors: ***N. ELANGOVAN**[1], A. MAHNAN[2], G. S. GODING, Jr[1], Y. ZHANG[4], P. WATSON[1], J. KONCZAK[3];
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Abstract: Spasmodic dysphonia (SD) is a primary focal dystonia characterized by involuntary laryngeal muscle spasms that cause disfluent speech with a strained-strangled voice quality. Lack of a permanent cure, unresponsiveness to traditional speech therapies, and only temporary relief through botulinum toxin injections have led researchers to evaluate alternate approaches to symptom relief. Specifically the finding of proprioceptive dysfunction in SD has allowed researchers to exploit the somatosensory afferent signals by manipulating mechanoreceptor responses in the laryngeal muscles and the skin above the thyroid cartilage. Recent work from our group applied a single session laryngeal vibro-tactile stimulation (VTS) to people with SD. Results showed both empirical and self-reported improvements in speech quality as well as changes in somatosensory-motor cortical activity. However, the optimal dosage and the long-term effects of laryngeal VTS in SD are unclear. In this study, we evaluate the effects of laryngeal VTS over a period of 3 months. People with SD were randomly assigned in 2 groups (high vs low frequency) x 2 dosage (high vs low dosage). Participants in the high frequency group received a 100 Hz VTS whereas people in the low frequency group received a 40 Hz VTS. Participants in the two groups were divided in two sub-groups: high dosage group received 3 sessions x 20 minutes VTS/week and the low dosage group received 1 session x 20 minutes VTS/week. Participants' voice quality and the cortical activity during vocalization of vowels were tested at baseline, at 6 weeks and at 12 weeks. We provide preliminary results on the long term effects of VTS on people with SD. These results provide first insights on the long-term efficacy of VTS as a non-invasive form of neuromodulation, determining effective VTS frequencies and the optimal treatment dosage to enhance the voice quality in people with SD.

Disclosures: **N. Elangovan:** None. **A. Mahnan:** None. **G.S. Goding:** None. **Y. Zhang:** None. **P. Watson:** None. **J. Konczak:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.16/H3

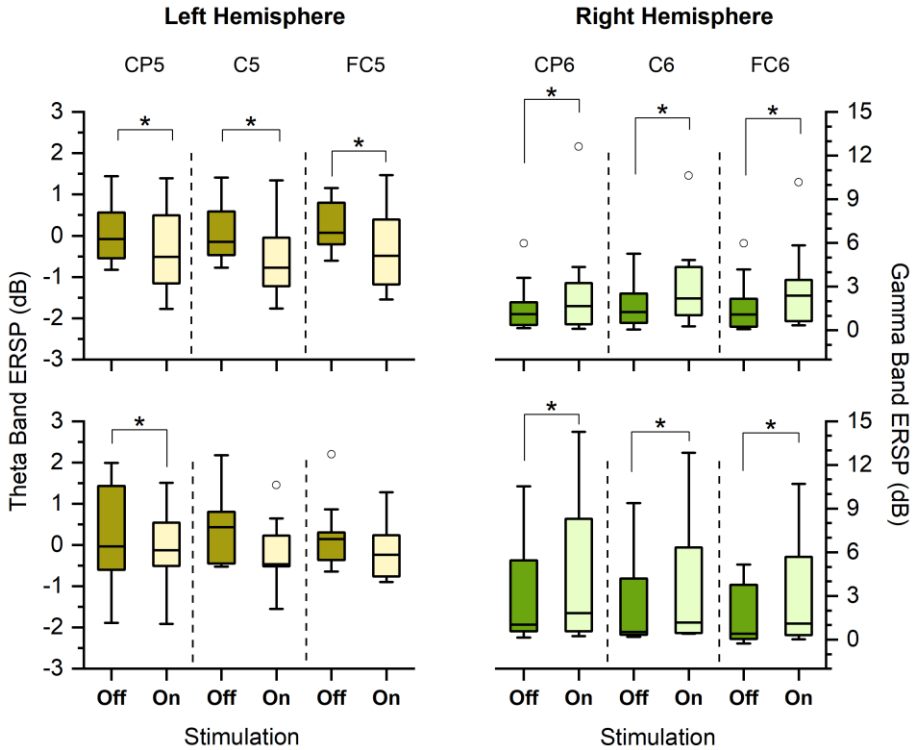
Topic: C.04. Movement Disorders other than Parkinson"s Disease

Support: NIH 1R21DC011841
NIH 1 R01 DC016315-01A1

Title: First evidence that laryngeal vibration can improve voice symptoms in the voice disorder spasmodic dysphonia

Authors: *A. MAHNAN[1], S. KHOSRAVANI[3], P. WATSON[2], Y. ZHANG[2], J. KONCZAK[1]; [1]Human Sensorimotor Control Lab., [2]Speech, Language, Hearing Sci., Univ. of Minnesota, Minneapolis, MN; [3]Harvard Univ., Boston, MA

Abstract: Spasmodic dysphonia (SD) is a focal dystonia affecting the larynx. It leads to a chocked or strained speech. SD is not responsive to traditional speech therapies. There is no cure for SD. Injection of Botulinum toxin to laryngeal muscles brings temporary voice symptom relief to some patients, but is not well tolerated by all. Proprioceptive deficits are an underlying feature of SD - a finding that opens an avenue for a missing behavioral treatment for the disease. Specifically, vibro-tactile stimulation (VTS) as non-invasive form of neuromodulation could be the suitable tool, given that it alters afferent signals from the mechanoreceptors in the vibrated muscles and skin. METHOD: Here, we examined the effect of laryngeal VTS on speech quality and cortical activity in 13 SD participants. The task involved the repeated vocalization of the vowel /a/ while receiving VTS for a total duration of 34 minutes. Cortical activity was monitored concurrently using EEG. RESULTS: In response to VTS, 8 participants (62%) exhibited a reduction of voice breaks and/or a meaningful increase in *smoothed cepstral peak prominence*, an acoustic measure of voice/speech quality. Symptom improvements persisted for 20 minutes past VTS. The application of VTS induced a significant suppression of theta band power over the left somatosensory-motor cortex and a significant rise of gamma rhythm over right somatosensory-motor cortex indicating that VTS reduces. CONCLUSION: Our results document that a one-time application of laryngeal VTS can effectively reduce the voice symptoms of SD. The suppression of theta band oscillations has been observed in patients with cervical dystonia who apply effective sensory tricks, suggesting that VTS in SD may activate a similar neurophysiological mechanism. Our results promote the development of wearable, non-invasive, voice-activated, user-programmable medical devices that could apply VTS on laryngeal muscles while monitoring its effect on speech production in real-time.



Disclosures: **A. Mahnan:** None. **S. Khosravani:** None. **P. Watson:** None. **Y. Zhang:** None. **J. Konczak:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.17/H4

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: R01 NS058487
R01 NS075012

Title: Cortical oscillations in cervical dystonia and dystonic tremor

Authors: C. W. HESS[1], ***B. GATTO**[2], J. W. CHUNG[5], R. L. HO[3], W. E. WANG[3], A. W. SHUKLA[4], D. E. VAILLANCOURT[3];
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Abstract: Dystonia is a movement disorder in which sustained or repetitive muscle contractions cause abnormal postures and/or movements. There are a variety of forms of dystonia that can differ by the region of the body affected, provoking triggers, and additional features such as tremor. In this study, we recorded 128 channel high-density electroencephalography (HD-EEG) and kinematic data during the performance of visually-guided motor control tasks. Normal control subjects and two separate groups of patients with cervical dystonia (-/+ dystonic head tremor) were studied to determine how task-related cortical oscillatory activity might differ between patients and controls. We also investigated whether any differences in cortical oscillations were 1) specific to motor tasks involving muscles that were clinically affected by dystonia or 2) influenced by associated features such as dystonic head tremor. In the first cohort we studied the cortical dynamics of horizontal head turning movements and in the second cohort we studied grip force production. We found that patients with cervical dystonia had increased beta-band power over the motor cortices in event-related spectral perturbations (ERSP) compared to controls during both head turning tasks and grip force tasks, and that this increase was consistent across both dystonia groups. These results suggest that motor control in patients with cervical dystonia is fundamentally abnormal, regardless of whether the muscles involved in the task are clinically affected by dystonia or whether dystonic tremor is present as an associated phenomenological feature.

Disclosures: C.W. Hess: None. **B. Gatto:** None. **J.W. Chung:** None. **R.L. Ho:** None. **W.E. Wang:** None. **A.W. Shukla:** None. **D.E. Vaillancourt:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.18/H5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RF1AG059867
NIH Grant R01AG048108
NIH Grant P01AG009975
NIH Grant R01AG017917
NIH Grant R01NS078009

Title: Neurofibrillary tangles accelerate the degradation of fractal motor regulation

Authors: P. LI[1,2], L. YU[3], A. S. P. LIM[4], A. S. BUCHMAN[3], J. A. SCHNEIDER[3], D. A. BENNETT[3], ***K. HU**[1,2];
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Abstract: Motor activity in health young humans possesses fractal temporal structures as characterized by similar fluctuation patterns across multiple time scales from seconds to hours. Advanced age and Alzheimer's disease (AD) perturb such fractal regulation (FR) and lead to increased randomness in the fluctuations especially at larger time scales (i.e., >2h), which is believed to reflect circadian disruptions according to animal studies. Here, we examined how AD pathology affects longitudinal FR changes. We studied 515 older adults (age: 85.0±5.9[SD]) in the Rush Memory and Aging Project who already came to autopsy and had post-mortem examinations of brain pathologies. All subjects had at least two antemortem motor activity assessments that were administered on a yearly basis. The average follow-up duration since the first motor activity assessment (baseline) was 4.8±2.8[SD] years. The temporal correlations in activity fluctuations at time scales from 2 up to 10h were examined to assess FR. Post mortem β-amyloid and phosphorylated tau were considered as pathological AD biomarkers. In a linear mixed effect model adjusted for age, sex, and education, increased burden of phosphorylated tau tangles was associated with accelerated FR degradation. Specifically, for each 1-unit increase in the burden of tangles, the annual rate of FR degradation (i.e., increased randomness in motor activity fluctuations at >2h) was increased by 4% (p=0.015). Interestingly, burden of tangles was associated with the degree of randomness in the activity fluctuations proximate to death in females (p=0.01) but not in males. In contrast, β-amyloid was not associated with either longitudinal FR changes or the FR proximate to death. These results indicate a link between tau pathology and fractal degradation.

Disclosures: **P. Li:** None. **L. Yu:** None. **A.S.P. Lim:** None. **A.S. Buchman:** None. **J.A. Schneider:** None. **D.A. Bennett:** None. **K. Hu:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.19/H6

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Loss of iron metabolism regulation causes motor and behavioral defects

Authors: *C. A. M. PORRAS[1,2], M. C. GHOSH[1], H. WILSON-OLLIVIERRE[1], T. A. ROUAULT[1];
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Abstract: Iron is an important cofactor for many proteins. It is used to create Fe-S clusters and heme prosthetic groups that enzymes use to catalyze redox reactions. This redox ability however can also result in the production of harmful radicals by Fenton chemistry causing oxidative stress during iron overload conditions. Therefore, iron homeostasis is critical for a cell to maintain. Proteins involved in the import, export, and sequestering/storage of iron are regulated by Iron Regulatory Proteins (IRP1/2). These two proteins become activated when the iron concentration in a cell falls too low. They bind to Iron Response Elements (IRE) present on mRNA transcripts of iron regulatory proteins. If the IRE is on the 5' end of the transcript, IRP1/2 prevent translation. Conversely if the IRE is on the 3' end, the transcript is stabilized by IRP1/2 and translation is promoted. This allows IRP1/2 to upregulate iron import proteins while simultaneously downregulating iron export and sequestering proteins during iron depletion. IRP1 and IRP2 are very similar proteins that are equally able to bind to IREs, but their expression profiles and regulation differ. IRP2 is the dominant IRP in neurons. Because of the high ATP demand necessary to maintain polarized membranes, neurons are particularly sensitive to changes in iron concentration which is used to create Fe-S clusters in the mitochondrial respiratory chain. We predict that loss of IRP2 causes a functional iron deficiency due to a decrease in iron import and an increase in iron sequestering. This results in mitochondrial dysfunction and eventually neurodegeneration. Recently, a patient with bi-allelic loss of function mutations in IREB2 leading to an absence of IRP2 protein has been identified. This patient has been diagnosed with dystonic cerebral palsy, epilepsy, and microcytic hypochromic anemia. The patient is also non-verbal and failed to achieve developmental milestones which could indicate a cognitive impairment. To investigate this further an $Irp2^{-/-}$ mouse model on a backcrossed C57Bl/6J background was created. The Irp2-null mice have a significant motor defect demonstrated by reduced performance on rotarod and hanging wire tests (approximately 45%

and 22% of wildtype respectively). Counterintuitively Irp2-null mice have more ambulatory movement in an open field test of spontaneous movement. The difference is greatest during the first 10 minutes of a 60-minute trial suggesting that the mice have delayed habituation to a novel environment. These results suggest that loss of Irp2 in mice causes motor and behavioral defects matching the IRP2 patient's neurodegenerative disorder.

Disclosures: C.A.M. Porras: None. **T.A. Rouault:** None. **M.C. Ghosh:** None. **H. Wilson-Ollivierre:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.20/H7

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01NS075012

Title: Loss of D2R-expressing torsinA impairs sensory-evoked brain activation and connectivity in a mouse model of dystonia

Authors: J. C. DESIMONE[1], ***H. LIU**[2], Y. LIU[3], Y. LI[3], D. E. VAILLANCOURT[4]; [1]Dept. of Applied Physiol. and Kinesiology, [2]Dept. of Biomed. Engin., [3]Dept. of Neurol., [4]Applied Physiol. and Kinesiology; Dept. of Neurology; Dept. of Biomed. Engin., Univ. of Florida, Gainesville, FL

Abstract: Early-onset generalized dystonia (DYT1 dystonia) is caused by a loss-of-function mutation in torsinA, leading to involuntary muscle co-contractions, and abnormal movements and postures. Dopamine type-2 receptor (D2R)-expressing medium spiny neurons and cholinergic interneurons of the striatum are centrally involved in the convergence of sensory input and mediation of motor output. Dysfunction of these neurons has been extensively implicated in dystonia pathogenesis. Providing *in vivo* markers of network-level brain function related to loss of D2R-expressing torsinA is fundamental to our understanding of dystonia pathophysiology and developing new therapies. The current study used *in vivo* functional and diffusion magnetic resonance imaging to examine brain function and microstructure in a mouse model of dystonia characterized by conditional knock-out of torsinA from striatum-specific D2R-expressing cells (Dyt1 d2KO mice). Until now, neuroimaging studies in dystonia mouse models have used resting-state paradigms to understand network-level brain dysfunction that may characterize the expression of dystonia. Sensorimotor deficits in dystonia highlight a critical need for brain imaging that can gain insight into the dysfunction of task-related sensorimotor pathways. To this end, a novel aspect of the current study is the use of sensory-evoked fMRI to understand how loss of D2R-expressing torsinA in striatal neurons influences blood-oxygen-

level dependent (BOLD) activation and functional connectivity within sensorimotor brain regions. Here, d2KO mice revealed widespread reductions in brain activation of the cortex, striatum, globus pallidus, and thalamus compared to controls, and BOLD signal in d2KO mice correlated with motor deficits. D2KO mice also revealed anti-correlated striatal activation during the sensory task. Resting-state fMRI in combination with striatal seed-based correlation revealed abnormalities in cortical and brainstem connectivity. Neurite orientation dispersion and density imaging (NODDI), a technique sensitive to microstructural properties of tissue and free-water compartments in the brain, revealed no volumetric or diffusivity differences between d2KO mice and controls. This study provides fresh evidence that loss of torsinA in striatal D2R-expressing neurons impairs the integration of exogenous sensory information, which may contribute to motor deficits in dystonia. Furthermore, this study establishes the utility of sensory-evoked fMRI to explore substrates of sensorimotor adaptations underlying dystonia pathophysiology in human and animal studies.

Disclosures: J.C. Desimone: None. **H. Liu:** None. **Y. Liu:** None. **Y. Li:** None. **D.E. Vaillancourt:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.21/H8

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Assessment of the effects of Kinesiotaping on musical motor performance in musicians suffering from focal hand dystonia. A pilot study

Authors: R. BRAVI[1], C. I. IOANNOU[2], S. CAPPELLI[1], A. ANDORLINI[1], E. J. COHEN[1], E. O. ALTENMULLER[2], ***D. MINCIACCHI**[1];
[1]Univ. of Florence, Florence, Italy; [2]Hanover Univ. of Music, Drama and Media, Hanover, Germany

Abstract: Purpose: Intensive training regimes can lead musicians to the manifestation of focal dystonia, a highly disabling movement disorder that often terminates their careers. In most cases, musician’s dystonia, only appears focally in the context of instrument playing as a painless muscular incoordination of refined movements. To date, this condition is not easily treated and the most effective therapies often fail to restore fine motor control in musicians. Given the limitations of the available treatment methods, alternative strategies are continuously explored. This pilot study explores the immediate and short-term effects of a Correction Kinesiotaping intervention (CKT) on fine motor control in musicians with focal hand dystonia (FHD). Kinesiotaping (KT) is a kinesthetic method, consisting of a tape having elastic properties and stretching capabilities, that recently has emerged in clinical practice as an interesting tool for

managing neurological disorders.
Methods: Seven male musicians with FHD performed musical exercises under the following conditions: without KT (baseline); during a CKT intervention and immediately after tape removal (block 1); during a Sham KT (SKT) intervention and immediately after tape removal (block 2). Blocks were randomly presented across participants. A tailored CKT intervention on affected fingers was provided based on the dystonic pattern that each patient manifested while playing. Motor performance was video-documented and four independent experts assessed blindly the general performance and fingers' posture on visual analogue scales. Also, musicians' self-reports of the musical abilities were evaluated. Finally, electromyographic activity and co-activation index of wrist antagonist muscles were analyzed.
Results: No significant differences of effects between CKT and SKT were reported by the experts, either for general performance or for fingers' posture; any subtle benefits observed during CKT were lost after the tape was removed. Musicians estimated that CKT was ineffective in improving their musical abilities. Also, no significant changes with respect to the co-activation index were found among the conditions.
Conclusions: CKT may not be useful to reduce dystonic patterns, nor to improve playing ability, in musicians with FHD.

Disclosures: R. Bravi: None. **C.I. Ioannou:** None. **S. Cappelli:** None. **A. Andorlini:** None. **E.J. Cohen:** None. **E.O. Altenmuller:** None. **D. Minciacchi:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.22/H9

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant NS109227
NIH Grant DK118480

Title: Overexpression of a torsina paralog rescues motor symptoms and neuropathology in a mouse model of DYT1 dystonia

Authors: *J. LI, S. PAPPAS, W. DAUER;
Univ. of Michigan, Ann Arbor, MI

Abstract: DYT1 dystonia is a dominantly inherited movement disorder caused by a loss-of-function mutation in the *TOR1A* gene that encodes the torsinA protein. ~30% of mutation carriers develop disease, and those who do exhibit mild to severely debilitating symptoms. A barrier to genetic therapy in DYT1 dystonia is that treatments based on increasing torsinA expression will simultaneously increase expression of the mutant protein, which may have

deleterious effects. TorsinB is a paralog of torsinA that that modulates the time course and tissue specificity of abnormal nuclear envelope budding, a pathologic hallmark of torsinA loss-of-function (Kim et al., 2010, Tanabe et al., 2016). Consequently, we hypothesized that torsinB overexpression would suppress torsinA loss-of-function-related motor and neuropathological phenotypes. We tested this hypothesis by generating a mouse line that expresses torsinB from the ROSA26 locus in a Cre-dependent manner. We then ingressed this allele into the Dlx-CKO model (Pappas et al., 2015) that exhibits developmental onset of abnormal twisting movements responsive to clinically used antimuscarinic drugs. These mice also exhibit selective degeneration of striatal cholinergic interneurons (ChIs). We find that torsinB overexpression essentially eliminates the abnormal twisting movements and ChI degeneration that are characteristic of the Dlx-CKO model. Critically, control mice that overexpress torsinB have no discernable behavioral or neuropathological phenotype, suggesting that overexpression of torsinB in the Dlx5/6-Cre field is not overtly deleterious. These findings indicate that torsinB is a potent modifier of torsinA loss-of-function-related behavioral and neuropathological phenotypes, identifying it as a potentially promising therapeutic target in DYT1 dystonia.

Disclosures: **J. Li:** None. **S. Pappas:** None. **W. Dauer:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.23/H10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RF1AG059867
NIH Grant R01AG048108
NIH Grant P01AG009975
NIH Grant R01AG017917
NIH Grant R01NS078009

Title: Increased randomness in motor activity predicts cognitive impairment in older adults

Authors: *P. LI[1,2], L. YU[3], A. S. P. LIM[4], A. S. BUCHMAN[3], J. A. SCHNEIDER[3], D. A. BENNETT[3], K. HU[1,2];
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Abstract: Human motor activity fluctuations are not random but possess strong temporal correlations across multiple time scales from seconds to hours. Such correlations are reduced with aging, and the reduction predicts faster cognitive decline and higher risk of Alzheimer’s

dementia independent of age. Here we examined the link between the reduction in motor activity correlations and brain pathologies, and their contributions to cognitive impairment/decline. We studied 580 older adults (age at death: 90.4±6.2 [SD]) who had post-mortem examinations of brain pathologies (including β-amyloid, tangles, Lewy body, gross chronic infarcts, chronic microinfarcts, cerebral atherosclerosis, cerebral amyloid angiopathy, arteriolosclerosis, hippocampal sclerosis, and TAR DNA-binding protein 43). For all subjects, antemortem motor activity recordings of up to 10 days and global cognition score based on 21 neuropsychological test batteries were collected annually for 4.8±2.8 [SD] years. The temporal correlations in activity fluctuations at time scales ~0.1-1.5h were examined and were quantified by an exponent α (i.e., α=0.5 indicates total randomness, and large values indicate stronger correlations). For the last assessed motor activity (2.2 years before death on average), α was closer to 0.5 in subjects with higher burden of arteriolosclerosis (p=0.0004), and female subjects with gross chronic infarcts had smaller α as compared to those females without infarcts (p=0.002). No significant associations were observed for the other 8 pathological measures. Total 37% variations in global cognition proximate to death were explained by the 10 measures of brain pathologies, age, sex, and education. The α close to death accounted for an extra 10% of the variations (p<0.0001). In addition, α at baseline (averagely 5.2 years before death) predicted faster cognitive decline (p=0.037) independent of the 10 measures of brain pathologies, age, sex, and education. These results indicate that increased randomness in motor activity fluctuations at <1.5h reflects/predicts cognitive impairment, and the effects cannot be fully explained by the known biomarkers of brain pathologies. Reduction in motor activity correlations may contribute to the etiology of dementia.

Disclosures: P. Li: None. **L. Yu:** None. **A.S.P. Lim:** None. **A.S. Buchman:** None. **J.A. Schneider:** None. **D.A. Bennett:** None. **K. Hu:** None.

Poster

385. Movement Disorders: Clinical and Preclinical Studies

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 385.24/H11

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: National Natural Science of Foundation of China 31490591

Title: The mechanism of motor dysfunction in Prrt2-deficient mouse

Authors: *S. LOU, Z. XIONG;
Inst. of Neuroscience, Chinese Acad. of Scie, Shanghai, China

Abstract: Paroxysmal kinesigenic dyskinesia (PKD), a brain disorder characterized by sudden attacks of involuntary movements, was caused by loss-of-function mutations in *PRRT2* gene. In

our previous study, genetically engineered animal models of PKD has been generated and they exhibited stimulus-triggered dystonia. However, how does the lost of PRRT2 contribute to motor dysfunction is still not fully understood. In this study, we found dystonia was closely accompanied with hyperexcitability in cerebellum of *Prrt2*-mutant mice. We found that optical stimulation induced more extended excitability in cerebellum of *Prrt2*-mutant mice compared to which in wild type mice. The carbamazepine, an effective medicine for preventing PKD attack in clinical, reduced cerebellar excitability and alleviated dystonia attack in animal model of PKD. Together, our findings provided persuasive evidence for the hypothesis that cerebellar hyperexcitability might be an underlying neuropathological mechanism in *Prrt2*-associated Paroxysmal kinesigenic dyskinesia.
Key words: Paroxysmal kinesigenic dyskinesia; *Prrt2*; cerebellum

Disclosures: S. Lou: None. **Z. Xiong:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 386.01/H12

Topic: C.06. Neuromuscular Diseases

Support: NIH-R01NS085161-01
Les Turner ALS Foundation
Wenske Foundation
NUCATS
NIH grant CA60553

Title: UCHL1 is necessary and sufficient for maintaining cytoarchitectural integrity of upper motor neurons

Authors: *B. GENC[1], J. H. JARA[1], S. S. SANCHEZ[1], A. K. B. LAGRIMAS[1], C. G. BROOKS[1], N. KOCAK[1], Y. ZHU[2], P. OZDINLER[1];
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Abstract: UCHL1 (ubiquitin C-terminal hydrolase-L1) is crucial for the maintenance of free ubiquitin in cells, especially in neurons. In the absence of UCHL1 function, corticospinal motor neurons (CSMN) show selective, progressive and most profound degeneration. Their cellular defects become evident very early in the *Uchl1*^{nm3419} (UCHL1$^{-/-}$) mice, which lack all UCHL1 function, as assessed by shrinkage of soma size, apical dendrite disintegration and massive spine loss. CSMN are the upper motor neurons in mice and this neuron population is central for numerous motor neuron disorders and neurodegenerative diseases, such as amyotrophic lateral

sclerosis (ALS), primary lateral sclerosis (PLS), and hereditary spastic paraplegia (HSP) in patients. Thus, it is important to determine whether UCHL1 function would be sufficient to improve upper motor neuron health and integrity, and whether upper motor neurons would be appropriate cellular targets for gene therapy approaches. We generated two lines of UCHL1 conditional knockout mice: one lacked UCHL1 only in layer 5 subcerebral projection neurons (SCPN) and the other lacked UCHL1 in spinal motor neurons (SMN). Knocking out UCHL1 only from layer 5 SCPN was sufficient to generate neuronal defects similar to that observed in the $UCHL1^{-/-}$ mice, and when UCHL1 activity was ablated from SMN, the CSMN remained intact. In addition, using AAV mediated gene delivery, we demonstrated that restoring UCHL1 activity specifically in CSMN of $UCHL1^{-/-}$ mice, successfully improved the soma size and integrity of apical dendrites of CSMN. Our results bring a novel mechanistic insight on the role of UCHL1 function for CSMN and establish upper motor neurons as proper cellular targets for gene delivery approaches.

Disclosures: B. Genc: None. **J.H. Jara:** None. **S.S. Sanchez:** None. **A.K.B. Lagrimas:** None. **C.G. Brooks:** None. **N. Kocak:** None. **Y. Zhu:** None. **P. Ozdinler:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 386.02/H13

Topic: C.06. Neuromuscular Diseases

Support: NIH F31 NS108632-01

Title: Transforming growth factor beta signaling in motor neurons in the $SOD1^{G93A}$ mouse model

Authors: *C. BRAINE[1], D. CASTRO[2], I. HUBBARD[3], M. CUEVAS[1], S. MANIATIS[3], R. BONNEAU[2], T. MANIATIS[1], H. PHATNANI[3];
[1]Columbia Univ., New York, NY; [2]New York Univ., New York, NY; [3]New York Genome Ctr., New York, NY

Abstract: Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gherig's Disease, is a fatal neurodegenerative disease caused by the death of motor neurons in the spinal cord and brain. ALS is a genetically complex disease; diverse mutations cause motor neuron death by disrupting various interrelated pathways. To date no therapy targeting a single factor can rescue motor neuron loss. Transforming Growth Factor Beta (TGF-β) is upstream of many of the pathways changed in disease and has been shown to be dysregulated in ALS. Upregulation of TGF-β signaling has been identified as neuroprotective in many neurodegenerative disease models; however, there is conflicting evidence about the role of TGF-β signaling in ALS.

In the first part of this study we use *in vitro* pharmacological approaches to manipulate different pathways within the TGF-β superfamily in co-cultured ES-derived motor neurons and astrocytes. Inhibition of the Classical arm of the pathway improved $SOD1^{G93A}$ motor neuron survival *in vitro*. Through RNAseq we identified drug induced changes in in MTOR, PI3k-AKT signaling, and P53 signaling in motor neurons.
We also seek to understand how disease related changes in TGF-β signaling in the spinal cord directly affect motor neuron survival and gene expression in the $SOD1^{G93A}$ model of ALS. We have preliminary data to suggest that disruption of Smad7, a key mediator of TGF-β signaling, in motor neurons contributes to their death. We will continue to explore how ablation of two different TGF-β receptors in motor neurons or glia in the $SOD1^{G93A}$ ALS animal model affects motor neuron survival. We will follow up these studies with spatially resolved gene expression profiling to understand how these manipulations affect expression in different cell types in the spinal cord.

Disclosures: C. Braine: None. **D. Castro:** None. **I. Hubbard:** None. **S. Maniatis:** None. **R. Bonneau:** None. **T. Maniatis:** None. **H. Phatnani:** None. **M. Cuevas:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 386.03/H14

Topic: C.06. Neuromuscular Diseases

Support: R21-NS085750
Les Turner ALS Foundation
Ellen McConnell Blakeman Fellowship

Title: MCP1-CCR2 and neuroinflammation in the ALS motor cortex with TDP-43 pathology

Authors: *N. KOCAK[1,2], J. JARA[1,2], M. GAUTAM[1,2], E. F. XIE[1,2], Q. MAO[3], E. H. BIGIO[3], P. OZDINLER[1,2];
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Abstract: The involvement of non-neuronal cells and the cells of innate immunity has been attributed to the initiation and progression of ALS. TDP-43 pathology is observed in a broad spectrum of ALS cases and is one of the most commonly shared pathologies. The potential involvement of the neuroimmune axis in the motor cortex of ALS patients with TDP-43 pathology needs to be revealed. This information is vital for building effective treatment strategies. We investigated the presence of astrogliosis and microgliosis in the motor cortex of ALS patients with TDP-43 pathology. prpTDP-43^{A315T}-UeGFP mice, corticospinal motor neuron

(CSMN) reporter line with TDP-43 pathology, are utilized to reveal the timing and extent of neuroimmune interactions and the involvement of non-neuronal cells to neurodegeneration. Electron microscopy and immunolabeling techniques are used to mark and monitor cells of interest. We detected both activated astrocytes and microglia, especially rod-like microglia, in the motor cortex of patients and TDP-43 mouse model. Besides, CCR2+ infiltrating monocytes were detected as they penetrate the brain parenchyma. Interestingly, Betz cells, which normally do not express MCP1, were marked with high levels of MCP1 expression when diseased. There is an early contribution of a neuroinflammatory response for upper motor neuron (UMN) degeneration with respect to TDP-43 pathology, and MCP1-CCR2 signaling is important for the recognition of diseased upper motor neurons by infiltrating monocytes. The findings are conserved among species and are observed in both ALS and ALS-FTLD patients.

Disclosures: **N. Kocak:** None. **J. Jara:** None. **M. Gautam:** None. **E.F. Xie:** None. **Q. Mao:** None. **E.H. Bigio:** None. **P. Ozdinler:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

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Program #/Poster #: 386.04/H15

Topic: C.06. Neuromuscular Diseases

Support: ARSLA
FRC
LabEx BRAIN ANR-10-LABX-43

Title: P2X4 ATP-gated receptor channels play key role in amyotrophic lateral sclerosis pathogenesis

Authors: ***E. BERTIN**[1], A. MARTINEZ[1], A. FAYOUX[2], P.-O. FERNAGUT[1], S. S. BERTRAND[2], E. BOUÉ-GRABOT[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal motoneuron (MN) disease characterized by protein misfolding and aggregation leading to cellular degeneration. To date neither biomarker, nor effective treatment have been found. ATP and upregulated P2X4 receptor (P2X4) expression, a non-selective cationic ATP-gated channel, are involved in various diseases such as ischemia, chronic pain, and several neurodegenerative diseases including ALS. In this context, ATP and P2X4 appeared as attractive novel targets for understanding and fighting the ALS disease. To unravel the implication of P2X4 in ALS pathogenesis, we generated innovating double transgenic mice carrying the human SOD1-G93A mutation and either lacking the P2X4

gene (SOD1/P2X4KO) or knocking for the AP2 binding site responsible for the constitutive internalization of P2X4 (SOD1/P2X4KI). In P2X4KI mice, the substitution of the endocytosis motif by the fluorescent protein mCherry prevents clathrin-dependent endocytosis of P2X4 specifically thus mimicking the pathological upregulation of surface P2X4 receptors. Analysis of the onset, progression and duration of the ALS-like disease in SOD1/P2X4KO and SOD1/P2X4KI compared to SOD1 mice shows that the ablation of the P2X4 gene as well as the expression of non-internalized P2X4 have a significant and positive impact on motor performances and animal survival. These results reveal that P2X4 are active and complex players in ALS progression in SOD1 mice. Immunostaining using nanobodies against P2X4 or antibodies against the mCherry protein in the mouse spinal cord confirmed the increased of P2X4 expression in SOD1 mice over the progression of the disease. This expression, initially restricted to MN, increased in microglia and peripheral macrophages during the symptomatic phase of ALS. In addition, heterologous expression of wild type mP2X4 and several mutants of the hSOD1 protein in *Xenopus* oocytes showed that both ATP-evoked current amplitude and surface P2X4 number are higher in cells expressing mutant hSOD1 indicating that misfolded mutant hSOD1 alters P2X4 surface trafficking. Co-immunoprecipitation and pull-down experiments from WT and SOD1 mice spinal cord demonstrated that misfolded SOD1-G93A protein interacts with AP2 and alters the interaction between AP2 and P2X4 as the disease progress. This result explains the increase in surface P2X4 in SOD1-G93A models over the time.

Disclosures: E. Bertin: None. **A. Martinez:** None. **A. Fayoux:** None. **P. Fernagut:** None. **S.S. Bertrand:** None. **E. Boué-Grabot:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 386.05/H16

Topic: C.06. Neuromuscular Diseases

Title: Role of arginine methylation in the intercellular transmission of FUS

Authors: *N. WATANABE[1], K. MATSUKAWA[1], M. SUAREZ-CALVET[2], D. DORMANN[3], T. HASHIMOTO[1], T. IWATSUBO[1];
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Abstract: Intercellular transmission of disease-related proteins is emerging as a key process in the spreading of neurodegeneration, although the underlying mechanisms still remain unclear. Cytoplasmic accumulation of fused in sarcoma (FUS) is a pathological signature of a subset of

patients with amyotrophic lateral sclerosis (ALS) or frontotemporal lobar degeneration (FTLD); however, the intercellular spreading of FUS has not been documented yet. To examine whether FUS can be transmitted interneuronally in brains, we generated an adeno-associated virus-based expression vector encoding dTomato-P2A-FUS (AAV-FUS), which expresses both dTomato and FUS in neurons. AAV-FUS-infected “donor” neurons express dTomato and FUS as individual proteins; this may result in the positivity of “recipient” neurons exclusively for FUS but not dTomato, if the intercellular protein transmission takes place. Four weeks after infection in newborn mice, nuclei and cytoplasm of a subset of neurons in the cerebral neocortex were positive for FUS and dTomato; notably, cytoplasm of neurons closely apposed to the FUS/dTomato-positive neurons were positive for FUS but not for dTomato, supporting the interneuronal transmission of FUS. Arginine residues in the carboxy-terminal region of FUS are highly methylated, whereas the cytoplasmic inclusions in FTLD brains were reported to be positive for unmethylated (UMA) FUS. We found that the cytoplasm of the “donor” and “recipient” neurons in the AAV-FUS-infected mice were UMA FUS-positive, which prompted us to speculate that FUS hypomethylation is causative to its interneuronal transmission.
To test this idea, we developed a cell-based transmission assay using NanoBiT split-luciferase complementation. We co-cultured the stably transfected HEK293 cell lines expressing LgBiT-(Lg-FUS) or SmBiT-tagged FUS (Sm-FUS), to detect the luminescence elicited by the reconstitution of luciferase through an interaction of Lg-FUS and Sm-FUS. Co-culture of the Lg-FUS and Sm-FUS stable cells caused an increase in the luminescence, suggesting the intercellular transmission of FUS. Treatment of the co-culture with a methyltransferase inhibitor, adenosine-2’,3’-dialdehyde, caused a significant increase in the luminescence compared with the control, suggesting that hypomethylation enhanced FUS transmission.
The parallel between the hypomethylated form of FUS and the possible interneuronal transmission of FUS *in vivo*, together with the increase in intercellular transmission and polymerization of FUS in cultured cells upon demethylation treatment, raise the possibility that hypomethylation is causative to the transmission of FUS.

Disclosures: **N. Watanabe:** None. **K. Matsukawa:** None. **M. Suarez-Calvet:** None. **D. Dormann:** None. **T. Hashimoto:** None. **T. Iwatsubo:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

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Program #/Poster #: 386.06/H17

Topic: C.06. Neuromuscular Diseases

Support: ERC
Academy of Finland
Erkko foundation

Title: Characterization of SOD1G93A crossed with CDNF KO mouse line

Authors: *F. DE LORENZO, A.-S. E. KORPELAINEN, M. LINDAHL, M. SAARMA, M. H. VOUTILAINEN;
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Abstract: Amyotrophic Lateral Sclerosis (ALS)´s etiology remains, to date, largely unknown: only about 5-10% of the cases are familial, while for the remaining 90-95% the disease occurs in a sporadic form, indicating the influence of multiple factors in ALS pathogenesis. Excess of free radicals or intracellular calcium, excitoxicity, loss of neurotrophic factors, oxidative stress and endoplasmic reticulum (ER) stress are few of the proposed causes that may lead to the degeneration, although the reason of the selective vulnerability of motor neurons (MNs) is still unclear. No effective therapy is currently available. We previously showed that a single intracerebroventricular injection of cerebral dopamine neurotrophic factor (CDNF) is able to halt the progression of the disease, increase survival and improve motor behavior in SOD1-G93A mice. Furthermore, CDNF rescues MNs by regulating the ER stress response. CDNF is normally expressed at high level in mouse skeletal muscles. CDNF KO mice are viable mice that lack CDNF ubiquitously.
The aim of this study was to evaluate whether SOD1-G93A mice lacking CDNF would develop symptoms and die earlier than classical SOD1-G93A.
The motor coordination and strength of the new line and controls were evaluated through an extensive battery of tests, including rotarod, grip strength, hanger test and locomotor activity, while gait impairment was analysed since pre-symptomatic stage utilizing a Digigait device. The animals were monitored for weight changes and development of symptoms until total hind-limbs paralyses. Post-mortem samples of gastrocnemius muscle, lumbar spinal cord and motor cortex were analysed for changes in the ER stress and autophagy markers.
Here we show that mice totally lacking CDNF indeed showed the first symptoms significantly earlier than the classical SOD1-G93A mice and SOD1-G93A mice expressing only partially CDNF (CDNF heterozygous crossed with SOD1-G93A). The CDNF KO – SOD1-G93A mice showed also more motor deficit. These mice, however, did not die earlier than their littermates. In the skeletal muscle, we found a higher upregulation of ER stress markers in the CDNF KO – SOD1G93A compared to transgenic and WT littermates. No significant difference in ER stress or autophagy markers was observed in spinal cord and motor cortex
Therefore our results suggest that lack of CDNF, especially in the muscle where CDNF is normally highly expressed, can exacerbate the symptoms in the SOD1-G93A mice.

Disclosures: F. De Lorenzo: None. **A.E. Korpelainen:** None. **M. Lindahl:** None. **M. Saarma:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventors of the CDNF-patent, which is owned by Herantis Pharma Plc. MS is a shareholder of Herantis Pharma Plc. **M.H. Voutilainen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of the CDNF-patent, which is owned by Herantis Pharma Plc..

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

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Program #/Poster #: 386.07/H18

Topic: C.06. Neuromuscular Diseases

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CIBERNED (CB06/05/0089)
GW Research Ltd.

Title: Preclinical evaluation of phytocannabinoid-based neuroprotective therapies for the treatment of amyotrophic lateral sclerosis using TDP43 transgenic mice

Authors: *L. GARCÍA TOSCANO[1,2,3], C. RODRIGUEZ-CUETO[1,2,3], W. HIND[4], R. GRAY[4], J. FERNÁNDEZ-RUIZ[1,2,3], E. DE LAGO[1,2,3];
[1]Inst. Universitario de Investigación en Neuroquimica, Dept. de Bioquímica y Biología Molecular, Facultad de Medicina, Univ. Complutense, Madrid, Spain; [2]Inst. Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain; [3]Ctr. de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain; [4]GW Res. Ltd, Cambridge, United Kingdom

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive and disabling disease characterized by muscle denervation, weakness and atrophy. It is caused by the progressive loss of upper and lower motor neurons triggered by different factors. The disease has no cure and only a few treatments (e.g. riluzole, edavarone, masitinib) are currently available for patients, but their efficacy is limited. Due to a currently unmet need, efforts are being done to find new neuroprotective agents which also includes cannabinoids. In this study, we have investigated classic phytocannabinoids, such as Δ^9-tetrahydrocannabinoid (Δ^9-THC) and cannabidiol (CBD), as well as other minor phytocannabinoids, such as Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabidivarin (CBDV) and cannabidiolic acid (CBDA) in experimental ALS.
We first compared the neuroprotective potential of these five phytocannabinoids (all at 10 mg/kg, i.p., and provided by GW Research Ltd, Cambridge, UK) in Prp-hTDP-43(A315T) transgenic mice. Next, we conducted a dose-dependent study with CBDA (0.1, 1 and 10 mg/kg, i.p.). Lastly, we compared the efficacy of CBDA with riluzole (both at 10 mg/kg, i.p), the most common drug for ALS patients. In all studies, male mice were daily treated from early to symptomatic stage (65th to 90th days of age) before being euthanized. Their neurological status was evaluated weekly using the rotarod test and analysis of limb clasping. Their spinal cords were collected for the histopathological evaluation, which included Nissl-staining for motor neurons and immunostaining for GFAP (astrocytes) and Iba-1 (microglia).
The neuroprotective efficacy of the five phytocannabinoids was in the following order: CBDA >

CBDV > Δ^9-THCV > CBD = Δ^9-THC. CBDA was the only one able to improve the neurological decline, to completely restore the loss of Nissl-stained motor neurons, and to attenuate the astroglial and microglial reactivity. Such activity was not dependent on its decarboxylation to CBD, as this phytocannabinoid was not particularly active by itself. The dose-response study of CBDA confirmed this robust neuroprotective activity at 10 mg/kg, being also more effective than riluzole, which only partially improved the neurological status and attenuated microglial reactivity, but with no effects on astroglial reactivity and the recovery of motor neurons. These results provide robust evidence towards the development of a CBDA-based neuroprotective therapy in ALS, either alone or in combination with other agents. However, additional research is still necessary to promote the translation of these results to patients.

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Poster

386. Motor-Neuron Disease Mechanisms

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Program #/Poster #: 386.08/H19

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS097231
NIH Grant R01NS096176

Title: C9orf72 promotes autophagy-lysosomal functionsand has dose-dependent roles against motor deficits in a mouse model of C9ALS/FTD

Authors: *C. LIANG[1], Q. SHAO[2], Q. CHANG[2], W. ZHANG[2], M. YANG[2], J.-F. CHEN[2]; [1]Univ. of Georgia, Athens, GA; [2]USC, Los Angeles, CA

Abstract: Hexanucleotide repeat expansions in C9orf72 intron are the most common cause of familial amyotrophic lateral sclerosis and frontotemporal dementia (collectively, C9ALS/FTD). C9orf72 homozygous knockout mice surprisingly exhibit no phenotype in motor neurons (MNs). In vivo significance of C9orf72 haploinsufficiency and downstream disease mechanisms remain to be established. We reported that C9orf72 null and haploinsufficiency in a mouse model of C9ALS/FTD exacerbate motor behavior deficits in a dose-dependent manner, and this occurs early in the course of pathogenesis (Acta Neuropathol Commun., 2019). Previously we identified a C9orf72/Smcr8-containing protein complex (Science Advances, 2016). Here we found that Smcr8 knockout (KO) mice exhibited motor behavior deficits. Mechanistic studies reveal that deficiency of Smcr8 disrupts axonal transport in MNs, impairs autophagy-lysosomal degradation, and leads to abnormal axonal swellings in spinal cords and neuromuscular junctions (NMJs) in mice. Furthermore, abnormal axonal swellings are also found in MNs derived from

C9ALS/FTD patient's induced pluripotent stem cells (iPSCs). Using a new mouse model with combined loss- and gain-of-function, we found that C9orf72 has critical and dose-dependent roles against motor deficits, and identified novel disease mechanisms based on C9orf72/Smcr8-mediated regulation of axonal transport and autophagy-lysosome functions.

Disclosures: **C. Liang:** A. Employment/Salary (full or part-time):; University of Georgia. **Q. Shao:** A. Employment/Salary (full or part-time):; University of Southern California. **Q. Chang:** None. **W. Zhang:** A. Employment/Salary (full or part-time):; University of Southern California. **M. Yang:** A. Employment/Salary (full or part-time):; University of Southern California. **J. Chen:** A. Employment/Salary (full or part-time):; University of Southern California.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 386.09/H20

Topic: C.06. Neuromuscular Diseases

Support: RY000152

Title: Comparative analysis of resistant and vulnerable neuromuscular junction in a mouse ALS model

Authors: *F. PROVOST[1,2], R. NADEAU[3], D. ARBOUR[1,2], M.-S. GAUTHIER[4], M. LAVALLÉE-ADAM[3], B. COULOMBE[4], R. ROBITAILLE[1,2];
[1]Univ. de Montréal, Montreal, QC, Canada; [2]Groupe de Recherche du Système Nerveux Central, Montreal, QC, Canada; [3]Univ. of Ottawa, Ottawa, ON, Canada; [4]Inst. de Recherches Cliniques de Montréal, Montreal, QC, Canada

Abstract: Introduction: Amyotrophic lateral sclerosis (ALS) is a motor neuron (MNs) disease characterized by the precocious loss of neuromuscular junctions (NMJs) and muscular paralysis. The denervation of NMJs at striated muscles is an early event that occurs before the loss of spinal cord MNs. Recent data revealed an alteration of synaptic transmission, morphological instability and inappropriate repair in NMJ of SOD1 mice model prior to motor symptoms. Interestingly, these mechanisms are known to be regulated by the Perisynaptic Schwann cells (PSCs), glial cells at NMJ, suggesting that the alteration of PSC functions may contribute to NMJ vulnerability. While numerous studies demonstrated a motor unit type-dependent susceptibility to denervation, the extraocular muscles (EOM) innervation show a prominent resistance to disease progression. We hypothesized that PSCs functions and properties at extraocular NMJs contribute to the resistance of disease progression. Methods: NMJ morphological analysis by immunostaining and confocal imaging, functional properties of PSCs by calcium imaging and a differential proteome analysis using Tandem Mass Tags coupled to

quantitative mass spectrometry was performed between the resistant muscle EOM and the vulnerable muscle, *soleus* (SOL) or *Extensor digitorum longus* (EDL), in $SOD1^{G37R}$ mouse model. Results: Although fewer denervated NMJs were observed in the EOM in comparison to the EDL, we also observed more abundant signs of glial repairs in the EOM in comparison to WT at symptomatic stage. Ca^{2+} responses elicited by ATP and muscarine in PSCs of EOM's were smaller than in the SOL. However, EOM PSC Ca^{2+} responses were larger in $SOD1^{G37R}$ in comparison to WT. Proteomic analysis between EOM and EDL at presymptomatic (P380) and symptomatic stages (P480) in $SOD1^{G37R}$ and WT is underway. Perspectives: Understanding the functional and molecular differences between vulnerable and resistant NMJs will help to provide insights into the denervation mechanisms involved and identify potential therapeutic targets.

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Poster

386. Motor-Neuron Disease Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: NIH RO1 NS091299 to DCZ

Title: Aberrant cap-dependent translation in TDP-43 model of ALS

Authors: *N. PENA, R. BEAR, D. ZARNESCU, B. ZAEPFEL, S. YAMADA;
Univ. of Arizona, Tucson, AZ

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disorder which targets motor neurons. An overwhelming majority of brain and spinal tissue samples from ALS patients contain TAR DNA-binding protein-43 (TDP-43) aggregates. A wide range of cellular processes have been studied in the context of TDP-43 pathology, including RNA metabolism. In particular, dysregulation of mRNA translation has been reported in several models of ALS. Little is known about how TDP-43 could exert toxic effects on protein synthesis. We investigated genetic interactions of TDP-43 with eukaryotic initiation factors (eIFs) in *Drosophila melanogaster*. TDP-43-positive RNA granules have previously been shown to contain eIFs. Our experiments provide evidence of TDP-43 disrupting the initiation step of translation, possibly through interactions with eIFs. In-vivo locomotor assays highlight the relationship between translation initiation and TDP-43 toxicity. Substantiating a role for TDP-43 in translation are findings that enhanced activity of eukaryotic initiation factor 4E binding protein (4EBP) worsened locomotor phenotypes in larvae expressing TDP-43. Furthermore, we directly tested translational activity via puromycin incorporation into nascent peptide chains.

Preliminary data from *Drosophila* ventral nerve cord (VNC) and neuromuscular junction (NMJ) samples, as well as human lymphoblastoid cells, suggest reduced translation in our ALS models of TDP-43 proteinopathy. Finally, we are using non-canonical amino acid tagging (BONCAT/FUNCAT) to measure translation in-vivo. This will allow for identification of newly synthesized proteins with tandem MS, or in situ visualization of global translation levels.

Disclosures: **N. Pena:** None. **R. Bear:** None. **D. Zarnescu:** None. **B. Zaepfel:** None. **S. Yamada:** None.

Poster

386. Motor-Neuron Disease Mechanisms

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Program #/Poster #: 386.11/H22

Topic: C.06. Neuromuscular Diseases

Support: ALS CANADA
QEII/BRC
NSHRF

Title: C-bouton activity is detrimental to muscle innervation in a mouse model of amyotrophic lateral sclerosis

Authors: T. L. WELLS, J. R. MYLES, *T. AKAY;
Med. Neurosci., Dalhousie University, Brain Repair Ctr., Halifax, NS, Canada

Abstract: Amyotrophic lateral sclerosis (ALS) is a disease characterized by the withdrawal of motor neuron axons from muscles followed by the death of motor neurons. Over time, patients with the disease lose their ability to move, talk, eat, and breathe. There is currently no cure for ALS, and patients usually die within five years of being diagnosed. C-boutons are cholinergic synapses on motor neurons that modulate motor neuron excitability in a task-dependent manner. Recently, we showed that the C-boutons are important for maintaining near-normal mobility despite muscle denervation and motor neuron loss during ALS disease progression (Landoni et al, 2019; Behav. Brain. Res.). Here, we aim to understand the relationship between C-bouton activity, muscle denervation, motor neuron survival, and physical capability in ALS. We show that, in ALS model mice ($SOD1^{G93A}$) in which C-boutons are silenced ($SOD1^{G93A}$*;Dbx1::cre;*$ChAT^{flox/flox}$) (SDC; n = 7), innervation in the gastrocnemius muscle after an age of P100 is higher when compared to $SOD1^{G93A}$ ALS mice with C-boutons (SOD1; n=7) (SDC = 81.18% ± 9.31, SOD = 57.18% ± 13.08; two-tailed student's t-test: $p \leq 0.01$). This suggests that the C-bouton modulation of motor neurons is detrimental to motor neuron health during ALS. We also show that, despite this effect, SDC (n = 6) and SOD1 (n = 11) mice reach the humane end-point of the disease at similar ages (SDC = P149 ± 10; SOD1 = P155 ± 10; two-

tailed student's t-test: p = 0.31). This suggests that C-boutons do not affect the overall health of ALS mice. By using an exercise regime to induce different levels of C-bouton activity, we are investigating how different levels of C-bouton activity influence the disease. These experiments will provide insight into how C-bouton activation affects ALS disease progression.

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Poster

386. Motor-Neuron Disease Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: ALS Association Grant 1028
Funding from the Lohengrin Foundation

Title: Knockdown of GADD34 ameliorates ALS in mutant SOD1 mice

Authors: *A. CAMARENA[1], G. D. GHADGE[2], Y. SONOBE[2], C. R. DRIGOTAS[2], R. P. ROOS[2];
[1]Univ. of Chicago Pritzker Sch. of Med., Chicago, IL; [2]Dept. of Neurology, Univ. of Chicago Med. Ctr., Chicago, IL

Abstract: Mutant (mt) Cu/Zn superoxide dismutase (SOD1) is thought to cause familial amyotrophic lateral sclerosis (FALS) because of misfolding and aggregation of the mutant protein. Here, we investigated whether GADD34 shRNA delivered by AAV9 can ameliorate disease in mtSOD1 transgenic mice through an enhancement of the unfolded protein response/integrated stress response (UPR/ISR), which is a normal cellular response to misfolded proteins. Initial studies showed that compared to control shRNA, GADD34 shRNA decreased GADD34 mRNA and protein expression and increased phosphorylated eIF2α in cultured NSC-34 cells with thapsigargin-induced ER stress. Additional experiments involved intravenous injections of neonatal C57BL/6J mice with AAV:control-shRNA or AAV:GADD34-shRNA followed 81 days later with tunicamycin intracerebroventricular injection to induce ER stress. Three days later, AAV:GADD34-shRNA-injected mice showed significantly less GADD34 mRNA in the spinal cord compared to AAV:control-shRNA-injected mice. Subsequent investigations showed that neonatal G93A and G85R mtSOD1 mice injected intravenously with AAV:GADD34-shRNA had a significantly increased lifespan compared to AAV:control-shRNA, along with reduced disease pathology, SOD1 load and aggregates, astrocytosis, and microgliosis. The results show that GADD34 shRNA is effective in ameliorating disease in mtSOD1 mice, and the feasibility of targeting the UPR/ISR as a therapy in mtSOD1-induced

FALS. The results also suggest that the UPR/ISR can be targeted in ALS in general and other neurodegenerative diseases in which misfolded proteins and ER stress have been implicated.

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Poster

386. Motor-Neuron Disease Mechanisms

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Title: Loss of function of astroglial exosomes in the SOD1G93A mouse model of ALS

Authors: *S. JIN[1], T. YANG[1], Y. MEN[1], J. M. YELICK[2], R. JARVIS[1], Y. YANG[3];
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Abstract: Exosomes are generated from endosomal compartment and known to be released by most cell types including neurons and glia cells in the CNS. These vesicles are proposed to be involved with the clearance and cell-to-cell spreading of toxic molecules. Recent research suggested that the majority of pathogenic proteins of neurodegenerative diseases such as amyloid beta, mutant SOD1, TDP43, phosphor-Tau are detected in exosomes fraction of patients CSF or in vitro cell model of disease. However, the role of cell type-specific exosomes in the spreading of pathogenic protein in neurodegenerative disease is still unclear. In the current study, we developed a cell-type specific exosome reporter mouse line (hCD63-GFP conditional knock-in, CD63-CKI) in which a GFP-fused CD63, can be induced in a particular cell type when bred with the cell-type specific Cre-driver mice or stereotaxic injection of Cre expressing AAV. By employing this mouse tool, we found that both neuronal and astrocyte-derived exosomes are widely present in the CNS. We performed stereotaxic injection AAV-CamkII-Cre and AAV-GFAP-Cre virus to ventral horn of lumber spinal cord in CD63f/+ or CD63f/+G93A SOD1 transgenic mice in disease on set (day90~100). We observed spread of astrocyte derived exosomes is significantly decreased in the direction of thoracic and sacral spinal cord in SOD1 transgenic (Tg) mice compare to non-Tg mice. The spreading of neuronal exosomes is not changed in SOD1 Tg mice compare to non-Tg mice. Moreover, we found a surprising loss-of-function effect of SOD1 Tg astrocyte's exosomes to cortical neurons. Non-Tg astrocyte's exosomes are able to significantly stimulate neuron dendrite morphological complexity, synapse number, and synapse activity (miniEPSC). In contrast, SOD1 Tg astrocyte's exosomes are

unable to stimulate neuron as non-Tg astrocyte's exosomes. On the another hand, both non-Tg and SOD1 Tg astrocyte's exosomes treated neurons are healthier and more active than non-treated control neurons. In summary, we investigated in vivo dynamics of astrocyte and neuron's exosomes in SOD1 Tg and non-Tg mice. Our preliminary data also suggests a potential loss-of-function of astroglial exosomes in the SOD1G93A mouse model of ALS.

Disclosures: S. Jin: None. **T. Yang:** None. **Y. Men:** None. **J.M. Yelick:** None. **R. Jarvis:** None. **Y. Yang:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 386.14/H25

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R15 NS074367-01A1 to E. N.O

Title: Mitochondrial dysfunction in the gastrocnemius-associated motorneurons of muscle-synthesized BDNF- deficient mice

Authors: M. L. CAWLEY, R. L. MUELLER, C. M. KIMBER, *E. N. OTTEM;
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Abstract: Mitochondria are essential for the high energy demands of the neuromuscular junction and leave motorneurons highly susceptible to potential dysfunction. Neuromuscular diseases (NMDs) are characterized by a loss of function between motorneurons and the muscles they innervate, and mitochondrial dysfunction is a common hallmark. A potential origin of progressive pathology associated with NMDs may be a reduction in brain-derived neurotrophic-factor (BDNF). We have shown that mice deficient in skeletal muscle-synthesized BDNF(msBDNF) demonstrate progressive motorneuron and muscle pathology at 120 days. Our recent studies have demonstrated a significant decrease in MitoTracker labeling at the pre-synapse of msBDNF homozygous knockout animals and no significant difference at the post-synapse (ANOVA; n=5, $P<0.001$). However, MitoTracker dyes are only sequestered by actively respiring mitochondria, and may limit estimates as dysfunctional mitochondria are unable to sequester the probe. We now aim to assess mitochondrial density and functionality in the gastroc-associated motorneurons of msBDNF deficient-mice. At 117 d, msBDNF deficient-mice received intramuscular injections of MitoTracker dye targeting the right gastrocnemius muscle. At 120 d experimental groups underwent a gastrocnemius harvest (n=8 per group) or a sciatic nerve ligation protocol (n=8 per group) prior to sacrifice. In combination with MitoTracker injections, we used immunohistochemical labeling to target the outer mitochondrial membrane protein TOM20. We used immunolabeling to further delineate potential pathology within

gastroc-associated motorneurons. We targeted the pre-synaptic vesicular protein synaptophysin, post-synaptic acetylcholine receptors, and retrograde transport-associated protein dynactin. To determine if experimental groups exhibit mitochondrial dysfunction, we used confocal microscopy and IMARIS 3D rendering software. Immunolocalization was measured throughout the axon and axon terminals of motorneurons. Preliminary results suggest mitochondria co-labeled with an activity-dependent dye and immunoassayed protein show a significant difference in colocalization. Results indicate that co-labeling mitochondria in a sciatic nerve ligation model illustrate mitochondrial density, as well as utility during transport. Ongoing studies suggest that mitochondrial mobility may be as dynamic as mitochondrial function and assisted by the coexistence of several signaling mechanisms. Co-labeling mitochondria in msBDNF deficient-mice further elucidates the role of growth factor signaling in the maintenance of motorneurons.

Disclosures: M.L. Cawley: None. **C.M. Kimber:** None. **E.N. Ottem:** None. **R.L. Mueller:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 386.15/H26

Topic: C.06. Neuromuscular Diseases

Title: Cortical interneuron-mediated inhibition delays the onset of amyotrophic lateral sclerosis in the SOD1 mouse model

Authors: C. S. KHADEMULLAH[1], A. J. AQRABAWI[2], K. M. PLACE[1], Z. DARGAEI[1], X. LIANG[1], ***J. C. PRESSEY**[1], S. BEDARD[1], J. YANG[1], D. GARAND[1], A. GASECKA[3], D. CÔTÉ[3], Y. DE KONINCK[3], J. KEITH[4], L. ZINMAN[4], J. ROBERTSON[5], J. KIM[2], M. A. WOODIN[1];
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Abstract: A balance between synaptic excitation and inhibition is essential for normal brain function. When this delicate balance is disrupted, it can lead to neuronal hyperexcitability, resulting in alterations in neuronal network activity and the onset of various neurological disorders. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects both lower and upper motor neurons. While hyperexcitability is a hallmark feature during the pre-symptomatic stages of ALS, there has yet to be a systematic analysis of inhibitory synaptic function during the progression of this disorder. Using electrophysiological patch-clamp recordings, we revealed parvalbumin (PV^+) interneurons to be hypoactive in the pre-symptomatic SOD1*G93A mouse model of ALS. We discovered that using AAV-mediated

delivery of chemogenetic technology targeted to increase the activity of the interneurons within layer 5 of the primary motor cortex (L5-M1), we were able to rescue intracortical inhibition and reduce pyramidal neuron hyperexcitability. Increasing the activity of interneurons in the L5-M1 was effective in delaying the onset of ALS-associated motor deficits, slowing symptom progression, preserving neuronal populations, and increasing the lifespan of SOD1*G93A mice. Taken together, this study provides novel insights into the pathogenesis and treatment of ALS.

Disclosures: **C.S. Khademullah:** None. **A.J. Aqrabawi:** None. **K.M. Place:** None. **Z. Dargaei:** None. **X. Liang:** None. **J.C. Pressey:** None. **S. Bedard:** None. **J. Yang:** None. **D. Garand:** None. **A. Gasecka:** None. **D. Côté:** None. **Y. De Koninck:** None. **J. Keith:** None. **L. Zinman:** None. **J. Robertson:** None. **J. Kim:** None. **M.A. Woodin:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

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Topic: C.06. Neuromuscular Diseases

Support: JSPS KAKENHI Grant JP15K19644
JSPS KAKENHI Grant JP17K16237

Title: Inhibition of collapsin response mediator protein2 phosphorylation ameliorates motor phenotype of ALS model mice expressing SOD1G93A

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Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurological disease characterized by the selective degeneration of motor neurons leading to paralysis and immobility. Missense mutations in the gene coding for the Cu2+/Zn2+ superoxide dismutase 1 (SOD1) accounts for 15-20% of familial ALS, and mice overexpressing ALS-linked SOD1 mutants have been frequently used as an animal model for ALS. Degeneration of motor neurons in ALS progresses in a manner called "dying back", in which the degeneration of synapses and axons precedes the loss of cell bodies. Phosphorylation of collapsin response mediator protein 2

(CRMP2) is implicated in the progression of neuronal/axonal degeneration of different etiologies. To evaluate the role of CRMP2 phosphorylation in ALS pathogenesis, we utilized CRMP2 S522A knock-in (CRMP2ki/ki) mice, in which the serine residue 522 was homozygously replaced with alanine and thereby making CRMP2 no longer phosphorylatable by CDK5 or GSK3B. We found that the CRMP2ki/ki/SOD1G93A mice showed delay in the progression of the motor phenotype compared to their SOD1G93ATg littermates. Histological analysis revealed that the CRMP2ki/ki/SOD1G93A mice retained more intact axons and NMJs than their SOD1G93A-Tg littermates. These results suggest that the phosphorylation of CRMP2 may contribute to the axonal degeneration of motor neurons in ALS.

Disclosures: **Y. Numata-Uematsu:** None. **S. Wakatsuki:** None. **S. Nagano:** None. **M. Shibata:** None. **K. Sakai:** None. **N. Ichinohe:** None. **K. Mikoshiba:** None. **T. Ohshima:** None. **N. Yamashita:** None. **Y. Goshima:** None. **T. Araki:** None.

Poster

386. Motor-Neuron Disease Mechanisms

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Program #/Poster #: 386.17/H28

Topic: C.06. Neuromuscular Diseases

Support: NIH RO1 NS091299
MDA 418515
HHMI Gilliam Fellowship
Undergraduate Biological Research Program

Title: Characterization of metabolic defects across multiple models of ALS

Authors: *H. BALL[1], E. MANZO[1], D. C. ZARNESCU[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that disrupts muscle function and has no cure. TAR DNA Binding Protein (TDP-43) is an RNA binding protein that has been found in cytoplasmic aggregates in 97% of ALS cases regardless of etiology. *Drosophila* is a well established genetic model for human disease that we used to develop a model for ALS based on TDP-43. Flies expressing either wild-type or mutant human TDP-43 (wild-type and mutant) recapitulate several symptoms of ALS, including motor dysfunction and a reduced survival. Recently, we found that glycolysis is upregulated in this model as a compensatory mechanism that improves locomotor function and increases lifespan. To determine whether glycolysis is similarly altered in other types of ALS we are using different fly models based on C9, SOD1 and a recently generated CRISPR model of TDP-43 proteinopathy. Preliminary results suggest that similar to the TDP-43 proteinopathy model based

on overexpression, the CRISPR model as well as SOD1 and C9 models can also benefit from increased glucose availability. We will report on the neuroprotective potential of increased glycolysis on key ALS phenotypes including locomotor function, neuromuscular junction morphology and lifespan across several *Drosophila* models of ALS.

Disclosures: H. Ball: None. **E. Manzo:** None. **D.C. Zarnescu:** None.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

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Program #/Poster #: 386.18/H29

Topic: C.06. Neuromuscular Diseases

Support: Italian Ministry of University and Research - MIUR (SIR project RBSI14B1Z1)

Title: Knocking down the metabotropic glutamate receptor 5 in the $SOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis ameliorates the reactive phenotype of spinal cord astrocytes

Authors: *C. USAI[1], C. TORAZZA[2], F. PROVENZANO[2], S. RAVERA[3], T. BONIFACINO[2], G. BONANNO[2,4,5], M. MILANESE[2,4];
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Abstract: The ultimate cause of disease progression in amyotrophic lateral sclerosis (ALS) is motor neuron (MN) death. However, there is a large consensus that MN damage is supported by degeneration of non-neuronal cells, such as microglia and astrocytes. Therefore, to unveil the precise role of each cell population will be crucial to design targeted therapies. Group I metabotropic glutamate (Glu) receptors (mGluR1, mGluR5) play a role in ALS, since their expression and function are altered in the disease. We demonstrated that mGluR1 and mGluR5 activation sustains abnormal Glu release in the spinal cord of the $SOD1^{G93A}$ mouse model of ALS (Neuropharmacol 66: 253-63. 2013) and that knocking-down mGluR1 or mGluR5 in $SOD1^{G93A}$ mice significantly prolongs survival and ameliorates the clinical progression of the disease (Neurobiol Dis. 64: 48-59. 2014; Neuropharmacol. 123: 433-445, 2017). We investigated here the effects of halving mGluR5 in $SOD1^{G93A}$ mice, on the reactive phenotype of spinal cord astrocytes cultured from 120 day-old symptomatic $SOD1^{G93A}$ mice, age-matched $SOD1^{G93A}Grm5^{+/-}$ (heterozygous for mgluR5) or WT mice. The basal and the mixed mGluR1 and mGluR5 agonist 3,5-DHPG-stimulated intracellular Ca^{2+} concentration was increased in astrocytes from $SOD1^{G93A}$ vs. WT mice and it was significantly reduced in astrocytes from

$SOD1^{G93A}Grm5^{+/-}$ mice. The expression of the astrocyte activation markers GFAP, vimentin, and S100β was increased in $SOD1^{G93A}$ and significantly reduced in $SOD1^{G93A}Grm5^{+/-}$ astrocytes. The same was true for accumulation of cytosolic misfolded-SOD1. $SOD1^{G93A}$ astrocytes showed increased oxygen consumption and reduced ATP synthesis, which were normalized in $SOD1^{G93A}Grm5^{+/-}$ astrocytes. $SOD1^{G93A}$ astrocytes were characterized by a significant increase of IL1β, TNFα e IL6 expression and release respect to WT astrocytes. The abnormal expression and release of the cytokines was strongly reduced in $SOD1^{G93A}Grm5^{+/-}$ astrocytes. Finally, to verify the impact of the mGluR5 down-regulation in astrocytes on MN viability, spinal MNs from $SOD1^{G93A}$ embryos were co-cultured with astrocytes from $SOD1^{G93A}$ or $SOD1^{G93A}Grm5^{+/-}$ adult mice. MN cell death was strongly reduced when they were seeded on $SOD1^{G93A}Grm5^{+/-}$ compared to $SOD1^{G93A}$ astrocytes. Thus, a low constitutive level of mGluR5 in $SOD1^{G93A}$ mice has a positive impact on the spinal cord astrocyte phenotype, supporting the idea that the *in-vivo* disease amelioration, observed after mGluR5 ablation, may rely on a shift toward less noxious reactive astrocytes.

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Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 386.19/H30

Topic: C.06. Neuromuscular Diseases

Title: Behavioral characterization of low copy SOD1 G93A transgenic mice

Authors: S. FLUNKERT, *R. RABL, A. KASZA, V. NIEDERKOFLER, E. AUER, B. HUTTER-PAIER;
QPS Austria GmbH, Grambach, Austria

Abstract: Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that results in the death of motor neurons in the brain and spinal cord. So far no effective treatments are available. Several causative genes are known and, of these, mutant superoxide dismutase 1 (SOD1) is by far the most frequent, accounting for up to 20% of familial ALS cases. A range of human mutant SOD1 transgenic mouse models has been developed that model the human disease. Of these, the most widely used is the SOD1(*G93A)1Gur mouse, which expresses a human SOD1 transgene with G93A mutation (Gurney et al., 1995). Mice become paralyzed in one or more limbs beginning around 12 weeks of age. Life expectancy is only four to six weeks beyond onset of symptoms. Although the SOD1G93A mouse has been a bedrock for ALS research and a well-used model for over a decade, there are some concerns about its use caused by its very strong and early phenotype. The low copy number SOD1 mouse

model might thus be a suitable alternative since it presents with a delayed onset of the ALS phenotype compared to the original high copy number strain because of a reduction in transgene copy number.
The aim of this study was to evaluate the behavioral phenotype onset and survival of low copy number SOD1G93A mice.
Method: SOD1G93A mice with a low copy number (JAX mouse #002300) of mixed sex were longitudinally analyzed from week 24 to 30 for motor deficits using the wire suspension test and grip strength test. Additionally, animals were analyzed in the Beam walk test, RotaRod and Pasta Gnawing test at the age of 24, 27 and 30 weeks.
Results: Behavioral tests are almost completed and will afterwards be statistically analyzed. So far, animals seem to develop a progressive motor phenotype that starts at 24 to 28 weeks of age and thus much later compared to the widely used SOD1G93A high copy number mouse model.
Conclusion/ Summary: The slow progressive pathology observed in SOD1G93A low copy number mice could provide a more appropriate model for studying early-stage pathological processes in ALS by having a longer treatment window for the development of new therapies.

Disclosures: **S. Flunkert:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **R. Rabl:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **A. Kasza:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **V. Niederkofler:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **E. Auer:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **B. Hutter-Paier:** A. Employment/Salary (full or part-time):; QPS Austria GmbH.

Poster

386. Motor-Neuron Disease Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 386.20/H31

Topic: C.06. Neuromuscular Diseases

Support: IRSC RNI00252
Packard Center for ALS Research RU000066

Title: Delayed disease onset and progression in SOD1^{G37R}/Thy1-YFP16 ALS mouse model

Authors: É. MARTINEAU[1,2], ***F. FIORE**[1,2], S. MARCHAND[1,2], R. ROBITAILLE[1,2]; [1]Neurosciences, Univ. De Montréal, Montréal, QC, Canada; [2]Groupe de Recherche sur le Système Nerveux Central (GRSNC), Montréal, QC, Canada

Abstract: In amyotrophic lateral sclerosis (ALS), neuromuscular junctions (NMJ) undergo denervation and re-innervation as part of a global degeneration process occurring early in the disease. In an effort to investigate this mechanism, we crossed SOD1^{G37R} transgenic mice to

thy1-YFP mice (line 16). To our surprise, SOD1-YFP16 mice showed a delayed disease onset and slower disease progression. To better understand this partial rescue of the ALS phenotype, we monitored the ALS progression and motor phenotype of SOD1-YFP16 mice, quantified the differences in NMJ denervation and motor neuron survival and performed electrophysiological recordings at the NMJ to compare synaptic activity. We observed that disease onset in SOD1-YFP16 mice was delayed by ± 49 days. Progression of the disease was slowed, resulting in an increase in SOD1-YFP16 mice survival of ± 133 days. In addition, these mice preserved their motor function for a longer period of time. Morphologically, they displayed reduced NMJ damage in both fast-twitch (EDL) and slow-twitch (SOL) muscles in comparison to their age-matched SOD1 littermate, as assessed by immunohistochemical staining. Spinal alpha-motoneuron survival was also increased in these mice. No differences in spontaneous or evoked synaptic transmission was detected at the NMJ between SOD1-YFP16 and SOD1 mice, suggesting that rescuing synaptic activity may not be necessary for restoring motor function and NMJ phenotype in this ALS mouse model. Importantly, this delay was not associated with a reduction in human SOD1 copy number. Finally, we identified the YFP16 transgene insertion site, on chromosome 5 at position 5qC2 (5' of the 4933402J10Rik gene). Altogether, we report a significant delay in ALS progression in this SOD1-YFP16 mouse model, possibly linked to the YFP transgene insertion in a novel disease-modifying locus. Further investigation into the molecular mechanism underlying this delay could be of significance for our understanding of ALS pathogenesis.

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Poster

386. Motor-Neuron Disease Mechanisms

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Program #/Poster #: 386.21/H32

Topic: C.06. Neuromuscular Diseases

Support: NIHR21NS102599

Title: Altered expression of circadian clock genes in mice expressing amyotrophic lateral sclerosis-linked mutant superoxide dismutase 1

Authors: *K. M. KILLOY, B. A. HARLAN, M. PEHAR, M. R. VARGAS;
Cell and Mol. Pharmacol., Med. Univ. of South Carolina, Charleston, SC

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset disease characterized by specific degeneration of motor neurons. Beyond progressive motor impairment, patients with ALS suffer from yet incompletely characterized defects in energy metabolism. Several endocrine factors that display circadian rhythmicity and participate in metabolic synchronization of

peripheral tissues are altered in ALS patients and animal models. In addition, circadian control of NAD^+ availability directly modulates mitochondrial oxidative metabolism and antioxidant defenses. In mammals, most physiological processes are subject to daily oscillations that are governed by a circadian rhythm. Circadian timekeeping is orchestrated at a molecular level by cell-autonomous molecular clocks found on almost every cell. A group of neurons located in the suprachiasmatic nucleus (SCN) synchronizes this multitude of oscillators across brain regions and the entire body. The SCN clock is itself synchronized to local time by phase-setting signals, the most salient of which is the daily light/dark cycle, and sustains rhythmicity in complete absence of external synchronization cues. In turn, the SCN synchronizes the oscillators in peripheral tissues through secreted factors, feeding-fasting rhythms and body temperature. Loss of circadian timekeeping has been associated with cellular and system-wide alterations in metabolism, redox homeostasis and inflammation. Here we examined the expression of circadian clock and clock-controlled genes in the central nervous system and peripheral tissues of mice expressing ALS-linked mutant human superoxide dismutase 1 ($hSOD1^{G93A}$). We found that over a 24-hour period, early symptomatic $hSOD1^{G93A}$ mice exhibit aberrant oscillation in the expression of circadian clock and clock-controlled genes in several tissues when compared to age-matched non-transgenic mice. This work identifies the molecular clock as a potential target for the development of future ALS therapies.

Disclosures: **K.M. Killoy:** None. **B.A. Harlan:** None. **M. Pehar:** None. **M.R. Vargas:** None.

Poster

386. Motor-Neuron Disease Mechanisms

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Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R56- NS092572-01
Robert Packard Center for ALS Research
Farber Family Foundation

Title: Elevated expression of the ALS-linked DNA/RNA-binding protein FUS in astrocytes induces reactivity and spinal motor neuron death *in vivo*

Authors: K. MCAVOY, ***B. K. JENSEN**, N. M. HEINSINGER, K. MAYER, T. WESTERGARD, A. C. LEPORE, A. R. HAEUSLER, D. TROTTI, P. PASINELLI; Thomas Jefferson Univ., Philadelphia, PA

Abstract: Mutations in the DNA/RNA binding protein Fused in Sarcoma (FUS) cause Amyotrophic Lateral Sclerosis. Ubiquitously expressed in all cells, FUS regulates gene expression at multiple stages of DNA/RNA processing, from transcription to translation. While

ALS ultimately targets the motor neurons, non-neuronal cells, like astrocytes, are also important drivers of disease. We hypothesized that in addition to causing motor neuron dysfunction, mutations in FUS may also invoke cellular alterations in astrocytes. Here, we explore the contribution of astrocytes to neurodegeneration in models of FUS-ALS *in vivo.* Previously, we found that astrocytes expressing mutant FUS (mFUS) are toxic to wildtype (WT) motor neurons *in vitro*. We identified that the mechanism of toxicity is via activation of the astrocytic NF-κB pathway and release of pro-inflammatory cytokine TNFα. In motor neurons, TNFα signaling alters AMPA receptors, sensitizing them to excitotoxic cell death. Now, we developed an *in vivo* model expressing WT and mFUS specifically in astrocytes by direct cervical intraspinal delivery of AAV9 vectors encoding GFP-tagged FUS variants under control of the gfa_{104} promoter in adult mice. GFP-alone and sham surgeries were controls. Immunocytochemistry and qPCR revealed that expression of FUS (WT or mFUS) caused astrocytic reactivity, upregulation of TNFα, and alteration of AMPA receptor subunits. We observed reduced grip strength and wire-hang endurance in FUS-expressing animals. This was correlated to significant loss of MMP9/ChAT+ motor neurons in the spinal cord. No differences were detectable between males and females, 8 animals per group were used to ensure reproducibility and appropriate power for statistical analysis. Finally, we tested whether FUS-mediated effects could be attenuated through prevention of TNFα signaling. mFUS intraspinal injections (5 animals/group) were performed with or without TNFα-neutralizing antibody (anti-TNFα). In animals receiving anti-TNFα, we observed significant rescue of grip strength performance, as well as reduction in astrocyte reactivity and downstream NF-κB targets. Loss of motor neurons was significantly prevented. Controls included IGG-antibody injections or sham surgeries. These studies further substantiate that astrocytes contribute to disease in genetic models of ALS. We provide novel findings that astrocytic expression of FUS is sufficient to induce pathogenic changes *in vivo* through activation of the NF-κB and TNFα pathway. We also provide evidence that therapeutics designed to diminish activation of this pathway may be effective in reducing motor neuron degeneration in FUS-ALS.

Disclosures: K. McAvoy: None. **B.K. Jensen:** None. **N.M. Heinsinger:** None. **K. Mayer:** None. **T. Westergard:** None. **A.C. Lepore:** None. **A.R. Haeusler:** None. **D. Trotti:** None. **P. Pasinelli:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.01/H34

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: A human induced pluripotent stem cell-derived neuronal model to assess developmental neurotoxicity upon exposure to chemical mixtures

Authors: *F. PISTOLLATO[1], E. MENDOZA[1], S. BOPP[1], C. NUNES[2], A. WORTH[1], A. BAL-PRICE[1];
[1]European Commission, Joint Res. Ctr., Ispra, Italy; [2]Univ. of Lausanne, Lausanne, Switzerland

Abstract: Human induced pluripotent stem cell (hiPSC)-derived neurons and astrocytes represent unique cellular models to study in vitro brain developmental processes, such as synaptogenesis, neurite outgrowth and neuronal network formation. These models are nowadays considered for the assessment of developmental neurotoxicity (DNT) in vitro, enabling mechanistic understanding of chemically-induced adverse effects. Moreover, since infants and children are co-exposed to more than one chemical at a time, novel mixture risk assessment strategies for the evaluation of DNT should be implemented. Here we used hiPSC-derived neural progenitors differentiated into mixed cultures of neurons and astrocytes to assess the acute and repeated dose effects of chemicals belonging to different classes (e.g., pesticides, industrial chemicals, heavy metals, polychlorinated biphenyls, endocrine disruptors, etc.) and that have all been associated with learning and memory deficits in children. Selected chemicals were grouped based on their mode of action (MoA) into 'similar' and 'dissimilar' MoA compounds and cells have been treated with both single compounds and different chemical mixtures to assess cumulative effects on DNT specific endpoints (i.e., synaptogenesis, neurite outgrowth, and brain derived neurotrophic factor (BDNF) protein levels), described as common key events in different DNT-related adverse outcome pathways. Data suggest that chemicals (in particular those working through similar MoA), individually present at non-toxic concentrations, can have neurotoxic effects in mixtures. This methodological approach represents a valuable conceptual framework to evaluate chemical mixtures with potential to cause learning and memory impairment in children, which nowadays represents an ever increasing public health concern.

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Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.02/H35

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIAAA

Title: NADPH oxidase and endoplasmic reticulum stress contribute to neuroinflammation and neurodegeneration in postmortem human alcoholic brain

Authors: *L. QIN, R. P. VETRENO, F. T. CREWS;
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Abstract: The endoplasmic reticulum (ER) is susceptible to oxidative stress that can activate cell death mechanisms like apoptosis. In the current study, real-time PCR and immunohistochemistry were used to study expression of NADPH oxidase (NOX) and ER stress markers. NOX and ER stress markers in post-mortem human orbital frontal cortex (OFC) were compared to markers of neurodegeneration in moderate drinking controls and alcoholics. In the alcoholic OFC, we found significant increases in NOX mRNA levels, specifically alcoholics compared to moderate drinking control subjects had increases in NOX2 subunit gp91^{phox} (272%), NOX3 (206%), and NOX4 (215%). Our previous studies have shown significant increases in the number of NOX2 gp91^{phox}+immunoreactive (IR) cells in the alcoholic OFC. We also observed significant increases in mRNA levels of ER stress markers: caspase-12 (181%), glucose-regulated protein 78 (GRP78 [150%]), activating transcription factor 6 (ATF6 [208%]), C/EBP-homologous protein (CHOP [204%]), and protein kinase R-like endoplasmic reticulum kinase (PERK TV1 [195%], PERK TV2 [168%]) in the alcoholic OFC, relative to moderate drinking controls. Immunohistochemistry (IHC) to assess protein expression of ER stress markers showed increases in the number of caspase-12, GRP78, ATF6 and CHOP+IR cells in the alcoholic OFC. To evaluate the association of increased NOX and ER stress with neurodegeneration in the post-mortem alcoholic OFC, we investigated caspase cell death cascade proteins; i.e. caspase-3, -7, -8, -9 mRNA, and activated caspase-3 IHC protein expression. We found significant increases in mRNA levels of caspase-3 (158%), -7 (193%), -8 (166%), and -9 (181%) as well as increased activated caspase-3+IR (155%) in the alcoholic OFC, compared to moderate drinking controls. Double fluorescent IHC showed that ER stress markers: caspase-12, GRP78, ATF6 and CHOP were colocalized with NOX gp91^{phox}+IR which was colocalized with activated caspase-3 and Neu-N+IR cells in the human alcoholic OFC, suggesting that the enhanced expression of NOX and ER stress in human alcoholic brain neurons could contribute to chronic ethanol-induced neuroinflammation and neurodegeneration.

Disclosures: L. Qin: None. **R.P. Vetreno:** None. **F.T. Crews:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.03/H36

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NCTR protocol E0767501

Title: Ketamine reduces reactive oxygen species in zebrafish *in vivo*

Authors: *J. KANUNGO[1], Q. GU[2], S. F. ALI[3], B. ROBINSON[4], M. DUMAS[5];
[1]Natl. Ctr. For Toxicological Research/Food and Drug Admin., Jefferson, AR; [2]FDA Natl. Ctr. for Toxicological Res., Jefferson, AR; [3]Neurochemistry Lab, Div. of Neurotoxicology, Natl. Ctr.

Toxicological Res/Fda, Jefferson, AR; [4]Natl. Ceter for Toxicological Research, US Food and Drug Admin., Jefferson, AR; [5]The Bionetics Corp., Jefferson, AR

Abstract: The anesthetic ketamine is a non-competitive antagonist of the calcium-permeable N-methyl-d-aspartate (NMDA) receptor. High doses have been implicated in cardiotoxicity and neurotoxicity. These toxicities are often thought to be mediated by reactive oxygen species (ROS). However, findings to the contrary showing ketamine reduces ROS in mammalian cells and neurons *in vitro* are emerging. Here, we determined the effects of ketamine on ROS levels in zebrafish larvae *in vivo*. Based on our earlier studies demonstrating a reduction in ATP levels by ketamine, we hypothesized that as a calcium antagonist, ketamine would also prevent ROS generation which is a by-product of ATP synthesis. To confirm that the detected ROS in a whole organism, such as the zebrafish larva, is specific, we used diphenyleneiodonium (DPI) which blocks ROS production by inhibiting the NADPH oxidases (NOX). After 20 h exposure, DPI (5 and 10 μM) and ketamine (1 and 2 mM) reduced ROS in the 72 hours post-fertilization zebrafish larvae *in vivo*. Exposure to the dietary supplement acetyl l-carnitine (ALCAR) that induces mitochondrial ATP synthesis, caused elevated ROS generation with increasing doses. Combined, ketamine and ALCAR counter-balanced ROS generation in the larvae suggesting that ketamine and ALCAR have opposing effects on mitochondrial metabolism. This may be key to maintaining ROS homeostasis in the larvae and affords ALCAR the ability to prevent ketamine toxicity. These results show, for the first time, ketamine's antioxidative and ALCAR's prooxidative effects in a live vertebrate.

Disclosures: J. Kanungo: None. **Q. Gu:** None. **S.F. Ali:** None. **B. Robinson:** None. **M. Dumas:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.04/H37

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NRF-2018R1D1A1B07043055
KGM4561811

Title: Micrornas expressed differentially in the hippocampus of female and male rodents after chronic alcohol treatment

Authors: *M. CHOI, J. HAN, D.-J. KIM;
Addiction Inst., Seoul, Korea, Republic of

Abstract: Alcohol abuse causes various health problems including liver disease, stroke, and cognitive impairments. Drinking the same amount of alcohol causes more severe brain damage in women compared to men. Therefore, it is necessary to investigate whether alcohol-induced epigenetic modification influences differentially gene expression related to brain damage depending on sex differences. This study aimed to identify microRNAs (miRNAs) expressed differentially in the hippocampus of female and male rodents in response to alcohol. After chronic administration of ethanol (3-3.5 g/kg/day) in the female (control, n=10; alcohol, n=12) and male (control, n=10; alcohol, n=12) Sprague-Dawley rats for 6 weeks, we measured body weights during the experiment and analyzed up- or down-regulated miRNAs using miRNA arrays. We found that increase of body weights between ethanol-treated male and control male groups during ethanol administration was significantly different, while there was little difference in the increase of body weights between ethanol-treated female and control female groups during ethanol administration. When comparing ethanol-treated male group with control male group, 118 miRNAs were more than 1.5-fold up- or down-regulated in ethanol-treated male group than control male group. Among them, 6 miRNAs were significantly up-regulated, while 3 miRNAs were significantly down-regulated in ethanol-treated group compared to the male control group. And 477 target genes of up- and down-regulated 9 miRNAs were identified. On the other hand, 64 miRNAs were more than 1.5-fold up- or down-regulated in ethanol-treated female group than female control group. Among them, 3 miRNAs were significantly up-regulated, while 4 miRNAs were significantly down-regulated. And 327 target genes of up- and down-regulated 7 miRNAs were identified. Therefore, our results suggest that exposure to chronic alcohol differentially affects weight gain and miRNA expression depending on sex differences.

Disclosures: **M. Choi:** None. **J. Han:** None. **D. Kim:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.05/H38

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: U.S. Army Research Office and the Department of Defense Research and Education Program under grant number W911NF-15-1-0432
NIH grant 5R25GM077634-04

Title: Early synaptopathology in organophosphate-exposed hippocampal explants is governed by neuron-specific β_1 integrin responses

Authors: M. ALMEIDA[1], B. A. BAHR[2], *K. G. FARIZATTO[1];
[1]Biol., [2]Biotech Ctr. / William C. Friday Lab., Univ. of North Carolina at Pembroke, Pembroke, NC

Abstract: Organophosphates account for the world's deadliest poisons, causing severe symptoms and death in exposed individuals and first responders to nerve agent attacks. These toxins inhibit acetylcholinesterase which leads to cholinergic crises, seizures, and long-term neurological problems. Besides being used in warfare atrocities, organophosphates are still widely used as agricultural pesticides, thus a public health concern. We previously found that the organophosphate paraoxon produces oxidative and synaptic toxicity, leading to behavioral deficits (Farizatto et al. 2017, *J. Mol. Neurosci*. 63:115). Hippocampal explants exposed to paraoxon were also found to exhibit unique synaptotoxicity associated with selective integrin dynamics, implicating a compensatory response through the β1 integrin-cofilin pathway (Farizatto et al. 2019, *Scientific Reports*: **9**:6532 nature.com/articles/s41598-019-42934-z). In this follow-up study, paraoxon-exposed hippocampal cultures exhibited declines in synaptic proteins, independent of overt cellular, neuronal, or dendritic pathology. Synaptotoxic profiles were evident in the dendritic zones of the CA1, CA3, and dentate subfields, and they corresponded with β1 integrin responses. Noting that integrins form a bridge in many types of cell-cell interactions, it is of interest the enhanced β1 integrin staining was only evident in the neuropil, with no induction of such staining found in the astrocyte-rich area extending from the paraoxon-treated tissue. The active conformation of β1 integrin was targeted to synapses in response to the exposure, co-localizing with prominently-stained, synapsin II-positive puncta, even though this synaptic labeling had an overall reduction across the full dendritic area examined. As previously suggested, the stochastic events of β1 integrin signaling may signify a compensatory pathway to explain the apparent protection of select synapses. When the β1 integrin was blocked in hippocampal slices with the highly potent $\alpha_4\beta_1$ inhibitor BIO 5192, the tissue exhibited enhanced vulnerability to paraoxon. Disrupting the specific integrin potentiated the observed declines in synaptophysin and GluR1 levels 24 h after exposure. These findings suggest that anticholinesterase toxins mediate distinct synaptic damage, and the integrin signaling pathway plays an important compensatory role. Synaptic compromise in the hippocampus can be detrimental to many brain functions and integrin-linked responses activated by toxic insults appear to govern the extent of the synaptotoxicity.

Disclosures: **M. Almeida:** None. **B.A. Bahr:** None. **K.G. Farizatto:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

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Program #/Poster #: 387.06/H39

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant 5R03NS075512-02

Title: Transient receptor potential channel 1 involved in blood brain barrier disruption in mice treated with chlorpyrifos

Authors: *W. LI, M. EHRICH;
Virginia Tech., Blacksburg, VA

Abstract: We previously demonstrated that chlorpyrifos (CPF), a cholinesterase inhibitor, compromised the integrity of the blood-brain barrier (BBB) when used at low concentrations *in vitro*. To further explore the underlying molecular mechanism of the transient and reversible BBB disruption induced by CPF, we analyzed the expression levels of genes and proteins associated with transient receptor potential canonical (TRPC) channels in mouse brains at different time points after CPF treatment. The BBB integrity and function after CPF treatment were also evaluated by measuring the brain water content and molecule leakage into brain tissues. The results demonstrated that TRPC1 mRNA expression was significantly decreased in mouse brain 2-8 hours after CPF treatment. The TRPC1 protein levels in mouse brain were significantly increased at 1 hour post-CPF treatment. Corresponding to these TRPC1 gene and protein alterations, mild BBB leakage was observed in mice treated with CPF as measured by FITC-dextran extravasation. Results suggest that CPF treatment temporarily increased BBB permeability by transiently affecting TRPC1 protein expressions. Such a transient and reversible disruption of BBB has potential implications for CNS drug delivery.

Disclosures: W. Li: None. **M. Ehrich:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.07/H40

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Hong Kong Research Grants Council
National Key Basic Research Program of China

Title: Accumulation of cytoplasmic DNA in ATM deficiency activates the microglial viral response system with neurotoxic consequences

Authors: *F. X. SONG[1,2], F. MA[1], K. HERRUP[2];
[1]Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong; [2]Dept. of Neurobio., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: ATM (ataxia-telangiectasia mutated) is prominently involved in DNA damage repair, especially in DNA double-strand breaks in the double helix. Mutations in ATM cause a rare progressive multi-systemic disease known as ataxia telangiectasia (A-T). The neurological

symptoms of ataxia are profound Purkinje cell degeneration in the cerebellum. In addition to the neurological symptoms, however, patients with A-T suffer from a wide variety of symptoms such as telangiectasia, cancer and dysfunction of the immune system. The latter is characterized by low levels of antibodies and other immunoglobulins. The immunodeficiency in A-T is a logical consequence of the inefficient repair of DNA breaks during the process of V(D)J recombination which is crucial for T-cells and B-cells maturation. Given these problems in serum lymphocytes, most immunobiological studies focus on the role of ATM in V(D)J recombination. This downplays the potential impact of ATM deficiency on the brain. Yet anti-inflammatory strategies targeting the innate immune system have proven effective in A-T. So we have begun to examine the effects of ATM deficiency on the cells of innate immune system - microglia in the brain. We show that ATM dysfunction causes an accumulation of cytosolic DNA to trigger the anti-viral immune response of brain microglia via DNA sensor, STING, and active inflammatory pathway. Meanwhile, cytosolic DNA promotes signaling through the AIM2-containing inflammasome, resulting in the enhanced processing of inactive precursors into their fully active forms. Interestingly, DNA-seq revealed a unique distribution pattern of the cytosolic DNA among the genome. The small fragments within the cytoplasm belong to the intergenic regions which are far from the genes. More importantly, they match with the features of retroelements within the genome which can be used to precisely modify gene expression. We propose that, the cytosolic DNA fragments are signals for the communication between the nucleus and cytoplasm rather than random cut of the genome. Together, these processes create an inflammatory environment with strong neurotoxicity. The present findings highlight the significance of microglia-neuron interactions in the progression of the neurodegenerative symptoms of A-T and show how defective DNA damage repair can lead to an inflammatory response that has lethal consequences for neurons. We propose that, beyond their clear implications for the neurological symptoms in A-T, the present findings suggest the involvement of microglial DNA damage as a trigger for neuroinflammation during the etiology of other neurodegenerative conditions.

Disclosures: **F.X. Song:** None. **F. Ma:** None. **K. Herrup:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

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Program #/Poster #: 387.08/H41

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant R01 GM084979

Title: Sevoflurane and propofol have different potency to provide dual effects of neuroprotection and neurotoxicity in Alzheimer's disease cells

Authors: X. GAO[1], X. WANG[2], L. ZHANG[1], **G. LIANG**[1], R. MUND[2], H. WEI[1];
[1]Dept Anesthesiol. & Critical Care, [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: General anesthetics have different potencies in causing neurotoxicity. We have compared the potency of propofol vs. sevoflurane on cell survival and its mechanisms via the effects on cytosol ([Ca2+]c) or on mitochondria ([Ca2+]m) concentrations, in SH-SY5Y human neuroblastoma cells overexpressing wild type PS1 or mutated PS1 M146V. The cells were cultured and exposed to propofol or sevoflurane at various concentrations and durations. Cell viability was measured by the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. We treated the cells to equivalent propofol or sevoflurane (1 MAC or 0.455 mM vs. 2 μM) then measured both [Ca2+]c and [Ca2+]m using jellyfish photoprotein aequorin-based probes. Only 200 μM Propofol caused cell damage with equal potency in two types of cells, (clinically relevant concentrations: 1-20 μM). Sevoflurane at 4% for 3 hrs or 2% for 12 hrs promoted cell survival, but at 1% for prolonged exposure (24 hrs) resulted in significantly and equally increased cell damage in both types of cells. Only sevoflurane at 1 MAC but not equipotent propofol at 2 μM for 6 hrs was able to cause significantly and equally increased cell survival in both types of cell. Both sevoflurane and propofol had significantly higher cell response rate to the elevation of [Ca2+]c or [Ca2+]m in the presence of extracellular Ca2+ influx than in its absence. Propofol at 2 μM only slightly increased [Ca2+]c but not [Ca2+]m, which was more obvious in the presence of extracellular Ca2+ influx. Sevoflurane at 0.455 mM (equipotent to 1 MAC) caused significantly greater increase of peak and overall [Ca2+]c in AD than in control cells. This was ameliorated by the absence of extracellular Ca2+ influx. Sevofluane, but not propofol, caused a more significant increase in overall [Ca2+]m in WT than AD cells, in the presence of extracellular Ca2+ influx. In the absence of extracellular Ca2+ influx, sevoflurane, but not propofol, caused more significant elevations of overall [Ca2+]m in AD than control cells. Both Ca2+ influx from extracellular space and Ca2+ release coming from the endoplasmic reticulum contributed to the sevoflurane mediated elevation of [Ca2+]c or [Ca2+]m. However, propofol at clinically relevant concentrations affected intracellular Ca2+ homeostasis primarily through Ca2+ influx alone. Sevoflurane has a greater potency to affect cell survival than propofol, which is likely associated its effects on the elevation of [Ca2+]c or [Ca2+]m. Sevoflurane, but not propofol, at clinically relevant concentrations and durations provide dual effects of both neuroprotection and neurotoxicity at in both normal and AD cells.

Disclosures: X. Gao: None. **X. Wang:** None. **L. Zhang:** None. **G. Liang:** None. **R. Mund:** None. **H. Wei:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.09/H42

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH R01MH109719
UR EHS Pilot Grant
P30 ES00247
NIH T32GM007356

Title: The impact of early developmental arsenic exposure on hippocampal synaptic plasticity

Authors: *K. F. W. FOLEY[1], D. A. CORY-SLECHTA[2], H. XIA[3];
[1]Neurosci., [2]Dept. of Envrn. Med., [3]Pharmacol. & Physiol., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Early life exposure to toxic chemicals and environmental pollutants is associated with learning deficits and behavioral changes. An estimated 200 million people worldwide are exposed to arsenic concentrations in drinking water that exceed the World Health Organization's recommended limit (10ppb). Increased arsenic exposure is associated with increased all-cause mortality and deficits in children's cognitive and motor functions. While arsenic levels are kept below 10ppb in municipal water supplies, private wells are unregulated and ground water commonly exceeds 10ppb, including 20 out of 37 principal aquifers in the United States. Previous studies on arsenic exposure and synaptic function have demonstrated a decrease in synaptic transmission and long-term potentiation (LTP) in adult rodents, but have relied either on *in vitro* exposure or extended, adulthood exposure. Here, we study the effects of gestational and early developmental arsenic exposure in juvenile mice. C57BL/6 females were exposed to arsenic (0, 50ppb, 36ppm) in their drinking water starting at two weeks of age. These mice began breeding at ~1 month of age and continued to be exposed to arsenic after parturition. We then performed field recordings in acute hippocampal slices from the pre-weaning, juvenile offspring (P17-P23). In this paradigm, the juvenile mice are only exposed to arsenic *in utero* and via the mother's milk. Surprisingly, even with relatively low arsenic exposure (50ppb), we observed changes in basal synaptic transmission and plasticity in the offspring. These results suggest that indirect, ecologically-relevant arsenic exposure in early development impacts hippocampal synaptic transmission and plasticity.

Disclosures: K.F.W. Foley: None. **D.A. Cory-Slechta:** None. **H. Xia:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

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Program #/Poster #: 387.10/H43

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: JSPS KAKENHI Grant 16K21207
JSPS KAKENHI Grant 18K15518

Title: Neurogenesis in the adult hippocampus is affected via glial cells in the rat model of delayed carbon monoxide encephalopathy

Authors: *S. OCHI[1], K. SEKIYA[2], N. ABE[2], T. NISHIHARA[2], Y. YOSHINO[1], J. IGA[1], S.-I. UENO[1];
[1]Neuropsychiatry, [2]Dept. of Anesthesia and Perioperative Med., Ehime Univ. Grad. Sch. of Med., Toon, Japan

Abstract: Delayed carbon monoxide (CO) encephalopathy occurs following recovery after several weeks from acute CO poisoning. However, the mechanism of delayed neuronal injury remains unknown. Previously, we reported that the rat model of delayed CO encephalopathy showed cognitive impairment and hippocampal cell death, especially in the lesions of dentate gyrus (DG) and also the number of microglial cells and the mRNA expressions of several neurotrophic factors in the hippocampus at 7 days after CO exposure were decreased. Neurogenesis in the adult hippocampus plays important roles in a developmental process of cognitive function. Glial cells, especially astrocytes, are known to play a key role in promoting adult neurogenesis. Therefore, in the current study, we hypothesized that neurogenesis in the hippocampus may be affected in delayed CO encephalopathy and investigated the effects of delayed neuronal CO poisoning on neural precursor cells and glial cells. Wistar male rats (6 weeks old) were exposed to 1000 ppm CO for 40 min and then 3000 ppm for 20 min until they lost consciousness. If they did not lose consciousness in this 60 min, rats were exposed to 10000 ppm until they lost consciousness. Behavioral effects on learning and memory function were measured by the passive-avoidance test in controls and CO treated rats until 3 weeks. The latencies were significantly shorter in the CO models. Immunohistochemical analyses revealed that cell numbers in Sex determining region Y-box 2 (SOX2) and glial fibrillary acidic protein (GFAP) positive cells and microglia in the lesions of DG were significantly decreased in the CO rats compared with those in controls. Flow cytometry analyses also revealed that the number of microglia in the hippocampus were significantly reduced in CO rats. Realtime-PCR revealed that the mRNA expressions of Sox2, Gfap, CD11b, Fgf2 and Gdnf were significantly lower in CO rats (n=8) than those in the controls. However, there were no significant differences in the expression of Slc1a2, Slc1a3 and Bdnf between CO rats and controls. These results suggested that delayed CO injury might damage especially microglia, immature astrocytes and neural precursor cells and impede the restoration of them for several weeks. Thus, the impairment of neural precursor cells via abnormalities of glial cells may have important role in the pathogenesis of delayed CO encephalopathy.

Disclosures: S. Ochi: None. **K. Sekiya:** None. **N. Abe:** None. **T. Nishihara:** None. **Y. Yoshino:** None. **J. Iga:** None. **S. Ueno:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.11/H44

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NCTR/FDA Protocol E0767501

Title: Ketamine-induced adverse effects on the dopaminergic and serotonergic neuronal development is prevented by N-acetylcysteine in zebrafish

Authors: *Q. GU, B. ROBINSON, J. KANUNGO;
FDA Natl. Ctr. for Toxicological Res., Jefferson, AR

Abstract: The pediatric anesthetic ketamine is a non-competitive antagonist of the calcium-permeable N-methyl-D-aspartate (NMDA) receptor. In laboratory models, prolonged exposure to ketamine can induce cardiotoxicity and neurotoxicity in mammals and zebrafish. Here, we evaluated ketamine's effects on the development of dopaminergic (DA) and serotonergic (5-HT) neurons in zebrafish embryos. We also examined whether the antioxidant, N-acetylcysteine (NAC), could prevent ketamine's effects on those monoaminergic neurons. Using whole mount immunohistochemistry, the effects of ketamine were monitored on the brain 5-HT and tyrosine hydroxylase-immunoreactive (TH-IR) DA neurons. Ketamine exposure began at 28 hours post-fertilization (hpf) embryos and static exposure continued for 20 h. We used a ketamine concentration (2 mM) that produces an internal embryo exposure level comparable to human anesthetic plasma concentrations. At this concentration, ketamine exposure significantly reduced the areas occupied by 5-HT neurons in the brains of 48 hpf embryos. Additionally, TH-IR neurons in the brain and TH-IR cells in the trunk were significantly reduced. Previously, we described that acetyl-l-carnitine (ALCAR) prevented ketamine-induced neurotoxicity. Our current data show that co-treatment with NAC (1 mM) prevented ketamine's adverse effects on development of DA and 5-HT neurons in zebrafish embryos.

Disclosures: Q. Gu: None. **B. Robinson:** None. **J. Kanungo:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.12/H45

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: R01HL139712 (N.I.)
R01HL128546 (R.A.J)
Children's Heart Foundation (N.I.)
Foglia and Hills families
NIH IDDRC grant U54 HD090257

Title: Primary cilia related genes and neonatal anesthesia neurotoxicity in congenital heart disease

Authors: F. SOMAA, ***N. SARIC**, L. WANG, R. JONAS, K. HASHIMOTO-TORII, N. ISHIBASHI;
Children's Natl. Med. Ctr., Washington, DC

Abstract: Protein-damaging de novo gene mutations have been demonstrated to be strong predictors of neurodevelopmental anomalies in congenital heart disease (CHD). At least 15% of these genes have been found to be associated with primary cilia structure and/or function. Recent clinical studies have demonstrated a strong link between anesthetic exposure and neurodevelopmental outcomes in children with CHD. Studying the combined effect of anesthesia and primary cilia gene mutations will determine whether genetic alterations identified in CHD patients with neurodevelopmental abnormalities affect the molecular/cellular programs underlying brain development. Using Emx1cre; Ift88 f/f, in which primary cilia are lost specifically in cortical excitatory neurons we administered either PBS or Ketamine (20mg/kg) at postnatal day 7 (P7). We examined caspase-3, fractin and cleaved tubulin at P8 in the medial prefrontal cortex (mPFC). At P30, mice were behaviorally tested using water T-maze to assess their spatial memory performance and cognitive flexibility. To assess pyramidal neuron dendritic morphology, we used two other inducible Cre/lox mice to specifically label layer II (Nes-Cre/ERT2) and layer V (ER81-Cre/ERT2) neurons. We found significant enhancement of caspase-3, fractin and cleaved-tubulin immunoreactivity in the Ift88 knockout mice that were exposed to ketamine (cKO+Ket) compared to the other three groups (HT+PBS, HT+Ket, and cKO+PBS). During the reversal learning paradigm, cKO+Ket mice showed a strongly reduced ability to learn the task compared to other groups, indicating a cognitive flexibility deficit in this treatment group. We found that ketamine injection only had an effect on dendritic morphology in the Ift88 cKO mouse. Primary cilia play a key role in protecting cortical neurons from anesthesia-induced neurodegeneration. Our current results suggest that primary cilia deficiency, due to a common genetic predisposition with CHD, exacerbates the toxicity of neonatal anesthesia, thereby exaggerating neurobehavioral deficits in children with CHD.

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Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.13/H46

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Duke University Superfund Research Center ES010356

Title: Long-lasting behavioral effects of embryonic exposure of zebrafish to retinoic acid receptor acting environmental pesticides

Authors: *Z. R. HOLLOWAY[1], A. B. HAWKEY[1], D. UNAL[2], C. L. DEAN[2], A. AKHIESH[2], S. W. KULLMAN[3], E. D. LEVIN[1];
[1]Duke Univ. Med. Ctr., Durham, NC; [2]Duke Univ., Durham, NC; [3]North Carolina State, Durham, NC

Abstract: Retinoic acid receptor (RAR) signaling is known to play key roles in neurodevelopment. Disruption of RAR signaling in early neurodevelopment has been shown to be a key factor for increasing the risk of autism. Our previous work with the zebrafish model showed that embryonic disruption of RAR signaling with excess vitamin A (retinol) or exposure to valproic acid (VPA) causes behavioral dysfunction in larval zebrafish and also causes lasting behavioral disruption into adulthood. Recent high throughput screening of environmental contaminants has revealed a variety of chemicals which act on the retinoic acid receptor, including select several pesticides (see below). Further work is needed to link their putative retinoid activity to adverse behavioral outcomes. The present study measured the behavioral effects of embryonic exposure to these compounds in larval zebrafish, and these fish were assessed again in adulthood to determine if the effects persisted. Zebrafish embryos were exposed to vehicle (DMSO), chlorothalonil (CTL, 10- 100nM), imazalil (IML, 0.1-1μM), endosulfan-I (ESF, 0.1-1nM) or buprofezin (BPF, 0.3-3μM) from 5-120 hours post-fertilization (hpf). Doses fell below the threshold for overt toxicity as measured by increased lethality and dysmorphogenesis. Larval motility was assessed at 144hpf. Adult testing took place at 5-7 months of age. Each of the selected compounds significantly altered larval motility. The high doses of BPF and IML reduced motility under both light and dark conditions, while the lowest dose of IML increased motility regardless of lighting condition. The highest dose of CTL reduced motility in the dark, as did the lowest dose. The highest dose of ESF enhanced motility in the dark. For select compounds, behavioral effects were also found to persist into adulthood. In a novel tank dive test the highest dose of CTL reduced total motility and fish exposed to the moderate dose spent more time near the top of the tank, suggesting altered anxiety processes or increased risk-taking. For IML, both low and moderate doses reduced overall motility, while all three doses led to an increase in the time spent near the bottom of the tank, implying an increase

in anxiety. Further research is underway to complement behavioral functions tested in zebrafish developmentally exposed to these compounds. Zebrafish can provide an economic model to screen compounds for neurobehavioral toxicity to help prioritize further testing with rodent models.

Disclosures: Z.R. Holloway: None. **A.B. Hawkey:** None. **D. Unal:** None. **C.L. Dean:** None. **A. Akhiesh:** None. **S.W. Kullman:** None. **E.D. Levin:** None.

Poster

387. Mechanisms of Neurotoxicity II

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Program #/Poster #: 387.14/I1

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: PAPIIT-UNAM (IN214118) to Jorge Guevara
VIEP-BUAP grant (No. DIFA-NAT18-I) to Samuel Treviño
VIEP-BUAP grant (TEMS-NAT18-I) to Alfonso Díaz

Title: Cd promotes an antioxidant response in rat hippocampus

Authors: *G. PULIDO-FERNANDEZ[1], S. TREVIÑO[1], E. BRAMBILA[1], R. A. VAZQUEZ[2], P. AGUILAR-ALONSO[1], J. MORÁN-PERALES[3], A. HANDAL SILVA[3], J. GUEVARA[4], G. FLORES[2], A. DÍAZ[1];
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Abstract: Cadmium (Cd) is a heavy metal, classified as a carcinogen whose exposure could affect the function of the Central Nervous System. Studies suggest that Cd modifies neuronal morphology in the hippocampus, consequently affecting cognitive tasks. The oxidative stress pathway is proposed as the mechanism of toxicity. However, this mechanism is not clear yet. The aim of the present work was to study the effect of Cd administration on oxidative stress markers in rat hippocampus for 2, 3 and 4 months. For this, one-month old male Wistar rats weighing between 80 to 100 g were used. Animals were randomly divided into two groups (n=30/ group): 1) control (drinking water) and 2) treatment with Cd. 32.5 ppm of Cd was added to the water in the form of cadmium chloride ($CdCl_2$) for the treatment group, while the control group received only purified water, provided daily *ad libitum*. Each group was subdivided into three groups (n = 10), with the purpose of evaluating the effect of Cd-exposure over two, three and four-month periods. The data were reported as the mean ± standard error (SE). A Student's t-Test where $p < 0.05$ was considered significant was used to analyze the results of Cd, ROS, MDA and glutathione (reduced and oxidized) concentration, and the activity of antioxidative

enzymes (superoxide dismutase, catalase, glutathione reductase, peroxidase and transferase). The results show that the oral administration of Cd increased the Cd concentration in hippocampus after 3 y 4 months. Likewise, it caused an increase in MDA (a marker of lipoperoxidation) and in the activity of glutathione enzymes. While the levels of ROS, the activity of catalase and superoxide dismutase and glutathione content didn't change through the study. These results suggest that oral administration of Cd promotes its accumulation in hippocampus. Accordingly, oxidative response events exacerbate, which in long term contribute to the hippocampal neurodegeneration and to deterioration of memory. Our results suggest that the exposure to Cd represents a critical health problem, which if not addressed quickly, could cause much more serious problems in the quality of life of the human population, as well as in the environment in which they develop.

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Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.15/I2

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH AG023084 to BVZ
NIH NS034467 to BVZ
Cure Alzheimer's Fund to BVZ

Title: Protein PICALM has an important role in neuronal health and brain homeostasis

Authors: *D. LAZIC[1], A. MONTAGNE[1], Z. DAI[1], Z. ZHAO[1], K. KISLER[1], A. SAGARE[1], T. MAEDA[2], B. ZLOKOVIC[1];
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Abstract: *PICALM*, a gene encoding phosphatidylinositol-binding clathrin assembly protein is associated with Alzheimer's disease (AD). Besides its well-known role in regulating intracellular trafficking of endocytic vesicles, studies in $APP^{Sw+/0}$; $Picalm^{+/-}$ mice revealed that PICALM is involved in the clearance of amyloid-β across the blood-brain barrier. Additionally, genetic analyses and magnetic resonance imaging (MRI) in humans suggested the positive correlation of protective *PICALM*-associated single-nucleotide polymorphism (SNP) $rs3851179^{A}$ with higher hippocampal volume and increased entorhinal cortical thickness, when compared to SNP $rs3851179^{G}$. Because PICALM is crucial for embryonal development and it plays essential roles

in regulating axonal growth and turnover of synaptic vesicles and receptors, a complete deletion of PICALM from neurons *in vivo* can be detrimental to neuronal homeostasis. In order to examine the role of neuronal PICALM *in vivo*, we generated tamoxifen-inducible neuron-specific PICALM knockout mouse line, *Picalm$^{lox/lox}$; Camk2a-CreER*. Four weeks after 4-hydroxy tamoxifen administration, *Picalm$^{lox/lox}$; Camk2a-CreER* mice showed impaired performances in hippocampal-dependent behavioral tests. Volumetric MRI studies and immunohistochemistry showed that *Camk2a-CreER* mice exhibit brain atrophy, most prominent in hippocampus, and enlargement of lateral and third ventricles. Studies in primary neuronal culture using *Picalm$^{-/-}$* embryos showed increased baseline activity, increased cell surface expression of glutamate receptors and higher susceptibility to glutamate toxicity. Altogether, our data suggest that PICALM plays an important role in cognition and neuronal health, and that the mechanism of neurotoxicity in PICALM-deficient neurons may involve altered localization of glutamate receptors.

Disclosures: D. Lazic: None. **A. Montagne:** None. **Z. Dai:** None. **Z. Zhao:** None. **K. Kisler:** None. **A. Sagare:** None. **T. Maeda:** None. **B. Zlokovic:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.16/I3

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: MOF 20180430

Title: Abnormal activation of NMDA receptors by glufosinate ammonium

Authors: *D. WOO, Y. KIM, D. KANG;
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Abstract: (GLA) (Glufosinate-ammonium) is widely known as an herbicide and is known to cause neurotoxicity and thereby affect neural development. However, much of the mechanism of GLA has not been studied. Because the structure of GLA is similar to that of Glutamate, the effect of GLA on N-Methyl-D-aspartic acid (NMDA) receptors was evaluated by monitoring Fura-2 binding intracellular Ca2+. 100 μM Glutamate / 50 μM Glycine activated NMDA receptors of the primary cortical neurons of postnatal rat day 2. The cells treated with 1 mM GLA / 50 μM Glycine showed 44.5 ± 6.34 % of NMDA receptor-activated Ca2+ transient responsive cells and 0.055 ± 0.007 peak ratio compared to 0.38 ± 0.31 mediated by the activation of NMDA receptor. On the other hands, we did not have Ca2+ transients of primary cortical astrocytes. These results suggest that GLA targets NMDA receptors of neuronal cells. We also tasted that D-2-amino-5-phosphonopentanoate (DAP5), competitive NMDA receptor antagonist

inhibited GLA/Glycine-induced Ca2+ transient by decrease of 20.5 ± 18.3 % peak ratio. In addition, free Mg2 + of external solution increased GLA/Glycine responsiveness by 41.8% in number and 37.4% in peak ratio compared to those of 2mM external Mg2+. The evidences suggest that GLA activates NMDA receptors therefore and affect critical time in brain development stage.

Disclosures: D. Woo: None. **Y. Kim:** None. **D. Kang:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.17/I4

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Beta-methylamino-L-alanine induces neuronal injury via kinase dependant mechanisms in rat primary neuronal cultures

Authors: C. DUCHEMIN-NEVEU, ***E. ANDRIAMBELOSON**, S. WAGNER;
Neurofit SAS, Illkirch, France

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that leads to the death of patients within 3-5 years after diagnosis. The only drug (riluzole) that exists prolongs the lifespan of patients by a few months. Although the pathophysiology of ALS is not completely understood, it shows close similarities with other neurodegenerative disease like AL-SPDC (Amyotrophic lateral sclerosis/parkinsonism-dementia complex) characterized by clusters of dysfunctional proteins within neurons. Indeed, several studies have pointed to the potential role of dietary exposure to the cyanobacteria-derived non-amino acid, β-Methylamino-L-alanine (L-BMAA) as a possible risk factor for ALS or ALS/PDC. Furthermore, post-mortem analyses indicate the presence of L-BMAA in the brains of patients that suffered from non-genetic progressive neurodegenerative disease, including ALS. The present study was undertaken to evaluate the neurotoxicity of L-BMAA in primary neuronal cultures prepared from the brain and spinal cord of rat embryos. In order to assess the putative role of protein phosphorylation in the neurotoxicity of L-BMAA, the potential neuroprotective effect of two candidate protein kinases (casein kinase 1-δ and Glycogen synthase kinase-3) inhibitors were investigated. The results showed that L-BMAA induces the death of different population of rat neurons in a dose-dependent manner. Neuronal damage was detected as early as 24h after exposure to L-BMAA but became substantial when the exposure was extended up to 7 days. Whilst the L-BMAA neurotoxicity was detected under the different cultures of neurons, those from the spinal cord appeared to be the most sensitive when compared to those from the brain. Furthermore, in the presence of a casein kinase 1 inhibitor (LH-846 or PF670462) and of GSK3 inhibitor (A1070722 or TCS-2002), the neurotoxicity of L-BMAA was suppressed. Thus, the above results suggest an

enhanced neurotoxicity of L-BMAA towards spinal motor neurons. It appears that L-BMAA neurotoxicity is mediated by protein phosphorylation mechanisms involving protein kinases such as casein kinase 1-δ or Glycogen synthase kinase-3.

Disclosures: C. Duchemin-Neveu: None. **E. Andriambeloson:** None. **S. Wagner:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.18/I5

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: R21DA047936 (MS)
R03DA043428 (MS)
R01DA035714(JZ)
NIH CA223956 (MW)

Title: Investigating interactions between microglia and astrocytes as models of hand

Authors: *D. A. ODHIAMBO;
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Abstract: While the deployment of combination antiretroviral therapy [CART] has seen to the decline in AIDS-associated deaths, HIV-associated neurocognitive disorders [HAND] remain increasingly high. The production of HIV-proteins mainly Tat and gp120 in the central nervous system is characterized by direct or indirect neuronal toxicity. Studies have reported heightened severity in HAND in combination with the abuse of drugs such as cocaine. In elucidating mediators of immune activation in the brain, studies have suggested a role for microglia and astrocytes in the production of toxic factors that promote neuronal damage and subsequent inflammation in the brain.
In this regard, we are investigating the role of HIV-1 regulatory protein, Tat in combination with cocaine, in microglia and astrocyte activation. We compared the direct effects of the HIV-Tat and cocaine, on cortical mixed neuronal and glial cultures, as well as the effects of conditioned medium produced by treated glial cultures on mixed neuronal cultures. Neurotoxicity of Tat and cocaine; and conditioned medium; and their effects on microglia and astrocytes activation were analyzed.
Astrocytes-microglial cells were isolated from adult rat, cultured and treated with Tat, cocaine and a combination thereof. Conditioned medium was collected from the treated astrocyte-microglial cultures and applied on mixed neuronal cultures. These astrocytes-microglial cultures were then immunostained for microglia and astrocyte specific markers. Primary cortical cultures were also analyzed for apoptosis/cell death. Effects of conditioned medium were compared with

the effects of direct Tat and cocaine treatment on mixed neuronal cultures.
Contrary to effects of HIV-Tat and cocaine on mixed neuronal cultures, there was no evident microglial activation; microglia activation is characterized by an increase in size and number of microglia. There was, however, a change in the standard astrocyte phenotype to the activated neurotoxic A1 phenotype. Neurons exposed to treated astrocyte-microglia conditioned medium showed an increase in cleaved Caspase3/7 and ethidium bromide positive cells, suggesting cell death.
Our lab has recently shown that Tat and cocaine dependent astroglia activation is inhibited by RK-33, a selective inhibitor of Dead Box RNA helicase 3 [DDX3]. We demonstrated that DDX3 inhibition protects neurons from combined toxicity of HIV proteins and cocaine. Combined, these results suggest a role for both astrocytes and microglia in the production of neurotoxins that cause neuronal damage with indication that astrocytes are possibly the primary target of HIV regulatory protein Tat.

Disclosures: D.A. Odhiambo: None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.19/I6

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Resveratrol prevents the ethanol-induced oxidative stress on hippocampus of Wistar rats

Authors: *D. JUÁREZ SERRANO[1], I. CESAR-ARTEAGA[1], F. RAMOS-COLLAZO[2], J. C. MORALES-MEDINA[4], A. D. DIAZ-FONSECA[3], P. AGUILAR-ALONSO[1];
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Abstract: Ethanol is a psychoactive substance with dependency-causing properties, a high intake is associated with memory loss and neuronal disturbance associates to oxidative stress. Oxidative stress is an imbalance between oxidants and antioxidants leaning towards an oxidative environment, which potentially leading to cell and molecular damage. Free radicals are eliminated by antioxidant endogenous, if these is not enough to counteract oxidative damage there are used antioxidant exogenous. It has been found that chronic ethanol intake favors deterioration of the hippocampus, inhibiting cell survival and altering neuronal morphological maturation. To counteract these effects, various antioxidant both natural and synthetic, have been described, one of which is Resveratrol, a polyphenol with antioxidant, anti-aging, anticancer and neuroprotective properties. The aim of this work is to analyze if resveratrol is able to prevents the ethanol-induced oxidative stress hippocampus Wistar rats. Male Wistar rats, 3 months old,

were divided into the following categories: Control (without treatment), 4 groups administered with high doses of ethanol (20, 30, 40 and 50% ethanol), respectively and other 4 groups administered with resveratrol 10 mg / kg / day. All groups were daily administered orally for 2 months and all groups have n=3 animals. Posteriorly, the hippocampus was obtained for quantification of nitrite production, malondialdehyde (MDA), MDA + 4-hydroxinonenal (4-HDA) and enzymatic activity of Catalase and Superoxide Dismutase. The levels of nitric oxide and lipoperoxidation products rise significantly when the concentration of ethanol increases compared to the control group, however, the treatment with Resveratrol significantly reduces the oxidative stress caused by a high consumption of Ethanol. The enzymatic activity studied (catalase and superoxide dismutase) did not present significant changes with respect to the controls. Results show that resveratrol prevents damage by acting as an independent scavenger to the endogenous system. In addition, there is a decrease in markers of oxidative stress when resveratrol is administered in high alcohol consumption. In conclusion, resveratrol prevents the ethanol-induced oxidative stress on hippocampus of Wistar rats decreasing cellular lipoperoxidation but not to the activation of the enzymes catalase and superoxide dismutase.

Disclosures: **D. Juárez Serrano:** None. **I. Cesar-arteaga:** None. **F. Ramos-collazo:** None. **J.C. Morales-medina:** None. **A.D. Diaz-fonseca:** None. **P. Aguilar-alonso:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.20/I7

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: VIEP- Haciendo Ciencia en la BUAP Primavera 2019

Title: Resveratrol protects from oxidative stress produced by chronic administration of ethanol in rat cerebellum

Authors: *C. GARCÍA-OLVERA[1], D. JUÁREZ SERRANO[2], I. CESAR ARTEAGA[3], G. PULIDO-FERNANDEZ[4], A. R. NAVARRO-CRUZ[2], L. G. GARCÍA-ALBARRÁN[2], P. AGUILAR-ALONSO[5];
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Abstract: The abuse of alcohol consumption is a public health problem. Chronic consumption of ethanol has been associated with permanent impact on the morphology, structural function and membrane lipid organization of brain. An important region affected is cerebellum, which is

responsible for regulating vascular tone, posture, spinal movements, balance and the execution of fine movements. During a chronic consumption of ethanol, both the execution of movements and the balance are affected. A mechanism to explain cerebellar degeneration is oxidative stress, where Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are involved. It is mediated by the endogenous antioxidant system. The use of natural antioxidants as Resveratrol have a neuroprotector activity. The aim of this work is evaluate oxidative stress induced by chronic consumption of ethanol in rat cerebellum. Male Wistar rats of 3 months of age divided into the following groups were used: control (without treatment), 8 groups treated with ethanol (2.5, 5, 7.5, 10, 20, 30, 40 and 50 %) and other 8 groups treated with 10 mg/ kg/day of resveratrol + ethanol concentrations mentioned above. All groups were daily administered oral via for 2 months (n=3). Cerebellums were obtain for quantification of nitrites and hydroperoxydes production. Results show a significant decrease in levels of nitric oxide in groups administered with ethanol compared with the control group, although this levels rise when ethanol increases, also it is observed that the combined administration of ethanol + resveratrol in high doses prevents the elevation of these levels. When lipoperoxidation intermediates was analyzed, hydroperoxide levels are increased from the 10% ethanol group and progressively maintained until 50%. In the resveratrol groups, the protective effect is observed that inhibits the formation of hydroperoxides when it is exceeded at 50% ethanol. The large amount of polyunsaturated fatty acids in cerebellums makes it susceptible to lipoperoxidation reactions. The reduction and degradation of hydroperoxides is carried out by the glutathione system coupled to other enzymes of the endogenous antioxidant system, such as catalase and superoxide dismutase. The daily intake of resveratrol favors the reduction of these lipoperoxidation intermediates generated during chronic consumption of ethanol. It also acts as a regulator of the production of nitrites, and could prevent the formation of RNS such as peroxynitrites, associated with various neurodegenerative diseases.

Disclosures: C. García-Olvera: None. **D. Juárez Serrano:** None. **I. Cesar Arteaga:** None. **G. Pulido-fernandez:** None. **A.R. Navarro-Cruz:** None. **L.G. García-Albarrán:** None. **P. Aguilar-Alonso:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.21/I8

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Vanadium pentoxide inhalation effects on motor performance and motor cortex cytology

Authors: O. MEJÍA-GARCÍA[1], M. RODRÍGUEZ-ALCÁNTARA[1], E. MONTIEL-FLORES[1], A. GUTIÉRREZ-VALDEZ[1], *L. REYNOSO-ERAZO[2], J. ESPINOSA-VILLANUEVA[1], J.

ORDOÑEZ-LIBRADO[1], M. AVILA-COSTA[1];
[1]Neurosci., [2]Hlth. Educ., Univ. of Mexico, Mexico, Mexico

Abstract: Vanadium (V) is a transition metal that which has three oxidations states in the environment. The population is daily exposed to low levels of V on food and water, however, the inhaled way is the most important route since this metal is emitted into the atmosphere after the burning of fossil fuels. For these reasons there have been studied different experimental models with inhaled exposition to Vanadium pentoxide (V_2O_5)in mice, which have showed that this metal causes CNS alterations, including dendritic spines alterations and neuronal necrotic-like cell death in hippocampus, as well as decrease in TH^+neurons in the Substantia nigra compacta (SNc). The neuronal alterations are related to the capacity of V to enter into the cells to cause oxidative stress, lipoperoxidation, besides the alterations in the intracellular pathways and cytoskeleton. Furthermore, it has been reported that V oral exposition produces motor and memory alterations in rats. Therefore we aim to assess if V_2O_5inhalation in rats induces motor alterations and morphological changes in motor cortex pyramidal neurons. 10 Wistar rats inhaled V_2O_5 [0.02 M] 1 h twice a week for 6 months and motor activity was evaluated in the beam-walking task once a month. All rats was sacrificed and perfused after the last inhalation and processed for Golgi stain to determine the dendritic spines number in pyramidal neurons of the primary motor cortex. Our findings show that after 6 months of V inhalation there were not significative differences in the motor activity compared to the control group. However, V causes significant cytological damage in the number of dendritic spines in experimental group (mean= 5) compared to the control group (mean= 9.1). We concluded that after 6 months, V_2O_5inhalation induces motor cortex pyramidal neurons alterations. Nevertheless, is not enough to cause motor activity impairments.

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Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.22/I9

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: The effects of different combinations of plant extracts and traditional chinese medicine on hydrogen peroxide- and thapsigargin-induced neurotoxicity

Authors: *K. SUEN[1], Y. CHEUNG[1], T. HSU[1], S. WONG[1], S. CHAN[1], H. CHENG[1], S. LAW[1], Y. CHONG[1], K. HUNG[1], H. WONG[1], K. WAN[1], H. LEUNG[1], K. LIU[1], T. LI[1], W. TANG[1], M. LIN[1], H. CHEUNG[1], R. C. CHANG[2];

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Abstract: Oxidative stress and endoplasmic reticulum (ER) stress have been known as some of the significant causes of neuronal cell death in many neurodegenerative diseases. Agents that can attenuate these stresses are believed to have neuroprotective effects. *Allium fistulosum* has been shown to have antioxidant and antimicrobial activities (Chang et al., 2016). The root extract of *Clitoria ternatea* has been found to improve memory in the chronic cerebral hypoperfusion rat model (Damodaran et al., 2018). *Hibiscus sabdariffa* aqueous extract has been shown to have protective effects against acute cerebral ischaemia (Owoeye and Gabriel 2017). *Sterculia lychnophora* has been reported to have neuroprotective effects against hydrogen peroxide-induced toxicity (Wang et al., 2013) while its activity against ER stress has not yet been studied. Traditional Chinese medicine is usually formulated as a complex of herbs which may have synergistic effects for a disease (Zhou et al., 2016). In the present study, the effects of different combinations of extracts of *Allium fistulosum*, *Clitoria ternatea*, *Hibiscus sabdariffa* and *Sterculia lychnophora* against hydrogen peroxide- and thapsigargin-induced neuronal cell death were studied. The extracts were prepared by dissolving the powder of ground dried *Allium fistulosum*, *Clitoria ternatea*, *Hibiscus sabdariffa* and *Sterculia lychnophora* in absolute ethanol and insolubles were filtered out by 0.22 micro-meter membrane filter. Undifferentiated SH-SY5Y cells were pre-treated with a single or multiple plant extracts for 24 hours in DMEM supplemented with 2% fetal bovine serum. Following the removal of the extracts, SH-SY5Y cells were treated with 400 micro-molar of hydrogen peroxide or 0.5 micro-molar of thapsigargin for 24 hours in DMEM supplemented with 2% fetal bovine serum. Results indicated that there were differential effects of the extracts against hydrogen peroxide- and thapsigargin-induced cytotoxicity, implying that the combined applications of these herbal extracts may have different effects on neuronal cell death induced by oxidative stress and ER stress.

Disclosures: K. Suen: None. **Y. Cheung:** None. **T. Hsu:** None. **S. Wong:** None. **S. Chan:** None. **H. Cheng:** None. **S. Law:** None. **Y. Chong:** None. **K. Hung:** None. **H. Wong:** None. **K. Wan:** None. **H. Leung:** None. **K. Liu:** None. **T. Li:** None. **W. Tang:** None. **M. Lin:** None. **H. Cheung:** None. **R.C. Chang:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.23/I10

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: CIHR CGS-D
CIHR Grant FDN-154312

Title: Double-hit of inflammation and general anesthesia causes persistent impairment in executive function

Authors: *S. KHODAEI[1], D. WANG[1], B. A. ORSER[2];
[1]Physiol., Univ. of Toronto, Toronto, ON, Canada; [2]Depts. Physiol & Anest, Univ. Toronto, Toronto, ON, Canada

Abstract: Cognitive impairment in patients during the postoperative period is common and associated with poor long-term outcomes, prolonged length of hospital stay, and increased mortality. The mechanisms underlying such cognitive impairments are likely multifactorial but poorly understood. Preclinical findings suggest that general anesthetic drugs and preoperative inflammation contribute to postoperative cognitive deficits; however, to date, the two factors have been studied in isolation. The consequences of a "double-hit" of inflammation and anesthesia on cognition are unknown. Here, we tested the hypothesis that a double-hit of inflammation and anesthesia causes greater impairment in cognitive performance than each factor alone. To induce inflammation, adult male C57BL/6 mice were injected with lipopolysaccharide (LPS; 1.0 mg/kg, i.p.) or vehicle. The mice were next treated with the general anesthetic drug etomidate (20 mg/kg, i.p.) or vehicle 24 hours later. Over the next 4 days, memory and executive function were studied using the novel object recognition (NOR) assay and the 3-day puzzle box assay, respectively. The results showed that in the NOR task, LPS but not etomidate alone impaired recognition memory. The combination of LPS and etomidate caused memory deficits that were no different than LPS alone. In contrast, in the puzzle box assay, mice treated with either LPS or etomidate alone showed no impairment in executive function, whereas the double-hit caused impairment. These results suggest that different cognitive domains are differentially affected by the combination of anesthesia and inflammation. Ongoing studies will examine other cognitive domains such as spatial memory, the effects of more commonly used anesthetic drugs such as sevoflurane and propofol, and the molecular mechanisms underlying the performance deficits.

Disclosures: S. Khodaei: None. **D. Wang:** None. **B.A. Orser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Orser is an inventor named on a Canadian patent (2,852,978), a US patent (9,517,265) and a pending US patent (62/268,137).

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.24/I11

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant ES024756 RO1

Title: Protective effects of astrocyte-specific deletion of yin yang 1 in the substantia nigra on manganese-induced dopaminergic neuronal injury in mice

Authors: *J. A. JOHNSON, Jr[1], E. PAJARILLO[1], A. RIZOR[1], M. ASCHNER[2], E. LEE[1]; [1]Col. of Pharm. and Pharmaceut. Sci., Florida A&M Univ., Tallahassee, FL; [2]Dept. of Mol. Pharmacol., Albert Einstein Sch. of Med., New York, NY

Abstract: Chronic manganese (Mn) exposure causes a Parkinson's disease (PD)-like neurological disorder referred to as manganism. Our previous studies have shown that Mn increased expression of transcription factor yin yang 1 (YY1) which repressed astrocytic glutamate transporters, glutamate transporter 1 (GLT-1) and glutamate aspartate transporter 1 (GLAST) in astrocytes. Since the reduced astrocytic glutamate transporter expression could result in Mn-induced excitotoxicity in vivo, in this study, we have tested if astrocytic YY1 mediates Mn-induced dopaminergic neurotoxicity via reduction of astrocytic GLT-1 and GLAST in the substantia nigra pars compacta (SNpc) of mouse brain. To test the hypothesis, we injected AAV viral vectors expressing Cre recombinase and green fluorescent protein under the control of glial fibrillary acidic protein (GFAP) promoter (AAV5-GFAP-Cre-eGFP) into the SNpc of YY1-loxP mice bilaterally to delete YY1 only in astrocytes of the SNpc region of YY1 loxP mice. AAV5-GFAP-eGFP vectors were used as controls. Three weeks after the injection, mice were exposed to Mn (or H_2O as a vehicle, intranasal instillation, 30 mg/kg, daily) for 21 days prior to behavioral tests such as open field and rotarod assessment, followed by immunohistochemistry (IHC) and protein/mRNA expression levels of tyrosine hydroxylase (TH), YY1, GLT-1 and GLAST in the midbrain of mice. Results showed that Cre recombinase was expressed and it deleted YY1 efficiently in astrocytes. The behavioral studies revealed that deletion of astrocytic YY1 in SNpc attenuated Mn-induced reduction of locomotor activity and motor coordination compared with control. Astrocytic YY1 deletion in SNpc also reversed the Mn-induced reduction of TH mRNA/protein levels. Moreover, astrocytic YY1 deletion in SNpc also attenuated Mn-induced repression of GLT-1 and GLAST in midbrain, suggesting that astrocytic YY1 is involved in Mn-induced dopaminergic neurotoxicity potentially via reduction of astrocytic glutamate transporters GLT-1 and GLAST. These findings suggest that astrocytic YY1 could serve as a potential target for the development of therapeutics to restore astrocytic glutamate transporter function and prevent excitotoxic neuronal injury.

Disclosures: J.A. Johnson: None. **E. Pajarillo:** None. **A. Rizor:** None. **M. Aschner:** None. **E. Lee:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.25/I12

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: CIHR Foundation Grant FDN-154312

Title: General anesthesia causes a persistent decrease in brain-derived neurotrophic factor in the hippocampus

Authors: *A. ALAVIAN-GHAVANINI[1], M. A. MANZO[1], D.-S. WANG[1], L. KAUSTOV[3], B. A. ORSER[1,3,2];
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Abstract: Postoperative neurocognitive disorder occurs in some patients and general anesthetic drugs may be a contributing factor. Our previous preclinical studies have shown that many commonly used anesthetic drugs trigger a sustained increase in cell-surface expression of extrasynaptic $GABA_A$ receptors in neurons and cause cognitive deficits. Co-treatment with the α2 adrenergic receptor agonist dexmedetomidine, prevents overexpression of extrasynaptic $GABA_A$ receptors and attenuates cognitive impairment. Dexmedetomidine is neuroprotective likely because it increases the levels of Brain-Derived Neurotrophic Factor (BDNF) in the brain. Based on these results, we hypothesize that general anesthetic drugs reduce BDNF, leading to overexpression of $GABA_A$ receptors and cognitive deficits. The goal is to determine whether a clinically relevant dose of a general anesthetic drug causes a persistent decrease of BDNF in the brains of adult mice. Male C57BL/6J mice (2-3 months old) were treated with isoflurane (1.3% for 1 hr) or air. One or 7 days later, BDNF mRNA and protein levels were measured in ex vivo hippocampi with qPCR and Western blotting, respectively. The results showed that BDNF mRNA levels were slightly reduced at Day 1 (74.2 ± 29.0% of control; mean ± SD, $n = 3$, $p = 0.34$ compared with control) and significantly reduced at Day 7 (22.3 ± 10.3% of control; $n = 3$, $p < 0.05$ compared with control). BDNF protein was 112.5 ± 16.2% of control at Day 1 ($n = 3$, $p = 0.39$ compared with control) and 65.6 ± 9.6% of control at Day 7 ($n = 3$, $p < 0.05$ compared with control). Thus, a clinically relevant dose of isoflurane downregulates BDNF gene expression and protein levels for at least 7 days. Interestingly, our previous results have shown that overexpression of $GABA_A$ receptors persists for one week after isoflurane, so the two mechanisms correlate in time. Ongoing studies will determine whether other anesthetic drugs alter the BDNF levels and the time course of BDNF recovery.

Disclosures: A. Alavian-Ghavanini: None. **M.A. Manzo:** None. **D. Wang:** None. **L. Kaustov:** None. **B.A. Orser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Orser is an inventor named on a Canadian patent (2,852,978), a US patent (9,517,265) and a pending US patent (62/268,137)..

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.26/I13

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant U54 NS079202
NIH Grant R01 ES016308

Title: Chlorpyrifos (CPF)-induced neurobehavioral deficits and neuropathology in adult male rat

Authors: *Y.-H. TSAI, N. LEVOE, J. VU, D. A. BRUUN, P. J. LEIN;
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Abstract: Organophosphate (OP) cholinesterase inhibitors are a family of potent neurotoxic chemicals that includes nerve agents and pesticides. Chronic low-level exposures to OPs are associated with cognitive deficits in humans but the mechanism(s) underlying chronic OP neurotoxicity remain speculative, although mechanism(s) other than acetylcholinesterase inhibition are widely postulated to be important. Here, we investigated the hypothesis that subchronic exposure to the OP chlorpyrifos (CPF) causes neurobehavioral deficits via oxidative stress and/or neuroinflammation. Using a rat model based on documented human occupational exposures to CPF, adult male Long Evans rats were exposed to 10 mg/kg/d CPF (s.c.) or vehicle (10% Ethanol+90% Neobee M-5) for up to 21 d. A subset of animals were pretreated with the antioxidant Trolox (1 mg/kg/d i.p.) beginning 5 d prior to and continuing throughout CPF exposure. Brains collected after 3, 10, 15, or 21 d of CPF exposure were evaluated for: (1) neurodegeneration as assessed using FluoroJade C staining; (2) microglial activation and astrogliosis, as assessed by IBA-1 and GFAP immunoreactivity, respectively; and (3) neuronal oxidative stress as determined by co-immunoreactivity for 3-nitrotyrosine (3-NT), a marker of protein nitration, and NeuN label neurons. Animals treated with CPF for 21 d exhibited deficits in cued fear conditioning test compared to vehicle controls. In contrast, performance in cued fear conditioning was not significantly different between CPF-exposed animals treated with Trolox and vehicle controls. Chronic CPF exposure did not increase FJC staining in multiple brain regions (such as hippocampus, thalamus, piriform cortex, and amygdala) at the time points examined, and levels of neurodegeneration were not altered by Trolox treatment. CPF exposure increased GFAP immunoreactivity in a brain- and exposure length-dependent manner, and these effects were attenuated in CPF-exposed animals pretreated with Trolox. Trolox treatment also altered CPF effect on 3-NT immunoreactivity. Collectively, these results identify neuroinflammation, and potentially oxidative stress, as novel mechanism(s) of chronic CPF-induced neurotoxicity, and suggest that antioxidants may be useful in protecting against the neurotoxic effects of OPs.

Disclosures: **Y. Tsai:** None. **N. Levoe:** None. **J. Vu:** None. **D.A. Bruun:** None. **P.J. Lein:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.27/I14

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: CNPq
INCT (EN 465671/2014-4)/CNPq

Title: Evaluation of glutamate homeostasis, ATPases and depressive status in rats subjected to mild hyperhomocysteinemia

Authors: *T. M. DOS SANTOS, C. SIEBERT, L. D. BOBERMIN, A. Q. DOS SANTOS, C. A. NETTO, A. T. S. WYSE;
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Abstract: Increased levels of homocysteine (HCY) can be associated with several disorders, including psychiatric diseases. However, the mechanisms related to these changes are not well-known. In the present study, we evaluate depressive-like parameters such as body weight gain, adrenal/body weight ratio, sucrose preference and forced swimming tests, as well as activity and protein abundance of catalytic (α1 and α3) and regulatory (β1) subunits of Na^+, K^+-ATPase in the amygdala and prefrontal cortex of male rats submitted to chronic mild hyperhomocysteinemia (HHCY) chemically-induced. Mg^{2+}-ATPase, glutamate uptake, and cell viability were also evaluated. Wistar male rats received a subcutaneous injection of HCY (0.03µmol/g of body weight) or saline (0.9% 0.5mL/g of body weight) twice a day for 30^{th} to 60^{th} days of life. From 61 days of life, rats were submitted to behavior analyzes (n=13 animals/group) and euthanatized for brain and adrenal dissection (n=7-8 animals/group). Statistical analyzes were performed by Student's t-test. Mild HHCY did not impair body weight gain, adrenal/body weight ratio, 1% sucrose preference and swimming tests (both $p>.05$). Chronic mild HHCY increased total ATPases activities (Na^+, K^+-ATPase + Mg^{2+}-ATPase) in amygdala ($t=2.155$, $p>.05$) and prefrontal cortex ($t=3.642$, $p>.01$). In the amygdala, Na^+, K^+-ATPase was increased ($t=3.84$, $p<.01$) without alterations on Mg^{2+}-ATPase ($p>.05$). In the prefrontal cortex, Mg^{2+}-ATPase was increased ($t=5.029$, $p<.001$) without alterations on Na^+, K^+-ATPase ($p>.05$). Na^+, K^+-ATPase subunits protein abundance (α1, α3 and β1), glutamate uptake, and cell viability were not altered by HHCY-induced model (both $p>.05$). The amygdala and prefrontal cortex presented HHCY-specific responses. Our findings suggest that although chronic mild HHCY may have promoted an adaptation, leading to ATPases increase (Na^+, K^+-ATPase and Mg^{2+}-ATPase) in the amygdala and prefrontal cortex, such modifications did not

cause depressive-like behavior, and not compromised glutamate uptake and cell survival. These alterations may be related to the risk for several disorders that leads to an increase in Na^+, K^+-ATPase activity in the amygdala. These finds help to better understand the involvement of brain structures in chronic mild HHCY. Supported by CNPq and INCT EN/CNPq.

Disclosures: **T.M. Dos Santos:** None. **C. Siebert:** None. **L.D. Bobermin:** None. **A.Q. dos Santos:** None. **C.A. Netto:** None. **A.T.S. Wyse:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.28/I15

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH DA042737

Title: Alcohol drinking decreases the glutamate aspartate transporter to enhance methamphetamine excitotoxicity

Authors: *A. L. BLAKER[1,2], E. R. MOORE[2], B. K. YAMAMOTO[2];
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Abstract: A high comorbidity exists between alcohol and methamphetamine (Meth) but the neurochemical consequences of their co-abuse are poorly understood. Alcohol and Meth individually increase glutamatergic transmission in the brain but the effects of their co-exposure on excitotoxicity and its possible role in mediating damage to dopamine is unknown. Male Sprague Dawley rats were allowed intermittent access to 10% ethanol (EtOH) every other day over 28 days. Rats underwent a binge Meth or saline regimen the following day. Rats that drank EtOH displayed decreases in the glutamate aspartate transporter (GLAST) and increases in basal glutamate collected by microdialysis in the striatum 24 hrs after the last day of drinking. Consistent with the known effects of ceftriaxone to increase the expression of glutamate transporter, ceftriaxone treatment during EtOH drinking attenuated the decreases in GLAST and increases in basal glutamate concentrations. EtOH drinking also enhanced the increases in extracellular glutamate, cleaved caspase-3 mediated spectrin proteolysis, and dopamine depletions 7 days later. Ceftriaxone attenuated these enhanced responses to the serial exposure to EtOH and Meth. These data indicate the EtOH-induced decreases in GLAST and the associated increases in extracellular glutamate converge with Meth-induced glutamate efflux in the striatum to enhance spectrin proteolysis through cleaved caspase-3. Furthermore, this vulnerability to Meth-induced excitotoxicity produced by EtOH drinking synergistically contributes to long-

lasting dopamine depletions produced by Meth. These results elucidate a novel mechanism of neurotoxicity after the serial exposure to EtOH and Meth that is different than either drug alone.

Disclosures: A.L. Blaker: None. **B.K. Yamamoto:** None. **E.R. Moore:** None.

Poster

387. Mechanisms of Neurotoxicity II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 387.29/I16

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH grant R01CA208371

Title: Nasal administration of mesenchymal stem cells reverses chemotherapy-induced peripheral neuropathy

Authors: *N. BOUKELMOUNE, J. MA, G. O. LAUMET, A. KAVELAARS, C. J. HEIJNEN; Symptom Res., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Background: Chemotherapy-induced peripheral neuropathy (CIPN) is one of the most commonly reported adverse side effects of cancer treatment. CIPN affects 30 to 80% of cancer patients and often persists after treatment completion, having detrimental effects on patient's quality of life. Currently, there are no effective FDA-approved drugs to prevent or reverse CIPN and the mechanisms associated with the pathogenesis of CIPN are not completely understood. However, growing evidence identifies mitochondrial dysfunction in the etiology of this condition. Mesenchymal stem cells (MSC) have been shown to stimulate tissue repair and ameliorate the outcome of various neurodegenerative diseases. We have shown previously that nasal administration of MSC resolves cisplatin-induced cognitive impairment (Chiu et al., Oncotarget, 9:35581, 2018).
Hypothesis/Goals: In this study we tested the hypothesis that nasal MSC treatment reverses central and peripheral neuropathy and restores mitochondrial bioenergetics in cisplatin-treated mice.
Methods: Male and female mice were treated with two cycles of cisplatin (2.3 mg/kg for 5 days), followed by nasal administration of MSC at 48 and 96 h after the last dose of cisplatin. Behavioral testing was performed prior to cisplatin and MSC treatment as well as 5-21 days after the last MSC dose. Mechanical allodynia was measured using von Frey hairs and spontaneous pain was tested using a conditioned place preference test. Mitochondrial function in the dorsal root ganglia (DRG) as well as peripheral nerve was determined by Seahorse Flux analysis.
Results: Nasal MSC administration resolved cisplatin-induced mechanical allodynia in both male and female mice, while mechanical allodynia persisted in mice treated with cisplatin only. MSC treatment also alleviated spontaneous pain. Furthermore, MSC administration normalized the

cisplatin-induced decrease in mitochondrial dysfunction in DRG neurons as well as in tibial nerve as compared to mice who received cisplatin alone.
Conclusions: Our results show that administration of two doses of nasal MSC is sufficient to reverse symptoms of cisplatin-induced peripheral neuropathy, including mechanical allodynia and spontaneous pain by restoring mitochondrial function in dorsal root ganglia and peripheral nerve.

Disclosures: N. Boukelmoune: None. **J. Ma:** None. **G.O. Laumet:** None. **A. Kavelaars:** None. **C.J. Heijnen:** None.

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.01/I17

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant R44ES026268-02
NIH Grant R43AG062012-01

Title: Alzheimer's and Parkinson's disease modeling using iPSC derived neurons, astrocytes and microglia for screening of neurotoxic and/or neuroprotective compounds

Authors: *S. ANKAM[1], K. GORDON[1], R. BASA[1], C. T. MARTIN, III[1], C. HANDLEY[1], S. L. FENG[1], J. V. KARPIAK[1], T. ROHN[2], J. PRICE[1,3], P. MCDONOUGH[1];
[1]Vala Sciences, Inc, San Diego, CA; [2]Boise State Univ., Boise, ID; [3]The Scintillon Inst., San Diego, CA

Abstract: The role of inflammation in neurodegenerative diseases is an emerging area of study. Microglia, the neuroimmune cell, can play a neuroprotective or a neurotoxic role depending on the specific disease circumstance. Recent advances have led to the development of various protocols for differentiating microglia, neurons, and astrocytes from human iPSCs. By using cells derived from either healthy or genetically susceptible patients, we can develop co-culture systems to study neuroinflammation and neurotoxicity in various disease conditions. Here we describe the use of Vala Sciences' imaging platform, the IC200 Kinetic Image Cytometer™ (KIC), to characterize and develop screening assays in co-cultures with microglia and neurons, as well as astrocytes. We developed advanced, custom algorithms for our CyteSeer image analysis software, and it is now possible for us to automate neurite tracing, nucleus mapping, and analysis of calcium activity for single cells in a co-culture system. In this study, microglia were derived from human iPSCs and were characterized using the biomarkers CX3CR1, TREM2, IBA1, P2RY12, and TMEM119 by immunofluorescence. We then assessed microglial functionality by performing an engulfment assay using pHrodoRed Zymosan beads. Increased

engulfment after stimulation with IL-4, but not after stimulation with lipopolysaccharide (LPS), demonstrated polarizability to different microglial activation states (M2 vs. M1, respectively). Next, we developed co-culture systems with microglia, dopaminergic neurons or glutamatergic neurons, and astrocytes to further examine the role of microglia in co-culture models of Parkinson's Disease (PD) and Alzheimer's Disease (AD). Addition of microglia to a glutamatergic neuron culture increased neurite outgrowth by 50%, suggesting a neuroprotective role for microglia in this co-culture system. Additionally, we have demonstrated differential engulfment of β-amyloid 1-40 and β-amyloid 1-42 (the AD-relevant β-amyloid fragment) by microglia in co-culture. Neurite outgrowth and calcium imaging assays are being performed on the co-culture systems with stressors for AD (ApoE and β-amyloid) or PD (paraquat) to determine if microglia protect neurons from or sensitize neurons to these specific disease-relevant insults.

Disclosures: S. Ankam: None. **K. Gordon:** None. **R. Basa:** None. **C.T. Martin:** None. **C. Handley:** None. **S.L. Feng:** None. **J.V. Karpiak:** None. **T. Rohn:** None. **J. Price:** None. **P. McDonough:** None.

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.02/I18

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Investigating the contrasting functional roles of NCOA7 and NCOA7B during neuroinflammation

Authors: *E. CASTROFLORIO[1], S. CORROCHANO[1], M. J. FINELLI[2], J. DEN-HOED[2], P. L. OLIVER[1];
[1]Mammalian Genet. Unit, MRC Harwell Inst., Harwell, United Kingdom; [2]Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: Although the underlying causes of neurodegeneration are still under investigation, the immune system likely plays a key role in disease onset and pathogenesis. However, the specific function of cytokines and other immune signalling pathways in disease pathogenesis is unclear. The nuclear receptor coactivator 7 (NCOA7) is a poorly characterized gene containing the Tre2/Bub2/Cdc16 (TBC), lysin motif (LysM), domain catalytic (TLDc) domain. This motif is present in a family of proteins known to protect against oxidative stress-related insults, including TBC1 domain family member 24 (TBC1D24), a protein mutated in a range of disorders characterized by epilepsy, hearing loss and neurodegeneration. The TLDc domain is highly conserved across species, although the structure-function relationship is unknown. Interestingly, a short isoform of NCOA7 (NCOA7B) - containing just the TLDc domain - has been shown to

be significantly induced by bacterial lipopolysaccharides (LPS) and interferons, as well as being able to modulate endosome-mediated viral entry. Here, using unique mouse models, we report for the first time that NCOA7 and NCOA7B act in different cell types in the brain, attenuating an exaggerated immune response and promoting proper neuronal connectivity in vivo.

Disclosures: E. Castroflorio: None. **S. Corrochano:** None. **M.J. Finelli:** None. **J. Den-Hoed:** None. **P.L. Oliver:** None.

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.03/I19

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH NINDS R01-NS109226
Packard Foundation Fellowship

Title: Gamma frequency sensory stimulation induces neuroimmune signaling cascade

Authors: *K. M. GARZA[1,2], B. BORRON[2], S. AMIGUES[2], J. DAVIS[2], A. PYBUS[2], S. SANKAR[2], M. ATTOKAREN[2], L. WOOD[3], A. C. SINGER[2];
[1]Emory Univ., Atlanta, GA; [2]Coulter Dept. of Biomed. Engin., [3]Woodruff Sch. of Mechanical Engin. and Inst. of Bioengineering and Biosci., Georgia Inst. of Technol., Atlanta, GA

Abstract: Interactions between the nervous system and the immune system protect neurons from pathogens. Since dysfunctions of the neuroimmune system underlie many neurodegenerative and neurological diseases, methods of manipulating neural immunity have strong therapeutic potential. The neuroimmune system includes neurons and microglia as well as cytokines, signaling proteins which allow communication between cells. Prior work has shown that exposing mice to lights flickering at 40Hz induces neural spiking activity at 40 Hz (within the gamma frequency) and recruits microglia in primary visual cortex. By recruiting both neuronal activity and immune cells, this paradigm has potential clinical applications, but the effect of 40Hz flicker on neuroimmune biochemical signaling remains unknown because the fields of neural oscillations and neuro-immunology are rarely examined together. We exposed mice to 40Hz flicker, and using a Luminex multiplex assay, we assessed differences in networks of cytokines and phosphoproteins known to play a role in immune function. We found that 40Hz flicker leads to significant increases in cytokine expression, such as IL-6 and IL-4, which promote microglial engulfing states, as well as microglial chemokines, such as M-CSF and MIG. To identify possible mechanisms underlying cytokine expression, we then quantified the effects of 40Hz flicker on internal signaling pathways known to control cytokine expression. We found that 40Hz flicker up-regulates phospho-signaling within the MAPK and NF-kB pathways, both

of which regulate cytokine expression. We found that while changes in cytokine levels occur after 1 hour of 40Hz flicker stimulation, changes in protein phosphorylation occur more rapidly, within a few minutes. These results are the first to show how gamma frequency neural spiking activity affects neuroimmune signaling. Identifying the effects of 40Hz frequency neural stimulation on immune signaling in the brain will enable new strategies to promote and interrogate healthy brain immune function.

Disclosures: **K.M. Garza:** None. **B. Borron:** None. **S. Amigues:** None. **J. Davis:** None. **A. Pybus:** None. **S. Sankar:** None. **M. Attokaren:** None. **L. Wood:** None. **A.C. Singer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognito Therapeutics Inc.

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.04/I20

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: FAPERJ
CNPq
CAPES
INNT
BNDES

Title: Modelling the *Toxoplasma gondii* infection in human neural cells: Role of metalloproteinases and neuroinflammation

Authors: *E. C. LOIOLA[1], R. M. MARIANTE[2], P. TRINDADE[1,3], L. O. PORCIUNCULA[4], S. K. REHEN[5,1];
[1]IDOR, Rio de Janeiro, RJ, Brazil; [2]Oswaldo Cruz Inst., Rio de Janeiro, RJ, Brazil; [3]UNIRIO, Rio de Janeiro, RJ, Brazil; [4]UFRGS, Porto Alegre, RS, Brazil; [5]UFRJ, Rio de Janeiro, RJ, Brazil

Abstract: The intracellular parasite *Toxoplasma gondii* is a common pathogen within worldwide population and latent toxoplasmosis is usually considered asymptomatic. However, there are evidences that toxoplasmosis could cause behavioral changes in humans and many studies highlight the link between *T. gondii* infection and increased incidence of psychiatric disorders. Neuroinflammation itself also plays a major role in several mental illness and high levels of pro-inflammatory substances as cytokines and metalloproteinases (MMPs) have been described in the blood and cerebrospinal fluid of schizophrenic and bipolar disorder patients. Activated glial cells are an important source of MMPs in CNS and toxoplasma infection could induce the production of these enzymes. Here, we used human induced pluripotent stem cells (hIPSC)

differentiated into neural stem cells, neurospheres and astrocytes. Individual predisposition to parasite infection could be accessed by *T. gondii* immunolabeling in infected cultures. This approach permits the study of molecular mechanisms involved in parasite infection and the outcome on human neural cells. The effect of MMP9 activity in the parasite infection were estimated after treatment with apigenin (a flavonoid that inhibits MMPs expression) and 1-10-phenathroline (a MMP inhibitor). Both treatments abrogate *T. gondii* infection in neurospheres. To evaluate the role of infection inhibitors drugs on MMP9 production, hIPSC derived astrocytes were treated with apigenin or 1-10-phenantroline. The treatment induced a decrease in MMPs activity evaluated by gel zymography. Taken together these results offer new methodologies to study parasite interactions and its effects in human neural cells and suggest mechanistic links between neuroinflammation and *T. gondii* infection.

Disclosures: **E.C. Loiola:** None. **R.M. Mariante:** None. **P. Trindade:** None. **L.O. Porciuncula:** None. **S.K. Rehen:** None.

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.05/I21

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: AMED 19be0304207j0103
MEXT 17K08330

Title: What happened to the *in vitro* blood-brain barrier (BBB) model with activated microglia in the brain side-activated microglia can trigger the BBB disruption and modulate the related chemokines and cytokines via the interactions with astrocytes and blood vessels

Authors: *K. SATO[1], K. HOSHIKAWA[2], Y. SHIGEMOTO-MOGAMI[1];
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Abstract: BBB disruption is associated with the various brain disorders with neuroinflammation. Although microglial activation and the subsequent changes in cytokines/chemokines (C/Cs) have already been reported to be important for the development of neuroinflammation, little data are available concerning whether it is cause or consequence. In this study, we investigated the interaction of activated microglia (LPS-MG) with BBB using in vitro BBB model composed of endothelial cells (EC), pericytes (Peri), and astrocytes (Ast). When we added LPS-MG to the abluminal side of the model, BBB functions were severely lowered as revealed by decreases in the trans-endothelial electrical resistance (TEER) and in the expression levels of tight junction (TJ) proteins. We also confirmed that the cell surface localizations of TJ proteins were

disappeared immunocytochemically. Furthermore, 19 C/Cs were markedly increased in the abluminal side. Unexpectedly, although LPS-MG alone released 10 of the 19 C/Cs, their real concentrations were rather lower compared with the C/C changes in the abluminal side of with LPS-MG. Co-culture of LPS-MG with Ast caused remarkable increases in 12 of the 19 C/Cs, while co-culture of LPS-MG with EC and Peri also resulted in a significant increase in fractalkine. These results suggest that activated microglia can trigger BBB disruption and also modulate the related C/Cs via the interactions with astrocytes and blood vessels.

Disclosures: K. Sato: None. **K. Hoshikawa:** None. **Y. Shigemoto-Mogami:** None.

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.06/I22

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Resverlogix Corp

Title: Apabetalone epigenetically inhibits monocyte adhesion to brain endothelial cells by downregulating key neuroinflammation markers *in vitro* and *in vivo*

Authors: *S. WASIAK[1], E. DAZE[1], L. M. TSUJIKAWA[1], S. DAS[1], L. FU[1], D. GILHAM[1], B. D. RAKAI[1], S. C. STOTZ[1], C. D. SARSONS[1], D. STUDER[2], K. D. RINKER[2], R. JAHAGIRDAR[1], N. C. W. WONG[1], M. SWEENEY[3], J. O. JOHANSSON[3], E. KULIKOWSKI[1]; [1]Resverlogix Corp., Calgary, AB, Canada; [2]Univ. of Calgary, Calgary, AB, Canada; [3]Resverlogix Inc., Calgary, AB, Canada

Abstract: Objectives and Rationale: Leukocyte infiltration across the blood brain barrier (BBB) contributes to the initiation and exacerbation of neuroinflammation, leading to neuronal injury. Cytokine-driven activation of endothelial cells enables monocyte binding to BBB. Thus, inhibiting this process is an attractive therapeutic approach. Epigenetic modulation of gene expression contributes to vascular inflammation. Here we evaluate anti-inflammatory properties of the clinical stage small molecule apabetalone that inhibits bromodomain and extraterminal domain (BET) proteins on models of brain inflammation. Methods: THP-1 monocyte transcriptional responses to TNF alpha with or without apabetalone were examined. Human brain microvascular endothelial cells (HBMVECs) stimulated with TNF alpha and IFN gamma with or without apabetalone were assayed for gene expression, cytokine secretion, surface adhesion protein level, and THP-1 adhesion under flow conditions. *In vivo* neuroinflammation was assessed in C57BL/6 male mice pretreated with 150 mg/kg apabetalone for 7 days and then injected with 10 mg lipopolysaccharide (LPS) intraperitoneally. Brain mRNA was analyzed 24h post LPS injection. Results: Apabetalone suppressed the expression of THP-1 genes induced by

TNF alpha, including IL-1 beta, chemokine MCP-1, chemokine receptors CCR1 and CCR2 and adhesion molecule VLA-4 (ranging from 40% to 90% reduction). In cytokine stimulated HBMVECs, apabetalone reduced the mRNA induction of vascular activation markers IL-6, MCP-1, VCAM-1, and E-selectin (55% to 97% reduction). Surface expression of adhesion proteins VCAM-1 and E-selectin as well as secretion of IL-6 and MCP-1 were also reduced. Consequently, apabetalone countered THP-1 adhesion to HBMVECs in laminar flow assays. In mice, apabetalone attenuated the LPS-induced brain mRNA expression of inflammation markers including E-selectin, ICAM, CCR2, and CD68. Conclusions: Apabetalone decreased neuroendothelium's activation and interactions with monocytes, potentially reducing immune cell transmigration in neuroinflammatory conditions. Apabetalone's effect on cognition in in diabetes patients following acute coronary syndrome older than 70 years is being evaluated by repeat MoCAs in the phase 3 BETonMACE trial (Q2 2019).

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Resverlogix Corp. **M. Sweeney:** A. Employment/Salary (full or part-time):; Resverlogix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Resverlogix. **J.O. Johansson:** A. Employment/Salary (full or part-time):; Resverlogix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Resverlogix. **E. Kulikowski:** A. Employment/Salary (full or part-time):; Resverlogix Corp. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Resverlogix Corp..

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.07/I23

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Ting Tsung and Wei Fong Chao Foundation in Houston, TX

Title: Therapeutic strategies for immunomodulation of peripheral myeloid cells in Alzheimer's disease

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Abstract: Inflammation is a hallmark of neurodegenerative disease and a significant component of the pathology of Alzheimer’s disease (AD). Patients present with extensive microgliosis along with elevated pro-inflammatory signaling in the central nervous system. Animal models of AD recapitulate these findings and neuroinflammation is now a potentially important target for therapeutic intervention. Peripheral immune cell entry into the CNS and the extensive neuro-immune talk between the periphery and the CNS are increasingly recognized as important modulators of neurodegeneration. However, the specific role of peripheral myeloid cells in mediating and influencing AD pathogenesis remains unresolved. Our study describes specific peripheral immune cell alterations during disease progression including immune cell populations, phenotypes, and gene expression profiles. Patients were evaluated for disease severity using Clinical Dementia Rating (CDR) and peripheral myeloid cells were isolated from peripheral blood of patients with prodromal AD (CDR0.5, n = 44), mild AD dementia (CDR1, n = 25), moderate/severe AD dementia (CDR2/3, n = 28), and age-matched controls (n = 54). During stages of AD dementia (CDR1 and 2/3) peripheral myeloid cells increase their pro-inflammatory gene expression while at early stages of disease (prodromal AD—CDR0.5) pro-inflammatory gene expression is decreased. Suppressive myeloid-derived suppressor cells

(MDSCs) are increased in number and function in prodromal AD compared to controls but lose their numbers and suppressive function as patients progress to AD dementia. This loss of suppression in late stages of AD provides a therapeutic opportunity whereby immunomodulation via anti-inflammatory cell-based therapies could ameliorate the pro-inflammatory phenotype of immune cells. We have initiated studies to determine whether macrophages differentiated to an anti-inflammatory (M2) phenotype could restore the impaired suppressive function. Prolonging the early protective suppression while also reversing the later loss of suppressive activity may offer a beneficial therapeutic strategy for halting the progression of disease.

Disclosures: **A.D. Thome:** None. **A. Faridar:** None. **D.R. Beers:** None. **J.R. Thonhoff:** None. **W. Zhao:** None. **S. Wen:** None. **M. Pascual:** None. **J.C. Masdeu:** None. **S.H. Appel:** None.

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.08/I24

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Dr Miriam and Sheldon G. Adelson Foundation Medical Research Foundation - AMRF
NEI R01EY027881
NEI R01EY024481

Title: Remyelination of regenerating CNS axons: The optic nerve model

Authors: *S. DE LIMA[1], N. M. S. PORTS[2], N. P. BISCOLA[3], N. ZHANG[3], L. A. HAVTON[4], P. A. ROSENBERG[5], L. BENOWITZ[1];
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Abstract: Several recent studies have achieved appreciable optic nerve regeneration by activating the growth potential of mature retinal ganglion cells (RGCs). Our lab reported that, with appropriate stimulation, some RGCs can extend axons from the eye to appropriate central target areas (de Lima et al., PNAS, 2012), and that some regenerating axons become myelinated and re-establish the electrically excitable domains that are important for action potential generation and propagation (Marin, de Lima et al., J. Neurosci, 2016). However, the re-formation of electrically excitable domains accompanying myelination progresses much more slowly than axon regeneration. Relatively few studies have addressed the problem of myelination of regenerating axons, which is critical for functional recovery. To gain insight into the possible

causes of its impairment, we have addressed the following questions: (1) how early does myelination begin after the onset of regeneration?; (2) Is myelination related to the size of regenerating axons?; and (3) is there a change in oligodendrocyte population dynamics after nerve injury and during regeneration? We induced regeneration in these studies by combining *pten* deletion in RGCs with the growth factor oncomodulin + cAMP elevation. Using pre-embedding immunogold labeling, we detected myelin wrapping regenerating axons (GAP-43+; CTB+) close to the injury site 2 - 4 weeks post-injury. However, whereas myelination starts when axons reach a diameter ≥ 300 nm during development, there was no clear relationship between axon diameter and myelination during regeneration. Surprisingly, we found a substantial increase in the number of oligodendroglia (olig2+ cells) along the regenerating optic nerve 3 weeks after injury and the onset of regeneration. For example, at 2.5 mm distal to the injury site, whereas in normal optic nerves we found 1927 +/- 131 olig2+ cells/mm2, in cases with optic nerve injury only we found 10% more cells 3 weeks after nerve injury (n.s; n = 4), while in cases with the pro-regenerative treatment we found an increase of 37% (p = 0.001; n = 9). Only 5-10% of these cells were preoligodendrocytes (PDGFRα+), whereas the vast majority were mature oligodendrocytes (CC1+). Thus, myelin wrapping starts shortly after the onset of axon regeneration close to the site of injury and oligodendrocyte population dynamics does not seem to be limiting in this region. However, myelination in regenerating axons does not seem to have the same dependence on axon diameter as during development, and nodal formation is delayed. These results suggest the possibility of a deficit in signaling between regenerating axons and axons in the setting of regeneration.

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Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.09/I25

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Robert's Program on Sudden Unexpected Death in Pediatrics

Title: Cerebrospinal fluid neopterin as a marker for central nervous system inflammation in sudden infant death syndrome (SIDS)

Authors: *M. RIEHS[1], H. HARB[2], B. W. OKATY[4], E. HAAS[5], B. PAUNOVIC[6], K. HYLAND[7], M. GORMAN[3], G. T. BERRY[8], R. D. GOLDSTEIN[9], R. L. HAYNES[1]; [1]Dept. of Pathology, [2]Dept. of Immunol., [3]Dept. of Neurol., Boston Children's Hosp., Boston, MA; [4]Dept. of Genet., Harvard Med. Sch., Boston, MA; [5]Rady Children's Hosp., San Diego, CA; [6]San Diego County Office of the Med. Examiner, San Diego, CA; [7]MNG Labs., Atlanta, GA;

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Abstract: The sudden infant death syndrome (SIDS) is the sudden death of an infant under 12 months of age that remains unexplained after a complete autopsy and death scene investigation. SIDS is likely multifactorial with the pathogenesis related to varying biological vulnerabilities, risk factors, and stressors or triggers of sudden death. Risk associations in SIDS have been linked to infection, with a subpopulation of SIDS infants having a history of acute illness preceding death. To test the hypothesis that SIDS involves central nervous system inflammation, we analyzed the levels of neopterin and the cytokine interferon-gamma (INF-γ) in the cerebrospinal fluid (CSF) of 66 SIDS infants and 15 non-SIDS controls dying of acute causes. Neopterin is produced by monocytes and macrophages upon stimulation with INF-γ and is considered a specific marker for central nervous system inflammation. There was no overall statistical difference in SIDS and controls in neopterin [SIDS, 55.9 +/- 67 nmol/L; controls, 45.0 +/- 31.4 nmol/L] or INF-γ [SIDS, 13.8 +/- 25 pg/mL; controls, 10.6 +/- 9.4 pg/mL]. There were, however, 7 SIDS cases with neopterin levels greater than 2 standard deviations above the mean of the controls (>107 nmol/L), levels considered as clinically significant for CNS inflammation. In controls cases, neopterin and INF-γ levels correlated suggesting an appropriate immune response to INF-γ. However, SIDS cases with high levels of neopterin generally had INF-γ levels within the control range, a finding that suggests an inappropriate or heightened immune response of these SIDS cases to an immunological trigger. Given the role of microglial response in immune activation within the brain, we are beginning to examine microglial phenotypes in SIDS using droplet-based single nuclei RNA sequencing. Preliminary data show differing expression patterns of microglial genes, including genes related to microglial activation and function. The CSF data support the hypothesis that a subpopulation of SIDS cases have a significant immune system response. Preliminary sequencing data support the potential role of microglia in this response.

Disclosures: **M. Riehs:** None. **H. Harb:** None. **B.W. Okaty:** None. **E. Haas:** None. **B. Paunovic:** None. **K. Hyland:** None. **M. Gorman:** None. **G.T. Berry:** None. **R.D. Goldstein:** None. **R.L. Haynes:** None.

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.10/I26

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: DOD grant PR151462
UAB MSTP grant 5T32GM008361-26

Title: IL-2 enhances IFNγ signals in subpopulations of T lymphocytes from treatment naïve relapsing remitting multiple sclerosis (RRMS) patients

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Abstract: BACKGROUND: Multiple sclerosis (MS) is a common, CNS demyelinating disorder and more than 85% of patients have a relapsing form of MS (RMS). Dysregulated lymphocytes within the adaptive immune system contribute to disease progression and severity in MS. IFNγ producing myelin-specific T lymphocytes correlate with functional impairment in MS (J. Neuroimmunol.141(1-2):132-140) and IFNγ administered to patients with RRMS worsens the disease. Regulatory T cells require interleukin-2 for lineage function and maintenance but this role in RMS has been underexplored.
OBJECTIVES: We tested the possibility that IL-2 would dampen IFNγ induced STAT activation in subpopulations of effector and regulatory T cells in a disease specific context. We sought to elucidate how IL-2 can alter IFNγ signaling in RMS to broaden our understanding of how these soluble mediators of disease resolution and inflammation act synergistically in the context of autoimmunity.
METHODS: We stimulated PBMC from treatment naïve RMS patients with IFNγ and/or IL-2. Using a multi-dimensional phospho-flow approach we evaluated the activation of STAT1, STAT3, and STAT5 in each subpopulation of T cells. PBMC from patients with rheumatoid arthritis (RA) was used as disease control. The data was analyzed using t-SNE and SPADE visualization tools to interrogate changes in the activation and proportion of these cell populations.
RESULTS: We found IFNγ stimulation broadly induced greater activation of STAT1 in MS than in RA within multiple lymphocyte populations. IL-2 co-stimulation in a subset of RMS patients further increased IFN-γ induced activation of STAT1 in multiple T cell populations and Treg cells. IL-2 induced activation of STAT5 was not altered by IFN-γ co-stimulation.
CONCLUSIONS: Our results suggest that in some MS patients, IL-2 will promote autoimmune neuroinflammation through activation of the IFNγ-STAT1 signaling axis in CD4 and CD8 T cell populations. This is in contrast to the role of IL-2 as a regulatory cytokine in inflammation/autoimmunity through attenuation of effector Th1 and Th17 cells and promotion of Treg cell activity. Low dose IL-2 is being explored as a therapy for autoimmune diseases, and our results indicate that in some MS patients, such treatment may exacerbate disease.

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Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.11/I27

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH NS097776
Emory University URC

Title: EP2 antagonism blocks monocyte brain recruitment after status epilepticus

Authors: *N. H. VARVEL[1], A. HSIEH[1], T. GANESH[2], R. J. DINGLEDINE[3];
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Abstract: Status epilepticus (SE) is a life-threatening medical emergency that triggers a succession of molecular and cellular events involving selective neuronal cell loss, blood brain barrier (BBB) leakage, activation of brain-resident microglia, recruitment of inflammatory blood-borne monocytes, and engagement of neuroinflammatory prostanoid signaling. Our group has identified brain-invading blood-borne monocytes and, independently, activation of prostaglandin receptor EP2 as a driver of SE-induced neuroinflammation and neuronal damage, contributing to mortality and neurobehavioral deficits. Blocking monocyte brain recruitment, via *Ccr2* knockout, or systemic EP2 antagonism, via a small-molecule, enhances weight regain and relieves multiple consequences of SE including erosion of the BBB, neuronal damage, andneuroinflammation, confirming that inflammatory pathways drive some of the deleterious consequences of pilocarpine-induced SE. Notably, EP2 antagonism after kainate-induced SE also enhances weight regain, quenches hippocampal inflammation, prevents deterioration of the BBB, provides neuroprotection, and, interestingly, nearly abolishes monocyte brain recruitment into the brain. Taken together these findings suggest that the beneficial consequences of EP2 antagonism are model-independent and might be attributed, in part, to inhibiting brain monocyte infiltration after SE.

Disclosures: **N.H. Varvel:** None. **A. Hsieh:** None. **T. Ganesh:** None. **R.J. Dingledine:** None.

Poster

388. Neurodegeneration and Injury: Neuroinflammation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 388.12/I28

Topic: C.10. Brain Injury and Trauma

Support: Kentucky Spinal Cord and Head Injury Research Trust, 15-12A
Kentucky Spinal Cord and Head Injury Research Trust, 18-8A
NIH/NIA P30 AG028383

Title: The role of mitochondria associate er membranes in the specific subcellular localization of inflammatory responsive microRNAs in the mammalian brain

Authors: *W.-X. WANG[1], P. PRAJAPATI[2], P. T. NELSON[3], J. E. SPRINGER[4];
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Abstract: Mitochondria associated ER membranes (MAMs) are specific ER domains that are attached to mitochondria. MAMs play a critical role in the communication between ER and mitochondria, facilitating processes such as lipid synthesis and exchange, calcium transport and homeostasis, and mitochondria dynamics. MAMs also play an important role in the formation of the autophagosome and inflammasome. Disruption of mitochondria and ER contact is associated with a variety of pathophysiological conditions and human diseases including inflammation, metabolic diseases, cancers, traumatic brain injury (TBI), and neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. Our new findings from human and rat brains indicate that the MAM is a subcellular site enriched for specific microRNAs (miRNAs). MiRNAs are an important class of regulators that mediate gene expression post-transcriptionally. We performed subcellular fractionation from human and rodent cerebral cortex and analyzed miRNA expression in purified MAM, mitochondria, ER, and cytosol fractions. The human brains were short-postmortem samples collected from research subjects of the University of Kentucky Alzheimer's Disease Center autopsy cohort. RT-qPCR analysis revealed that MAM contains a substantial number of miRNAs and enriched for several inflammatory miRNAs including miR-146a, miR-142-3p, and miR-142-5p; enrichment of miR-223 was only observed in human brains. Previously, we showed that these same inflammatory responsive miRNAs are highly associated with mitochondria fractions isolated from rat hippocampus and translocate to cytoplasm following TBI. Here we report that, events that compromise mitochondrial function, such as mitochondria uncoupling and TBI, result in MAM to ER translocation of these inflammatory miRNAs and down-regulation of miRNA-targeted genes involved in inflammatory signaling. These findings shed new light on the role of MAMs and suggest the shuttling of

cellular miRNAs as a potentially novel gene expression regulatory paradigm relevant to neuroinflammation in TBI and neurodegenerative diseases.

Disclosures: **W. Wang:** None. **P. Prajapati:** None. **P.T. Nelson:** None. **J.E. Springer:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.01/I29

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: FAPESP 2018/03482-0
CAPES/COFECUB Me928/19
Investissements d'Avenir ANR-10-IAIHU-06

Title: L-DOPA-induced dyskinesia in mice leads to the production of astrocyte-derived TNF-alpha through a glutamate-dependent mechanism

Authors: *M. DOS SANTOS PEREIRA[1], G. DIAS DE ABREU[1], J. ROCCA[2], S. HAMADAT[3], R. RAISMAN-VOZARI[3], P. MICHEL[3], E. DEL BEL[1];
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Abstract: Here we addressed the hypothesis that L-DOPA-induced dyskinesia (LID) could be favored by the striatal pro-inflammatory environment resulting from abnormal glutamate (GLU) release from the motor cortex induced by L-DOPA dyskinesiogenic treatment. To this aim, we used 6-OHDA-lesioned C57BL/6 dyskinetic mice (L-DOPA 25 mg/kg + benserazide 10 mg/kg i.p. for 21 days) to quantify striatal inflammatory markers. LID development was accompanied by GFAP (astrocyte) and IBA-1 (microglia) activation and by an elevation of TNF-α, IL-1β and IL-6 in in the denervated dorsal striatum. Interestingly, the anti-dyskinetic treatment with cannabidiol (CBD) and capsazepine (CPZ) (30 mg/kg, 5 mg/kg, i.p.) partially reduced TNF-α production in L-DOPA-treated mice while having no effect on IL-1β and IL-6 levels. Using purified microglial cells and astrocytes in culture, we tested the possible role of L-DOPA/DA and GLU in the induction of neuroinflammatory events in LID. While L-DOPA or DA failed to induce a pro-inflammatory response in both types of glial cells, GLU increased the expression of the astrocytic marker GFAP and the production of TNF-α by astrocytes. GLU failed, however, to stimulate cytokine production in pure microglial cell cultures. A treatment combining cannabidiol (CBD) and TRPV1 antagonist capsazepine (CPZ; dos Santos Pereira et al., 2016) abolished the increase of GFAP expression and TNF-α production triggered by GLU in astrocyte cultures. By using purified cortical neurons in culture, we also demonstrated that a chronic treatment with TNF-α (50 ng/ml) was able to stimulate GLU release. Interestingly, this effect

was blocked by a pretreatment with CPZ+CBD (0.1 µM). Overall, we conclude that TNF-α produced by GLU-activated astrocytes might have a crucial role in promoting inflammatory-type reactions in the dyskinetic striatum and this event may occur through a vicious circle mechanism.

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Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.02/I30

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: MY-NORTH Project
Mahidol University

Title: The involvement of NG2 expressing cells in methamphetamine induced neurotoxicity and neuroprotective role of melatonin in rat brain

Authors: *Z. M. HEIN, N. KRAIWATTANAPIROM, B. CHETSAWANG;
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Abstract: Among the residential cells in the brain, Neuron-Glial2 (NG2) expressing cells are described as the new member of the glial cells and are evenly distributed in both white and grey matter of the brain. Previously they had been assumed as oligodendrocyte precursor cells (OPCs) and they can present into oligodendrocytes and promote myelination of axons in demyelination diseases. Moreover, NG2 cells become activated in pathological conditions and they may have other functions than OPCs which have not been elucidated yet. Therefore, the aims of this study are to investigate the possible involvement of NG2 cells under methamphetamine (METH)-induced neurotoxicity in rat. In addition, the neuroprotective capacity of melatonin against this neurotoxicity was also investigated. The results showed that the levels of NG2 expression in rat brain gradually increase from postnatal day 0 to postnatal day 8 and then the lower levels of NG2 expression is shown in adults when compared with postnatal period. However, in adult rats, the levels of NG2 and COX-2 expression in brain was significantly increased in lipopolysaccharide-treated when compared to control-untreated rats. Taken together, intra-peritoneal injection of 5 mg/kg METH for 7 days significantly increased the levels of NG2 expression in brain when compared to control-untreated rats. Pretreatment of 10 mg/kg melatonin prior to treat with METH was able to reduce an increase in the levels of NG2 expression. Thus, these findings would extend the current knowledge of NG2 expressing cells in adult brain during pathological condition such as neuroinflammation.

Disclosures: **Z.M. Hein:** None. **N. Kraiwattanapirom:** None. **B. Chetsawang:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.03/I31

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH/NINDS Grant (NS099007)

Title: The effects of nadph oxidase inhibitor, diapocynin, treatment in diisopropylfluorophosphate (DFP)- induced long-term neurotoxicity

Authors: *M. PUTRA[1], M. GAGE[1], G. GASSER[2], S. SHARMA[1], V. ANANTHARAM[2], T. THIPPESWAMY[1];
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Abstract: Organophosphate (OP) compounds are among the most catastrophic threats to the military and civilian population. Upon exposure of OP, cholinergic crisis and *status epilepticus* arise as a result of irreversible inhibition of acetylcholinesterase that can be life-threatening if left untreated. As for OP survivors, long-term morbidity develops over time which includes progressive neurodegeneration and neuroinflammation and delayed cognitive impairment. Currently available medical countermeasures (atropine, oxime, and diazepam) fail to rescue these pathologies. Therefore, many efforts are directed to target neuroinflammation and oxidative stressors. This study examined the antioxidant properties of diapocynin (DPO), an oxidative metabolite of apocynin, in the rat diisopropylfluorophosphate (DFP) model. Adult Sprague-Dawley rats were intoxicated with DFP (4mg/kg, sc), treated with atropine sulfate and pralidoxime, and two hours later SE was terminated with diazepam. Further two hours later, DPO (300mg/kg) or 10% ethanol was administered orally, twice daily for the first three days. Neurobehavioral tests, electroencephalographic profile, immunohistochemistry, Western blots, multiplex assays were performed to evaluate the effects of DPO. DPO significantly improved DFP-induced motor impairment. Electrographically, there was no difference in epileptiform spike rate between the vehicle and DPO groups. Although DFP-induced reactive microgliosis was not significantly suppressed by DPO, several pro-inflammatory cytokines and chemokines such as IL-1α, IL-2, IL-17A, Leptin, IP-10 were significantly reduced in the hippocampus. Importantly, DPO significantly attenuated the DFP-induced neurodegeneration in the hippocampus and piriform cortex. However, DPO did not reduce DFP-induced nitro-oxidative stress markers such as iNOS, 3-NT, and 4HNE. Collectively, these data support the neuroprotective effects of DPO, possibly by dampening the upregulation of proinflammatory cytokines, in the rat model of DFP-induced neurotoxicity.

Disclosures: **M. Putra:** None. **M. Gage:** None. **G. Gasser:** None. **S. Sharma:** None. **V. Anantharam:** None. **T. Thippeswamy:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.04/I32

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: National Agency for Scientific and Technological Promotion PICT 2731 BID 2016

Title: Enriched environment housing protects the visual pathway alterations against experimental glaucoma in adult rats

Authors: ***M. GONZÁLEZ FLEITAS**, J. DEVOUASSOUX, M. L. ARANDA, H. DIEGUEZ, D. DORFMAN, S. CALANNI, M. CHIANELLI, R. E. ROSENSTEIN;
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Abstract: Glaucoma is a leading cause of blindness, characterized by retinal ganglion cell (RGC) loss and optic nerve (ON) damage. Increased intraocular pressure (IOP) is the most accepted risk factor for glaucomatous neuropathy, however many patients with successful IOP control continue to lose vision. Enriched environment (EE) consists of a manipulation in which animals are exposed to complex conditions through adaptations in the physical and social environment. Since it has been shown that EE provides a better recovery from different neuropathologies, the aim of this work was to analyze whether the exposure to EE is able to prevent glaucomatous damage. Adult male Wistar rats received 30% of chondroitin sulfate in the anterior chamber of one eye and vehicle in the contralateral eye, once a week, and were housed in standard environment (SE) or EE for 10 weeks. Animals were subjected to functional (electroretinogram and flash visual evoked potentials (VEPs)), and histological analysis. EE housing which did not affect IOP, prevented the decrease in VEPs and oscillatory potential amplitude, as well as the RGC loss (assessed by Brn3a-immunoreactivity). The axon number (assessed by toluidine blue staining) was also preserved by the exposure to EE. Moreover, EE housing prevented the decrease in the immunoreactivity for myelin basic protein (MBP) and luxol fast blue staining in the ON, as well as the increase in Iba1 (a microglia/macrophage marker) positive area in the retina and ON. These results suggest that the EE housing, a non-invasive strategy, protects the visual pathway against retina and optic nerve damage induced by experimental glaucoma. Although care must be taken when extrapolating data obtained in experimental models to humans, the protective effect of EE could reflect a scenario in which a physically and mentally active lifestyle promotes the visual pathway resiliency to damage induced by glaucoma.

Disclosures: **M. González Fleitas:** None. **J. Devouassoux:** None. **M.L. Aranda:** None. **H. Dieguez:** None. **D. Dorfman:** None. **S. Calanni:** None. **M. Chianelli:** None. **R.E. Rosenstein:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.05/I33

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: The Children's Brain Diseases Foundation

Title: Transcriptomic changes associated with neuroinflammation in a mouse model for cLINCL: Role of microglia

Authors: ***M. S. DOMOWICZ**[1], W.-C. CHAN[2], C. B. WARE[1], J. ANDRADE[2], N. B. SCHWARTZ[1];
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Abstract: Neuronal Ceroid Lipofuscinoses (NCLs), are a group of inherited, early onset, fatal neurodegenerative diseases associated with mutations in 13 genes and characterized by lysosomal accumulation of fluorescent storage material. Using a well characterized mouse model of classical late infantile NCL (cLINCL), in which the tripeptidyl peptidase 1 (*Tpp1*) gene is disrupted by gene targeting resulting in loss of detectable TPP1 activity, we have analyzed the progression of the neurodegenerative process by global RNA-sequencing at different ages (2, 3 and 4 months) in forebrain/midbrain and cerebellum (n=3). Our data indicate that a progressive neurodegenerative-inflammatory response involving microglia, astrocytes and endothelial cell are taking place, accompanied by activation of leukocyte extravasation signals and upregulation of NO and ROS production. Several astrocytic (i.e., *Gfap, C4b, Osmr, Serpina3n*) and microglial (i.e., *Ctss, Itgb2, Itgax, Lyz2*) genes were identified as good markers for assessing disease progression as they showed increased expression levels *in vivo* over time. Based on these gene expression changes we concluded that neuroinflammation starts, for the most part, after 2 months in the *Tpp1*$^{-/-}$ brain and that activation of microglia and astrocytes occur more rapidly in cerebellum than in the rest of the brain; confirming increased severity in this region. As in other neurodegenerative diseases, upregulation of *Csf1* and Csf1 receptor, possibly associated with astrocytic and microglial sources, respectively, were observed in *Tpp1*$^{-/-}$ cerebellum. Thus, we analyzed how the treatment with an inhibitor of Csf1 receptor, PLX5622, which have been reported to eliminate microglia *in vivo*, affected disease progression. Two-week treatment of 3-month old *Tpp1*$^{-/-}$ animals with PLX5622 in their diet, downregulated expression of microglial genes, but markedly upregulated expression of astrogliosis markers like Aquaporin 4 and *Gfap*. Furthermore, long term exposure to PLX5622 accelerated lethality in *Tpp1*$^{-/-}$ mice. This increase

in astrogliosis indicates that microglia play a protective role during the neuroinflammation process in *Tpp1*$^{-/-}$ mice and that microglia-astrocyte communication is essential in controlling the progression of the neurodegenerative process.

Disclosures: M.S. Domowicz: None. **W. Chan:** None. **C.B. Ware:** None. **J. Andrade:** None. **N.B. Schwartz:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.06/I34

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant HL140182

Title: Bacterial pneumonia elicits structural damage to neurons

Authors: *M. T. LIN[1], A. C. JAGER[1], M. GREENE[1], A. ANDREW-FORTUNE[2], A. M. SCOTT[1];
[1]Univ. of South Alabama, Mobile, AL; [2]Dillard Univ., New Orleans, LA

Abstract: Patients who develop pneumonia are at increased risk of cognitive decline. The risk may linger for more than a year even after the patients have fully recovered from pneumonia. While the mechanisms underlying bacterial pneumonia initiated cognitive dysfunction remain elusive, our recent studies show that *Pseudomonas aeruginosa,* an opportunistic bacterium that causes nosocomial pneumonia, induces cytotoxic amyloid production and release from pulmonary endothelial cells. Lung infection with this bacterium results in detectable amyloids in the blood, even in the absence of bacteremia. When injected into the cerebral ventricle, the endothelial derived amyloids impair mouse performance in hippocampal-dependent tasks. In this study, we determined the neurotoxic effects of endothelial derived amyloids on hippocampal CA1 pyramidal neurons. We performed stereotaxic intracerebral ventricular, intratracheal, and intraperitoneal injections of endothelial amyloids and quantified dendritic spine morphologies using Golgi staining. Our results showed that endothelial derived amyloid neurotoxicity is dependent upon the *P. aeruginosa's* virulence factor PcrV. Intracerebral ventricular injection and intratracheal instillation reduced spine density, and spine density reduction was greater in apical dendrites, compared to basal dendrites, in a time-dependent manner. Thus, in patients who contract bacterial pneumonia, lung infection may elicit endothelial amyloids that are capable of neurocognitive impairment.

Disclosures: M.T. Lin: None. **A.C. Jager:** None. **M. Greene:** None. **A. Andrew-Fortune:** None. **A.M. Scott:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.07/I35

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Tisch MS Research Center of New York (private funds)

Title: Expression profiles of noncoding RNAs in the CSF of PPMS and RRMS patients

Authors: *A. IACOANGELI, N. FAVRET, C. ARNDTSEN, S. A. SADIQ;
Tisch MS Res. Ctr. of New York, New York, NY

Abstract: Studies of the expression profiles of noncoding RNAs (ncRNAs) have been of diagnostic and prognostic value in several diseases. Multiple sclerosis (MS) is an inflammatory neurodegenerative disease and the two main clinical manifestations are primary progressive MS (PPMS) and relapsing remitting MS (RRMS). Interestingly, there is no single molecular test for the diagnosis of PPMS and RRMS. Our aim was to identify molecular markers for PPMS and RRMS patients by analyzing the expression of ncRNAs in cerebrospinal fluid (CSF) samples of these patients. We performed an exploratory differential strand-specific RNA sequencing analysis of small and long ncRNA. Total RNA was isolated from CSF samples of (n=4) PPMS patients, (n=4) RRMS patients, and (n=4) healthy donors. Using the DEseq2 package, statistically significant expression levels were determined as ncRNAs with an adjusted p-value of less than 0.1 and a log2fold change greater than 1. Gene ontology and splice variant expression analyses were performed on the statistically significant ncRNAs. The RNA sequencing analysis of CSF samples identified 78 ncRNAs that were differentially expressed in PPMS patients in comparison to RRMS patients. A bioinformatic analysis reported that the expression levels of 7 ncRNAs were significantly different. Notably, noncoding vault RNAs — vtRNA 1-1 and vtRNA 3-1P — were upregulated in CSF samples of PPMS patients. The vtRNAs are an integral part of the vault complex, a large ribonucleoprotein complex. Although their functions are still obscure, recent studies have shown their involvement with multidrug resistances and autophagy modulation. In conclusion, these results highlight the expression profiles of 7 newly identified ncRNAs as potential molecular markers for the diagnosis of PPMS and RRMS. Further investigations will also elucidate the functional meaning of the unique expression levels of vtRNA 1-1 and vtRNA 3-1P in PPMS and RRMS, two very diverse clinical manifestations of multiple sclerosis.

Disclosures: A. Iacoangeli: None. **N. Favret:** None. **C. Arndtsen:** None. **S.A. Sadiq:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.08/I36

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Obesity impairs cognition and leads to morphological and neurogenic alterations

Authors: C. FERNANDES[1], L. FORNY-GERMANO[1], S. FERREIRA[1], J. JÚNIOR[2], F. DE FELICE[1];
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Abstract: Overweight and obesity are public health problems that affect 30% of the world's population. It is known that this accumulation of body mass causes increased blood pressure, insulin resistance, diabetes, among other comorbidities. But overweight also affects the nervous system which increases the propensity to develop neurodegenerative diseases. However, it is not clear yet how and when such changes occur. We used, as an obesity model C57/Bl6 transgenic ob/ob mice, and C57/Bl6 wild type as a control. Magnetic resonance imaging (MRI) was initially performed for in vivo brain volume analysis, from which was possible to measure a 10% decrease in the total brain volume of the obese when compared to controls. To investigate if mass loss impairs cognition, we performed an object recognition test. Ob/ob mice exhibit low learning performance and locomotion. Therefore, we evaluate markers of neurogenesis and cell proliferation. A 50% decrease in neuroblast-labeled cells (doublecortin), as well as cell cycle markers (ki-67 and BrdU), were seen in the hippocampus and lateral ventricle regions of ob/ob. Then, we check microglia and astrocytes in the hippocampus region. The ob/ob animals showed not only an increase in the number of microglial cells but a severe modification in their structure. Increased microglial activation and a greater number of amoeboid cells were observed. Astrocytic cells also presented increased numbers in obese. Our data suggest that obesity may impair cognitive function and bring early neurodegeneration in mice.

Disclosures: C. Fernandes: None. **L. Forny-Germano:** None. **S. Ferreira:** None. **J. Júnior:** None. **F. De Felice:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.09/I37

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Postdoctoral Grant 3180553 (SBC)
FONDECYT 1171645 (RvB)

Title: Serine racemase expression in hippocampal neurons and astrocytes is modified in aging and by inflammation

Authors: *S. BELTRAN-CASTILLO, M. TRIOLO, R. VON BERNHARDI;
Neurol., Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: Neuroinflammation is frequently observed during aging and in neurodegenerative diseases. Our "glia-dysregulation" hypothesis proposes that aging results in the impairment of glial cell regulation leading to neuroinflammation and a neurotoxic environment, which in turn could result in neurodegenerative diseases as the late-onset Alzheimer disease (LOAD). D-serine is a dextro amino acid synthesized in mammals' brain by serine racemase (SR). It acts as a co-agonist of the glutamate N-methyl-D-aspartate receptor (NMDAR). Whereas D-serine can facilitate memory formation through NMDA-mediate long-term potentiation (LTP), it can also facilitate glutamate-induced excitotoxicity, leading to synaptic impairment and neurodegeneration. Here, we assessed the expression and distribution of SR in the hippocampus in aging and during systemic inflammation. We induced systemic inflammation administering a single i.p. injection of LPS (0.5 mg kg-1), or vehicle (PBS) in juvenile, adult and old mice (3-, 12- or 20-month old C57/BL6/j mice, respectively). After 24 h, mice were perfused with Hank's buffer for obtaining brain lysates, or with PBS and fixative (4% PAF) for obtaining 20 µm brain cryosections. SR content in the brain lysates was measured by western blot, and its expression in the hippocampus was evaluated by immunofluorescence, together with NeuN and GFAP immunolabeling for neurons and astrocytes identification, respectively. We observed an increased expression of SR in brain lysates in aging, reaching a 1.5- to 2.0-fold the level observed in juvenile mice at 12- and 20-month-old mice, respectively. SR expression increase was conspicuously associated with NeuN labeling in the stratum pyramidale of CA3 in 12- and 20-month old mice. Systemic inflammation induced a substantial increase in SR content in brain lysates from juvenile and adults' mice, although the already high content of SR in old mice was not further increased. Increased SR content after LPS was mainly associate to GFAP positive astrocytes at all ages. Our data reveal an aging- and neuroinflammation-associated increase of SR in the brain, which could facilitate D-serine- and glutamate-induced excitotoxicity, impairment of synaptic plasticity, neurodegeneration, and thus, be part of LOAD pathogenesis.

Disclosures: S. Beltran-Castillo: None. **M. Triolo:** None. **R. von Bernhardi:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.10/I38

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: The effect of chronic neuroinflammation on the kinetics of Dextran clearance from the brain parenchyma

Authors: *S. SURESH, J. LARSON, K. JENROW;
Central Michigan Univ., Mount Pleasant, MI

Abstract: Clearance of macromolecular waste from the brain parenchyma is achieved by a complex circulation of cerebral spinal fluid (CSF) within perivascular spaces, facilitating the diffusion of these ‘waste’ products through interstitial space and their transit to the cervical lymphatics where they are ultimately deposited into the serum.To investigate the influence of chronic neuroinflammation in this context, we induced a persistent inflammatory condition in the rat brain using a single high-dose administration of lipopolysaccharide (LPS, 5 mg/kg, i.p.). At eight weeks post-induction we measured the distribution kinetics of macromolecular florescent tracers following their injection into the subarachnoid CSF in healthy and chronically-inflamed rat brains. Two fluorescently-labeled dextran tracers, (3 and 10 kilodaltons, respectively), dissolved in a single 10 µl volume of artificial CSF, were infused directly into subarachnoid space via the cisterna magna and subsequently assayed in both parenchyma and serum at 15-, 30-, and 45-minutess post-infusion. From the subarachnoid space (Compartment 1), these dextran can be taken up directly into the dural/cervical lymphatics and deposited in the serum (Compartment 5). Alternatively, they can penetrate the brain by convection within perivascular space (Compartment 2) and diffuse within the parenchyma (Compartment 3). Clearance of these tracers from the parenchyma involves diffusion back into perivascular space (Compartment 4) and convective transport to the cervical lymphatics and removal to the serum. Our data show that the distribution kinetics of these dextran tracers is significantly impacted by neuroinflammation, and also by the size of the dextran. Here we have modeled these distribution kinetics using compartmental analysis. Our results reveal that the critical rate constant impacted by neuroinflammation involves the convective transport of tracer out of the parenchyma.

Disclosures: S. Suresh: None. **J. Larson:** None. **K. Jenrow:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.11/I39

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Using patient iPSC derived motor neurons as a model for leukoencephalopathy with brainstem and spinal Ccord Involvment and lactate elevation

Authors: *B. O'BRIEN[1], C. L. NEMETH[2], P. HUBO[1], S. N. TOMLINSON[1], A. SMITH[3], A. FATEMI[4];
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Abstract: Leukoencephalopathy with Brainstem and Spinal Cord Involvement and Lactate Elevation (LBSL) is a rare autosomal recessive disease most commonly affecting white matter regions of the brain, pyramidal tract, and dorsal column. Disease onset typically occurs during childhood or adolescence, but has also been reported during infancy and adulthood with phenotypes more severe and mild, respectively. Symptoms include slowly progressive ataxia, dysarthria and spasticity. Individuals with LBSL are compound heterozygotes for mutations in the gene *DARS2*, which encodes for the Class II Mitochondrial Aspartyl-tRNA Synthetase and plays an essential role during the translation of proteins in the mitochondria by charging aspartic acid to its cognate tRNA. Due to the large number of pathogenic variants found in LBSL patients, establishing a genotype/phenotype correlation has shown to be difficult. The most frequently observed pathogenic variant presents at the end of intron 2 on one allele, affecting the splicing efficiency of exon 3 resulting in nonsense mediated decay of the mature mRNA transcript. This splice site mutation pairs with a more variable missense mutation on the second allele. Despite the ubiquitous expression of *DARS2* throughout the body, mutations in this gene appear to only affect cell types found in the central nervous system (CNS), likely due to the extreme energy demands of the brain. To date, conditional knockout mouse models for *DARS2* have been established; however, these models fail to illustrate the true nature of the mutations found in LBSL due to the differences in murine splicing mechanisms and failed attempts to induce missense mutations. Patient derived induced Pluripotent Stem Cells (iPSCs) provide an excellent model for any disease, as they harbor the potential to rapidly differentiate into cell types of all three germ layers and exhibit the true pathology caused by disease triggering mutations. Using motor neurons rapidly derived from patient iPSCs, we show cell-type specific differences in the efficiency of splicing of *DARS2* in motor neurons compared to undifferentiated iPSCs. RT-qPCR and Western Blot analysis show an increase in abnormally spliced mRNA transcripts and a severe decrease in *DARS2* protein expression in motor neurons. In addition, live cell monitoring and electrophysiology analysis of motor neurons help to elucidate both

functional and developmental differences between LBSL and control cells. Establishing a patient derived neuronal cell model for LBSL allows for the opportunity to understand cell-type specific mechanisms for this disease, and serves as a test bed for potential therapeutics.

Disclosures: B. O'Brien: None. **C.L. Nemeth:** None. **P. Hubo:** None. **S.N. Tomlinson:** None. **A. Smith:** None. **A. Fatemi:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.12/I40

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: tcMEP and tcSEP in myelin oligodendrocyte glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE) in mice

Authors: Y. SHULMAN[1], Y. LEVI[1], L. FINKELSTEIN[1], I. LEVI[1], A. SCHAUDER[1], D. CASTEL[2], ***S. B. MEILIN**[1];
[1]MD Biosci., Nes Ziona, Israel; [2]Tel Avivi Univ., Tel Aviv, Israel

Abstract: Myelin Oligodendrocyte Glycoprotein (MOG) induced EAE is one of the most common murine models for multiple sclerosis. Yet, very little is known about the electrophysiology of the motor system and even less is known about the electrophysiology of the sensory system. EAE was induced by a single inoculum injection on study commencement. The inoculum injection consists of a homogenate emulsion mixture of MOG and CFA (200 μg MOG / 300 μg CFA). This was followed by pertussis toxin (PT) intraperitoneal injections (300 ng/mouse) on two occasions: once at the time of EAE induction and again 48 hours later on study day 2. In this study we assessed trance cranial motor evoked potential (tcMEP) and trance cranial sensory evoked potential (tcSEP) as a quantitative marker for *in vivo* monitoring of corticospinal tract damage. This study shows, for the first time, unique electrophysiology sensory (SEP) and motor (MEP) phenomena in early stages of EAE. The results show that in animals with mild EAE symptoms, the duration of MEP and connotative potentials increased without any change in the amplitude. However, the SEP amplitude increased significantly. As the disease progressed, a decrease in MEP and SEP amplitude was recorded. These results demonstrate a-synchronized neural motor activity at early stages followed by complete loss of signal in sever EAE disease. Additionally, the data show that early stage of the disease is also characterize by hyper-sensitivity of the sensory system. These significant findings can serve as a basis for a new drug screening model for treating early Multiple Sclerosis symptoms.

Disclosures: Y. Shulman: None. **Y. Levi:** None. **L. Finkelstein:** None. **I. Levi:** None. **A. Schauder:** None. **D. Castel:** None. **S.B. Meilin:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.13/I41

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Characterization of relevant mouse models for new biomarker

Authors: V. NIEDERKOFLER, T. LOEFFLER, E. AUER, S. FLUNKERT, *J. NEDDENS, B. HUTTER-PAIER;
QPS Austria GmbH, Grambach, Austria

Abstract: Introduction: For pre-clinical and clinical assessment of neurodegenerative diseases peripheral biomarker are needed to determine disease state and progression in a minimally invasive way. Recently established biomarkers like Neurofilament-light chain (NF-L) or sTREM2, are of importance to ensure early diagnosis and monitor disease progression in humans. Use of the same biomarkers in preclinical and clinical studies may thus be an option to overcome translational issues.
The aim of this study was to evaluate NF-L and sTREM2 levels in plasma and CSF of well-described mouse lines used for neuropharmacological studies in different indications.
Method: 5xFAD, Line61, NPC-/- and TDP43 mice as models of Alzheimer's, Parkinson's, Niemann-Pick Disease and Amyotrophic lateral sclerosis, respectively, were used at different ages. To explore Neurofilament light chain levels in CSF and plasma, the NF-Light® ELISA by UmanDiagnostics was used. For the detection of sTREM2 an immunosorbent assay specific for mouse TREM2, developed on the MSD (Mesoscale Discovery) platform was used. Immunohistochemical evaluation of neuronal loss and neuroinflammation was additionally performed.
Results: Besides other markers for neuroinflammation, also an increase in TREM2 levels was observed in 5xFAD mice. NF-L, a marker for neurodegeneration was found to be significantly increased in plasma already at an age of 6 months, before neuronal loss can be determined in the Alzheimer's disease 5xFAD mouse model. In contrast, in the alpha-synuclein overexpressing Line61, NF-L levels started to rise very late, reflecting data obtained from PD patients. Evaluations of additional mouse models are ongoing.
Conclusion/ Summary: Recently developed peripheral biomarkers for neurodegeneration, like NF-L, are also a valuable tool in preclinical mouse models. Additionally to their potential translational value, these models may also be a tool to longitudinally monitor neurodegeneration and neuroinflammation in the same animals by analyzing samples obtained from *in vivo* bleedings.

Disclosures: **V. Niederkofler:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **T. Loeffler:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **E. Auer:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **S. Flunkert:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **J. Neddens:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **B. Hutter-Paier:** A. Employment/Salary (full or part-time):; QPS Austria GmbH.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.14/I42

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: Universidad de Guanajuato. División de Ciencias de la Salud e Ingenierías. Departamento de Enfermería y Obstetricia

Title: Effect of Myrtillocactus geometrizans (garambullo) concentrations on the memory and the glucose, lipid peroxidation and neurotransmitter levels in the frontal cortex of rats high sugar diet fed

Authors: *C. SANDOVAL SALAZAR[1], A. ALAMO LEON[2], S. N. JIMENEZ GARCIA[2], X. S. RAMIREZ GOMEZ[2], V. BELTRAN CAMPOS[2];
[1]Salud e Ingenierías, Univ. De Guanajuato, Celaya, Mexico; [2]Salud e Ingenierías, Univ. de Guanajuato, Celaya, Mexico

Abstract: Obesity reduces life expectancy due to the factors like food habits. The consumption of high sugar diets promote weight gain, oxidative stress, increased glucose levels and affects learning and memory processes. Given this, it is necessary to explore prevention strategies through fruits such as *Myrtillocactus geometrizans*(garambullo); that contain antioxidant properties, which could decrease the oxidative.The aim of this study was to explore the antioxidant effect of three different garambullo concentrations on the memory, glucose, lipid peroxidation, neurotransmitter levels (glutamine, glutamate and GABA) in the frontal cortex of rats high sugar diet fed. A total of 50 healthy male rats were divided equally into five groups: 1)Standard diet+water; 2)High sugar diet+water; 3)High sugar diet+garambullo 150mg; 4)High sugar diet+garambullo 300mg 5)High sugar diet+garambullo 450mg. All the groups were fed over a six month period, but in the fifth month the third, fourth and fifth group received garambullo starting at four weeks. Subsequently, the water Morris maze test was performed. Tissues were obtained and the plasma glucose levels were measured using a spectrophotometric method. Lipid peroxidation levels were analyzed by measuring thiobarbituric acid reactive substances. The neurotransmitters concentration were quantified using HPLC electrochemical detection.The rats fed with a standard diet consumed approximately 50% more higher than the

rats who consumed a high sugar diet and the garambullo have no effect. Glucose and lipid peroxidation levels were high in the groups who consumed sugar compared to the standard diet and garambullo did not decrease the concentrations. With respect to the neurotransmitters, GABA levels decreased in the high sugar diet rats, however the glutamine and glutamate levels increased and the garambullo's only effect showed to decrease glutamate levels in the 450mg concentration. Likewise, the sugar diet impairs spatial memory. Accordingly, it is necessary to probe more doses of garambullo, as well as, subchronic or chronic administration to determine the optimal dose and time to understand how the treatment with fruits like garambullo may have effects on the brain and improve the negative effects of high sugar diets intake.

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Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

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Program #/Poster #: 389.15/I43

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH NS064934

Title: Rab10 mediated regulation of endocytic vesicle trafficking

Authors: *Z. LIU[1], A. B. WEST[2];
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Abstract: Common genetic variants in the *Rab10* gene have been identified as protective in genome-wide association studies in Alzheimer's disease (AD), and Rab10 expression is elevated in AD compared to controls. Recently, Rab10 has been discovered as a substrate for phosphorylation by the Parkinson's disease-associated protein leucine-rich repeat kinase 2 (LRRK2), further implicating the Ras-family Rab10 in neurodegeneration. Proteomic studies reveal that professional phagocytic cells in the innate immune system have some of the highest LRRK2 expression in humans, although the function of Rab10 in vesicle trafficking is not clear. Here we find that Rab10 regulates clathrin independent endocytosis in macrophages and microglia. Knock down of Rab10 reduces the uptake and clearance of a variety of soluble proteins and polysaccharides. We are further characterizing the role of Rab10 positive endosomes in macrophage activation and polarization, and how phosphorylation of Rab10 via the LRRK2 protein kinase affects Rab10 function. Overall we hypothesize that Rab10 mediates important aspects of upregulation of endolysosomal maturation in macrophage pro-inflammatory polarization.

Disclosures: **Z. Liu:** None. **A.B. West:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.16/I44

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NSF STC CBET 0939511
NIH 1R01 HL109192

Title: Reactive oxygen species-responsive drug delivery system for balanced anti-oxidation of inflamed brain tissue

Authors: Y. SEO[1,2], **Y.-T. HONG**[1], J. W. MITCHELL[2,3,4], *M. U. GILLETTE[2,3,4], H. KONG[1,2,3];
[1]Dept. of Chem. & Biomolecular Engin., [2]Neurosci. Program, [3]Beckman Inst. for Advanced Sci. and Technol., [4]Dept. of Cell and Developmental Biol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Overproduced reactive oxygen species (ROS) are closely related to various neuroinflammation and neurodegeneration. Abnormally high ROS levels can cause serious oxidative damage to biomolecules, cells, and, tissues. A series of nano- or micro-sized particles has been developed to reduce the oxidative stress level by delivering antioxidant drugs. However, most systems are often plagued by slow molecular discharge which reduces therapeutic efficacy or uncontrollably fast release which abolishes crucial physiological roles of ROS entirely. Herein, this study demonstrates the polymeric particles whose internal pressure can increase upon exposure to H_2O_2, one of the ROS, and, in turn, discharge antioxidants when needed. The on-demand pressurized particles are assembled by simultaneously encapsulating water-dispersible manganese oxide (MnO_2) nanosheets and green tea-derived epigallocatechin gallate (EGCG) molecules into a poly(lactic-co-glycolic acid) (PLGA) spherical shell. In the presence of H_2O_2, the MnO_2 nanosheets in the PLGA particle generate oxygen gas by decomposing H_2O_2 and increase in the internal pressure. The pressurized PLGA particles release antioxidative EGCG actively and, in turn, protect vascular and brain tissues from oxidative damage more effectively than the particles without MnO_2 nanosheets. This H_2O_2 responsive, self-pressurizing particle system would be useful to improving therapeutic efficacy of a broad array of neuromedicine.

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Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.17/J1

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: CB2015-1/257849 from National council of Science and Technology (CONACYT)
grant support to graduate students from CONACYT with CVU number: 888624

Title: Effect of metabolic syndrome on the clearence of amyloid beta peptide through the blood brain barrier and neuroinflammation in rat hippocampus

Authors: *C. F. AGUILAR-GAMAS, Sr, E. MARTINEZ-ABUNDIS, E. DE LA CRUZ-HERNANDEZ, N. GOMEZ-CRISOSTOMO;
Univ. Juarez Autonoma de Tabasco, Comalcalco, Mexico

Abstract: Obesity and the metabolic syndrome (MS) are a growing health problem worldwide due to the consumption of high-calorie diets (high sugar and high fat content diets) in combination with a sedentary lifestyle. Several studies shown that obesity and the metabolic syndrome are accompanied by the state of systemic inflammation, the nervous system is not exempt, where the activation of the vascular endothelium, microglia and astrocytes predominates, promoting the production of β-amyloid peptide (AB), as well as a dysfunction in the blood-brain barrier (BBB) -involved in the clearance of this protein-, which could lead to cognitive impaired and increased risk of developing Alzheimer's disease. The objective of this study is to evaluate the effect of MS on the function of the BBB through the evaluation of the clearance of AB such as the neuroinflammation process and its participation in cognitive impairment. Newly weaned male Wistar rats were fed chow with high sugar or high fat diets during 8, 10 and 12 months. Blood pressure, glucose, cholesterol, triglyceride levels and the percentage of retroperitoneal fat were determined to confirm the presence of MS. The memory was evaluated with Morris water maze. The hippocampus was dissected and the expression of amyloid beta, glial fibrillary acidic protein (GFAP) and Ionized calcium binding adaptor molecule 1 (IBA1) protein were determined to evaluate neuroinflammation and the elimination of AB through BBB. The results shown that the animals present the alterations of the metabolic syndrome at 10 and 12 months with both diets and an increased level of GFAP observed in both hypercaloric diets and is more significantly after 12 months consumption of the high-fat diet. The expression of IBA1 also was increase at 10 and 12 months of consumption of both diets, correlating with an increase in the expression of AB levels observed in all treatment times, being more remarkable at 12 months. These results indicate astrocytic and glial activation and therefore neuroinflammation in addition to the increase in AB hippocampal levels suggest an alteration in

the clearance process through the BBB. It is observed that at 8, 10 and 12 months with both diets there is a significant increase in the amount BA this is more marked in the diet high in fat, in the amount of IBA-1 the increase at 10 and 12 months is more notorious at 12 months, in both diets. These events could explain, at least in part, the association between MS and the neurodegenerative process that characterizes AD.

Disclosures: C.F. Aguilar-Gamas: None. **E. Martinez-Abundis:** None. **E. De la Cruz-Hernandez:** None. **N. Gomez-Crisostomo:** None.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.18/J2

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Anti-inflammatory effects of SM07883, a novel, potent, and selective oral DYRK1A inhibitor in mouse models of neuroinflammation

Authors: *S. ANDERSON, M. W. AKHTAR, K. DUONG-POLK, C. LAI, B. GUNER, S. HABROUN, B. MELCHIOR;
Samumed, LLC, San Diego, CA

Abstract: Neuroinflammation contributes to many neurodegenerative disorders, including Alzheimer's disease and Multiple Sclerosis. Inhibition of dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) activity reduced tau and amyloid pathology as well as associated gliosis in AD transgenic mice. This study dissected the mechanisms by which SM07883, an oral DYRK1A inhibitor, inhibited neuroinflammation *in vitro* and in mouse models innate and adaptive CNS immune responses. To measure inflammation *in vitro*, cytokine concentrations were measured with MSD platform in supernatants from BV2 microglial cells and primary mouse astrocytes challenged by lipopolysaccharide (LPS). STAT3 and NFATc1 phosphorylation and nuclear translocation were measured by Western blot, ELISA, and imaging. In 3 experiments, sorted BALB/C mouse splenocytes were stained with CFSE and stimulated for 5 days with anti-CD3/CD28 antibodies. Cell division was analyzed by flow cytometry. Brains from BALB/c mice challenged with intracerebral or intraperitoneal LPS were analyzed after SM07883 (10mg/kg, QD, 5 days, n =5) or vehicle administration. C57BL/6 wild type mice with induced experimental autoimmune encephalomyelitis (EAE) after MOG_{35-55} immunization were treated with SM07883 (5-10mg/kg, QD and 5mg/kg, BID, 35 days) or vehicle (n =15 for each group). Clinical scores were measured daily and cytokines in spinal cord tissue were analyzed by Milliplex assays. *In vitro*, SM07883 inhibited LPS-induced cytokine secretion compared to DMSO control in microglial cells (e.g., TNFα EC_{50} =71nM) and in primary astrocytes (EC_{50}: TNFα =932nM; IL-6 =698nM; KC/GRO =2.1uM). These effects were associated with dose-

dependent reductions in phosphorylation and nuclear translocation of NFATc1 and STAT3 ($p<0.05$). Potent reduction in T cell proliferation was associated with decrease in proinflammatory cytokines (EC_{50} =15nM, IFNγ =41nM, TNFα =46nM) with SM07883 compared to DMSO. Innate responses, measured by proinflammatory cytokines TNFα, IFNγ, IL-1β, and IL-6 as well as the chemokine KC/GRO, were increased in brains from LPS-challenged mice and significantly reduced with SM07883 treatment compared to vehicle. EAE mice treated with SM07883 improved clinical scores with reduced adaptive immune responses measured by decreased cytokines ($p<0.05$) and lymphocyte count in spinal cord compared to vehicle. SM07883, an oral DYRK1A inhibitor, significantly reduced proinflammatory mediators and associated inflammation in both innate and adaptive inflammatory mouse models compared to vehicle. SM07883 may potentially modulate neuroinflammation in neurodegenerative diseases.

Disclosures: **S. Anderson:** A. Employment/Salary (full or part-time):; Samumed, LLC. **M.W. Akhtar:** A. Employment/Salary (full or part-time):; Samumed, LLC. **K. Duong-Polk:** A. Employment/Salary (full or part-time):; Samumed, LLC. **C. Lai:** A. Employment/Salary (full or part-time):; Samumed, LLC. **B. Guner:** A. Employment/Salary (full or part-time):; Samumed, LLC. **S. Habroun:** A. Employment/Salary (full or part-time):; Samumed, LLC. **B. Melchior:** A. Employment/Salary (full or part-time):; Samumed, LLC.

Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.19/J3

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIHS Grant NS092803

Title: Aberrant miRNA expression in multiple system atrophy contributes to neuroinflammation and may affect oligodendrocyte maturation

Authors: T. KIM[1], **L. AKHMETOVA**[1], L. E. CROMBERG[1], *P. A. DESPLATS[2]; [1]Neurosciences, [2]Neurosciences and Pathology, UCSD, La Jolla, CA

Abstract: Multiple System Atrophy (MSA) is a neurodegenerative disease characterized by the accumulation of α-synuclein (α-syn) in oligodendrocytes; resulting in demyelination, neuronal damage and inflammation. Micro-RNAs (miRNA) regulate gene expression by triggering the degradation of their target mRNA molecules. There is ample evidence for the involvement of miRNA deregulation in neurodegeneration. The purpose of this study was to perform a comprehensive appraisal of miRNA alterations in *post mortem* striatum samples from MSA cases and a parallel transcriptome analysis of genes associated with neurodegeneration. In addition, since immature oligodendrocytes are increased in MSA brains, we evaluated whether

miRNA alterations mediate the effects of α-syn on oligodendrocyte maturation using an *in vitro* model.
We profiled 800 miRNAs using the nCounter® Human v3 miRNA panel and 770 genes related to neuropathology using the nCounter® Human Neuropathology panel in 18 brain samples. For the *in vivo* model, we differentiated neuronal progenitor cells from adult rat hippocampus (ARHNPC) into oligodendrocytes and used lentiviral constructs to overexpress α-syn. We applied the nCounter® Rat v1.5 miRNA panel to profile the expression of 420 miRNAs at different time points of the maturation protocol. Data was analyzed using nSolver® software v4.0, Ingenuity Pathway Analysis and Diana mirPath v3.
We identified 60 miRNAs with abnormal levels in MSA brains that are involved in extracellular matrix receptor interactions, prion disease, inflammation, ubiquitin-mediated proteolysis and addiction pathways. We also identified 96 differentially expressed genes, related to inflammation, myelination, autophagy and vesicle transport pathways. Importantly, based on the correlation between miRNA expression and the abundance of their known targets, miR-124-3p, miR-19a-3p, miR-27b-3p, and miR-29c-3p were identified as key regulators altered in MSA, mainly contributing to neuroinflammation.
Analysis of the *in vitro* model uncovered distinct miRNA profiles associated with oligodendrocyte maturation that were altered by α-syn overexpression.
We provide evidence for the role of miRNA deregulation in MSA pathology, increasing inflammation and altering oligodendrocyte maturation.

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Poster

389. Neurotoxicity, Inflammation, and Neuroprotection: Preclinical Studies II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 389.20/J4

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: AG055700
5T32HL091816-07

Title: Delirium is associated with inflammation and neuronal injury

Authors: *C. P. C. CASEY[1], H. L. LINDROTH[2,3], R. MOHANTY[4,6], T. BALLWEG[6], Z. FARAHBAKHSH[1], S. TWADELL[6], B. M. KRAUSE[5], V. PRABHAKARAN[7], K. BLENNOW[8], H. ZETTERBERG[8,9,10,11], R. D. SANDERS[12];
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WI; [6]Anesthesiol., Univserity of Wisconsin - Madison, Madison, WI; [7]Dept Neurosci, Univ. of Wisconsin Madison, Madison, WI; [8]Dept. of Psychiatry and Neurochemistry, Sahlgrenska Acad. at the Univ. of Gothenburg, Mölndal, Sweden; [9]Clin. Neurochemistry Lab., Sahlgrenska Univ. Hosp., Mölndal, Sweden; [10]Dept. of Neurodegenerative Dis., UCL Inst. of Neurol., London, United Kingdom; [11]UK Dementia Res. Inst., UCL, London, United Kingdom; [12]Univ. of Wisconsin, Madison, Madison, WI

Abstract: While delirium is associated with cognitive decline and dementia, there is limited evidence to support causality for this relationship. Clarification of the mechanism through which delirium could cause cognitive decline would enhance plausibility for a causal relationship. We tested whether delirium is associated with neuronal injury in 114 surgical patients recruited to a prospective biomarker cohort study. Patients underwent perioperative testing for changes in neurofilament light (NfL), a neuronal injury biomarker, as well as a panel of 10 cytokines, with contemporaneous assessment of delirium severity and incidence as well as sampling for inflammatory. A subset of patients underwent preoperative magnetic resonance imaging. Initially we confirmed prior reports that NfL levels correlated with markers of neurodegeneration (hippocampal volume [$\Delta R^2 = 0.129$, $p = 0.015$] and Fractional anisotropy of white matter [$\Delta R^2 = 0.417$, $p<0.001$]) in our cohort and that surgery was associated with increasing NfL from preoperative levels ([mean difference = 0.240 [0.178, 0.301] $\log_{10}$(pg/mL), $p < 0.001$]), suggesting putative neuronal injury. Next, we tested the relationship with delirium. NfL rose more sharply in participants with delirium compared to non-sufferers ([mean difference = 0.251 [0.136, 0.367] $\log_{10}$(pg/mL), $p < 0.001$]). This relationship showed dose-dependence, such that NfL rose proportionately to delirium severity [$\Delta R^2 = 0.199$, $p < 0.001$]. Given that inflammation is considered an important driver of postoperative delirium, next we tested whether NfL, as a potential marker of neurotoxicity, may contribute to the pathogenesis of delirium independent of inflammation. From a panel of 10 cytokines, the pro-inflammatory cytokine IL-8 exhibited a strong correlation with delirium severity ($\Delta R^2 = 0.208$, $p < 0.001$). Therefore, we tested whether the change in NfL contributed to delirium severity independent of IL-8. NfL was independently associated with delirium severity after adjusting for the change in inflammation ($\Delta R^2 = 0.040$, $p = 0.038$). These data suggest delirium is associated with exaggerated increases in NfL and that this putative neurotoxicity may contribute to the pathogenesis of delirium itself, independent for changes in inflammation. Dose-dependence with delirium severity enhances the biological plausibility for this relationship.

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Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.01/J5

Topic: C.08. Ischemia

Support: Grants-in-Aid for Scientific Research from the Ministry of Education, Sports, Science, and Culture of Japan

Title: Neuroprotective effects of a novel carnosine-hydrazide derivative on hippocampal CA1 damage after cerebral ischemia

Authors: *M. MORIOKA[1], K. NOGUCHI[2], T. F. ALI[3], M. OHTSUKA[4];
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Abstract: Ischemia-reperfusion injuries produce reactive oxygen species that promote the peroxide lipid oxidation process resulting in the production of an endogenic lipid peroxide, 4-hydroxy-trans-2-nonenal (4-HNE), a highly cytotoxic aldehyde that induces cell death. We synthesized a novel 4-HNE scavenger - a carnosine-hydrazide derivative, L-carnosine hydrazide (CNN) - and examined its neuroprotective effect in a model of transient ischemia. PC-12 cells were pre-incubated with various doses (0-50 mmol/L) of CNN for 30 min, followed by incubation with 4-HNE (250 μM). An MTT assay was performed 24 h later to examine cell survival. Transient ischemia was induced by bilateral common carotid artery occlusion (BCCO) in the Mongolian gerbil. Animals were assigned to sham-operated (n = 6), placebo-treated (n = 12), CNN pre-treated (20 mg/kg; n = 12), CNN post-treated (100 mg/kg; n = 11), and histidyl hydrazide (a previously known 4-HNE scavenger) post-treated (100 mg/kg; n = 7) groups. Heat shock protein 70 immunoreactivity in the hippocampal CA1 region was evaluated 24 h later, while delayed neuronal death using 4-HNE staining was evaluated 7 days later. Pre-incubation with 30 mmol/L CNN completely inhibited 4-HNE-induced cell toxicity. CNN prevented delayed neuronal death by >60% in the pre-treated group ($p < 0.001$) and by >40% in the post-treated group ($p < 0.01$). Histidyl hydrazide post-treatment elicited no protective effect. CNN pre-treatment resulted in high heat shock protein 70 and low 4-HNE immunoreactivity in CA1 pyramidal neurons. Higher 4-HNE immunoreactivity was also found in the placebo-treated animals than in the CNN pre-treated animals. Our novel compound, CNN, elicited highly effective 4-HNE scavenging activity *in vitro*. Furthermore, CNN administration both pre- and post-BCCO remarkably reduced delayed neuronal death in the hippocampal CA1 region via its induction of heat shock protein 70 and scavenging of 4-HNE.

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Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.02/J6

Topic: C.08. Ischemia

Support: NIH 1 R01 NS091099

Title: Hyperoxic resuscitation following canine cardiac arrest increases cerebellar Purkinje neuronal death

Authors: *G. FISKUM[1], D. LEE[2], T. PEARSON[2], J. PROCTOR[2], R. E. ROSENTHAL[2]; [1]Anesthesiol., [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Maintaining physiologic O_2 levels (normoxia) immediately after restoration of spontaneous circulation (ROSC) from cardiac arrest (CA) results in less hippocampal neuronal death compared to animals ventilated during the first hr of ROSC. However, other brain regions, e.g., those that undergo post-ischemic hypoperfusion, might actually benefit from hyperoxic reperfusion. This study tested the hypothesis that when compared to hyperoxic reperfusion, pulse-oximetry guided normoxic resuscitation following ventricular fibrillation (VFib) CA reduces short-term cerebellar neuronal death and inflammatory microglial activation. Female beagles were anesthetized and ventilated. CA was induced by electrical myocardial stimulation and cessation of ventilation. Ten min later, dogs were ventilated with 100% O_2 and resuscitated using 3 min of open chest CPR followed by electrical defibrillation. Dogs were then ventilated for one hr with 100% O_2 (hyperoxia) or with O_2 titrated rapidly to maintain hemoglobin saturation at 94 to 96% (normoxia). Following 24 hr critical care, dogs were euthanized and the cerebellum analyzed for neuronal death (Calbindin D) and microglial activation (Iba1). StereoInvestigator and GraphPad software were used for stereologic quantification of neuronal death and microglial activation. Calbindin-stained Purkinje cells were classified morphologically into "healthy" and "damaged" neurons. Healthy neurons displayed an intact morphology, with darkly stained nuclei and more lightly stained axons and dendrites. Dying neurons were characterized by the lack of nuclear staining and loss of intact morphology. Hyperoxic resuscitation significantly increased the number of dying Purkinje cells by 278%. The morphology of Iba-1 immunopositive microglia was classified as either "resting", with small cell bodies and extensive dendritic processes, or "activated", with large cell bodies and retraction of cell processes. Hyperoxic resuscitation increased the number of activated microglial/macrophages by 18% in comparison to values obtained after normoxic resuscitation. Hyperoxic resuscitation increased the number of dying Purkinje cells by almost 300% and the number of activated microglial by 20%. These results indicate that the favorable histopathologic outcomes following CA and normoxic compared to hyperoxic resuscitation are observed in the

cerebellum and therefore not limited to the hippocampus. These findings emphasize the importance of avoiding unnecessary hyperoxia during at least the first hr following CA and ROSC. Supported by NIH 1 R01 NS091099

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Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.03/J7

Topic: C.08. Ischemia

Support: AIMS award from Atomwise
AHA Career Development award 18CDA34110011
START PPOC

Title: Search of novel purinergic P2x4 receptor antagonists for the treatment of ischemic stroke

Authors: *P. SRIVASTAVA[1], V. K. RAO[1], B. T. LIANG[1], T. O'BRIEN[2], R. VERMA[1]; [1]Neurosci., Uconn Hlth., Farmington, CT; [2]Atomwise, San Francisco, CA

Abstract: Stroke remains a leading cause of disability in the United States. Despite recent advances, interventions to reduce damage and enhance recovery after stroke are lacking. As the both resident and peripheral immune response (specifically myeloid origin cells) contributes to secondary tissue damage and poor recovery even after several hours of stroke onset, targeted inhibition of myeloid cell response has become an area of major therapeutic interest. We have recently shown that P2X4R, a purinergic receptor for adenosine triphosphate (ATP), regulates activation of myeloid immune cells (infiltrating monocytes/macrophages and brain-resident microglia) after stroke injury (Verma et al., 2017). Over activation of P2X4Rs, due to ATP released from dying or damaged neuronal cells, contributes to ischemic injury. Motivated by this work, we hypothesize that acute inhibition of P2X4R is beneficial after stroke. As commercially available P2X4R inhibitor has several limitations in their in vivo use e.g. insoluble in biological vehicle and limited blood brain barrier (BBB) permeability. Using cutting edge artificial intelligence technique available through the AIMS program at Atomwise, a high throughput virtual screen was used to identify novel small molecular inhibitors of P2X4R. The best scoring compounds from the screen were then clustered for scaffold diversity and then filtered for drug-like properties to arrive at a final subset of 72 potential P2X4R inhibitors. These 72 compounds and the standard P2X4R inhibitor
5-BDBD were tested using Ca++ influx assay in Human P2X4R expressing cell line (HEK293). We found 6 active compounds with inhibitory activity in μM concentration. Out of these six one

compound was comparable with 5-BDBD in its P2X4R inhibitory activity. In summary, we identify a total of six potential small molecule inhibitors of P2X4R, which are presumably soluble in biological solvent and are BBB permeable. In next series of experiments, we will confirm their P2X4R inhibitory activity in primary microglial culture as well as in *in-vivo* model of ischemic stroke in rodents.

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Poster

390. Ischemic Stroke III

Location: Hall A

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Program #/Poster #: 390.04/J8

Topic: C.08. Ischemia

Support: NIH Grant R01HL139712
NIH Grant R01HL128546
DC-IDDRC U54HD090257

Title: Intra-arterial mesenchymal stromal cell delivery through cardiopulmonary bypass for neuroprotection in a juvenile porcine model

Authors: *T. MAEDA[1], K. SARKISLALI[1], N. SARIC[1], F. A. SOMAA[1], C. LEONETTI[1], G. R. STINNETT[1], Z. DHARI[1], B. LEWIS[5], C.-H. HSU[6], K. PANCHAPAKESAN[2], R. ULREY[3], K. GRECCO[4], P. VYAS[4], Y. IMAMURA KAWASAWA[7], T.-W. TU[6], K. HASHIMOTO-TORII[8], P. J. HANLEY[3], J. A. FRANK[5], R. A. JONAS[1], N. ISHIBASHI[1];
[1]Children's Natl. Heart Inst. and Ctr. for Neurosci. Res., [2]Ctr. for Genet. Med., [3]Ctr. for Cancer and Immunol. Res., [4]Radiology and Nuclear Med., Children's Natl. Hlth. Syst., Washington, DC; [5]Radiology and Imaging Sci., US Natl. Inst. of Hlth. (NIH), Bethesda, MD; [6]Mol. Imaging Lab., Howard Univ., Washington, DC; [7]Pharmacol. and Biochem. and Mol. Biology, Inst. for Personalized Med., Pennsylvania State Univ. Col. of Med., Hershey, PA; [8]Ctr. for Neurosci. Res., Children's Natl. Hlth. Syst., Washington, DC

Abstract: Neurodevelopmental impairment is an important challenge for survivors after pediatric cardiac surgery. Cardiopulmonary bypass (CPB) can cause substantial systemic inflammation and trigger prolonged microglial activation in the brain. Mesenchymal stromal cells (MSCs) have significant immunomodulatory properties. We hypothesize that MSC delivery through CPB is neuroprotective by modulating systemic and neuro-inflammatory responses. Two-week old piglets (n=16 total) were randomly assigned to one of 3 groups: (1) Control, (2) Deep hypothermic circulatory arrest (DHCA), (3) DHCA followed by MSC administration. In group 3, ^{18}F-FDG or superparamagnetic iron oxide (SPIO)-labeled MSCs ($10x10^6$ per kg) were

delivered through CPB. Positron emission tomography (PET) was performed 1hr after MSC delivery to determine the whole body distribution of cells. Animals were sacrificed 3hrs after CPB for analysis with magnetic resonance imaging (MRI) and immunohistochemistry. Total cortical RNA was extracted and sequenced, followed by differential expression analysis. Gene ontology (GO) was assessed using Enrichr on differentially expressed genes (DEGs) whose normalized read count displayed a rescue profile upon MSC delivery. Plasma cytokine/chemokine levels were determined by multiplex immunoassay. Clinically-relevant physiological biomarkers determined the effect of MSC delivery on multi-organ function. PET study showed that intra-arterial delivery through CPB uniformly distributed MSCs to all organs analyzed such as the brain, heart, and kidney except that lungs and intestine showed lower uptake. T2* weighted brain MRI showed diffuse distribution of SPIO particles throughout the entire brain. Immunohistochemistry revealed an even distribution of delivered MSCs within the cortex and white matter. MSCs were noted to have migrated into the extra-vascular space. Analysis of the RNA sequencing data identified 262 DEGs between the DHCA and DHCA/MSC groups. Of these, 53 upregulated genes were significantly enriched for WNT signaling in the GO analysis, indicating a potential mechanism for MSC-mediated neuroprotection. MSC delivery through CPB also modulated plasma cytokine/chemokine expression following surgery. In the brain MSC treatment reduced microglia expansion and caspase activation resulting from CPB. Various biomarkers after MSC delivery did not differ compared with CPB group. No evidence of either embolic events or microstrokes was observed by MRI and histology. MSC delivery during CPB has the translational potential to minimize systemic inflammation and reduce microglial expansion and caspase activation in children undergoing CPB.

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Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.05/J9

Topic: C.08. Ischemia

Title: 4R, a cembranoid compound, improves sensorimotor and cognitive functions after ischemic stroke

Authors: *J. WANG, J. WANG, J. HAO;
Dept. of Pharmaceut. Science, Col. of Pharm., Univ. of Cincinnati, Cincinnati, OH

Abstract: *Objective:* Our previous reports revealed 4R neuroprotective effects on rodents' cerebral ischemic disease model or 6-hydroxydopamine-induced Parkinson's disease model. Present studies focused on determine the effects of 4R on cognitive functions after ischemic stroke in mice, and elucidating relative mechanisms.
Methods: In vivo study: 4R (5 mg/kg/day) or same volume of DMSO or PBS was Intraperitoneally injected as early as 1 h after 60 min transient Middle Cerebral Artery Occlusion and up to 4 days. The sensorimotor functions (corner test) and cognitive functions (Y-maze test) were evaluated following the treatment. In vitro study: To determine the regulating mechanism of 4R on N9 cells (murine-derived microglia cell line) polarization under lipopolysaccharide (LPS)-induced inflammatory or oxygen glucose deprivation (OGD) conditions. And to evaluate the effects of conditioned medium derived from 4R-treated post-OGD N9 cells on viability of neuro2a cells (a murine neuroblastoma cell line).
Results: Corner test results indicated that 4R treatment improved corner test score to 0.41 compared to 0.86 of DMSO group at 24 h post ischemic stroke. Y-maze test results illustrated that 4R treatment group presented better memory retrieval ability than vehicle control group. In vitro results revealed the expression of N9 cell inducible nitric oxide synthase (iNOS), a Microglial M1 phase marker, was attenuated by 1uM 4R treatment to 69.4% and 56.4% of vehicle control under LPS or OGD conditions, respectively. The expression of N9 cell Argnase-1, a M2 phase marker, was increased by 1uM 4R treatment to 192.7% and 188.0% of vehicle control under LPS or OGD conditions, respectively. Furthermore, the viability of Neuro2a cells increased by 54.5% after treatment with the conditioned medium of 4R-treated post-OGD N9 cells, which associated with inhibiting pro-inflammatory cytokine TNF-α release and promoting anti-inflammatory cytokine IL-10 segregation.
Conclusions: 4R improves sensorimotor and cognitive functions after ischemic stroke in mice, which may due to the anti-inflammatory mechanism of 4R regulated microglia polarization.

Disclosures: **J. Wang:** None. **J. Wang:** None. **J. Hao:** None.

Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.06/J10

Topic: C.08. Ischemia

Support: 1R01NS096225-01A1
17GRNT33660336
19CDA3466032
17POST33660174
19POST34380784
19PRE34380808

Title: Neuroprotective effects of palmitic acid methyl ester on neuroinflammation and mitochondrial function in cerebral ischemia

Authors: *C. Y.-C. WU[1], A. COUTO E SILVA[2], G. A. CLEMONS[2], R. H.-C. LEE[1], H. LIN[1];
[1]Neurol., [2]Cell. Biol. and Anat., LSU Hlth. Sci. Ctr. Shreveport, Shreveport, LA

Abstract: We previously discovered that palmitic acid methyl ester (PAME) is a potent vasodilator released from the superior cervical ganglion. Post-treatment with PAME can enhance cortical cerebral blood flow and functional learning and memory, while it inhibited neuronal cell death in the CA1 region of the hippocampus under pathological conditions (i.e. cerebral ischemia). Since mechanisms underlying PAME-mediated neuroprotection remain unclear, we investigated the possible neuroprotective mechanisms of PAME after 6 min of asphyxia cardiac arrest (ACA, an animal model of global cerebral ischemia). Our results from capillary-based immunoassay suggest that PAME (0.02 mg/kg) can decrease neuroinflammatory markers, such as ionized calcium binding adaptor molecule 1 (IBa1, a specific marker for microglia/macrophage activation), interleukin-2 (IL-2), and monocyte chemoattractant protein-1 (MCP-1/CCL2) after cardiopulmonary resuscitation. Additionally, the oxygen consumption rate (OCR) and mitochondrial respiratory function in the hippocampal slices were restored following ACA (via Seahorse XF24 Extracellular Flux Analyzer) suggesting that PAME can ameliorate mitochondrial dysfunction. Finally, PAME improved memory-related long-tern potentiation (LTP) in the hippocampus reducing neurological deficits after ACA. Altogether, our findings suggest that PAME can provide neuroprotection in the presence of ACA to alleviate neuroinflammation and ameliorate mitochondrial dysfunction.

Disclosures: C.Y. Wu: None. **A. Couto E Silva:** None. **G.A. Clemons:** None. **R.H. Lee:** None. **H. Lin:** None.

Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.07/J11

Topic: C.08. Ischemia

Support: National Research Foundation of Korea grants 2015M3C7A1031395

Title: Effects of deletion of peroxiredoxins II on bilateral common carotid artery occlusion-induced impairments of hippocampal function

Authors: *Y.-S. JANG, J.-S. HAN;
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Abstract: Peroxiredoxins II (Prx II) act as an antioxidant enzyme that reduce peroxide levels. Evident immunoreactivity of Prx II is observed in hippocampal neurons. Prx II is expected to inhibit ROS accumulation in the hippocampus. Previous studies demonstrated that aged Prx II knock-out mice showed spatial memory impairments and altered levels of the proteins associated with learning and memory in the hippocampus. Therefore, it is suggested that Prx II play a neuroprotective role against brain injury through scavenging intracellular ROS. The present experiment was conducted to examine whether Prx II knock-out make more vulnerable to a sudden injury. Transient bilateral common carotid artery occlusion (BCCAo), one of the experimental animal models for transient global ischemia, was conducted. Mice were divided into 4 groups: sham-operated wild type (WT), WT with 20 min BCCAo, sham-operated Prx II KO, and Prx II KO with 20 min BCCAo. Cognitive status and neuronal death of these mice was evaluated using the hippocampus dependent tasks including Morris water maze task and novel object recognition test, and Fluoro-jade C. Severe significant memory impairments were found in the Prx II KO mice with BCCAo, compared with the other groups. But, no difference between WT with BCCAo and Prx II KO with BCCAo was observed in the Fluoro-jade C intensities of the hippocampus CA1. These results indicate that the Prx II play a protective role against brain injury, though its neurobiological mechanism is needed to be revealed. Supported by the National Research Foundation of Korea grants 2015M3C7A1031395 to J.S.H.

Disclosures: **Y. Jang:** None. **J. Han:** None.

Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.08/J12

Topic: C.08. Ischemia

Support: MAECI
NICHD - 1R03HD094608-01A1 (DJG)

Title: LFP based analysis of brain injured anesthetized animals undergoing closed-loop intracortical stimulation

Authors: A. AVERNA[1], ***F. BARBAN**[1,2], D. J. GUGGENMOS[3], R. J. NUDO[4], M. CHIAPPALONE[1];
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Abstract: Acquired brain injuries, such as stroke, are a major cause of long-term disability worldwide. When an injury occurs within primary motor cortex (M1), as is common in stroke,

there is an initial loss of descending information to the spinal cord, which leads to hemiparesis and other motor dysfunctions. In addition, it results in global changes in functional connectivity between spared, interconnected regions throughout the brain. Recently, activity-dependent stimulation (ADS), a closed loop intracortical neurostimulation technique, was shown to significantly improve behavioral recovery in rodent models that suffered a traumatic brain injury in the primary motor cortex. While the behavioral benefits are established, the neurophysiological changes underlying this effect remain unclear. In this study we attempt to understand how local field potential signal are modified in response to the ADS treatment following an injury to primary motor cortex. We used a rodent (Long Evans) model of induced ischemic injury in which endothelin-1, a potent vasoconstrictor, was injected unilaterally through the extent of the forelimb area of primary motor cortex. A total of two 4-shank, 16-site intracortical microelectrode array (MEA) were inserted in the ipsilesional rostral forelimb area (RFA) and forelimb-responsive somatosensory cortex. Rats were randomly assigned to either a non-stimulation control group (N=5) or ADS treatment group (N=7). Neural activity was recorded from all electrode sites in both groups. In the ADS group, therapeutic stimulation was initiated one hour after the lesion, with a user-identified single unit in RFA set to trigger the center channel in the S1 array. Stimulation was turned off after one hour, with recording continuing an additional 30 minutes. We performed analyses addressing power and connectivity changes in the LFP domain. Functional connectivity was measured using a power envelope correlations method developed by Hipp et al. (2012). Ischemic injury to M1 led to an overall decrease in LFP power within RFA. The number of functional connections between RFA and S1 increased after the stroke. Treatment with ADS induced a broadband increase in LFP power in both RFA and S1, while functional connections between the two areas decreased, which may be the one mechanism in which ADS operates.

Disclosures: A. Averna: None. **F. Barban:** None. **D.J. Guggenmos:** None. **R.J. Nudo:** None. **M. Chiappalone:** None.

Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.09/J13

Topic: C.08. Ischemia

Support: NIH/NINDS 1R01NS096225
American Heart Association 17GRNT33660336
American Heart Association 19CDA34660032
Louisiana State University Research Council/Grant in Aid

Title: Neuropeptide Y modulation via protein arginine methyltransferase in cerebral ischemia

Authors: *H. POSSOIT[1], A. COUTO E SILVA[2], C. Y.-C. WU[3], C. T. CITADIN[2], R. H.-C. LEE[3], H. LIN[3];
[2]Cell. Biol. and Anat., [3]Neurol., [1]LSU Hlth. Sci. Ctr. Shreveport, Shreveport, LA

Abstract: According to the American Heart Association, there are more than 350,000 Americans affected by cardiopulmonary arrest (CA) each year. CA is one of the leading causes of morbidity and mortality for those affected. No neuroprotective therapies are currently available to decrease neuronal cell damage and improve the cognitive outcomes of those affected; therefore, our goal is to identify novel targets that provide potential neuroprotective therapies.
The consequences of CA triggers the enhancement of the sympathetic nervous system (SNS) due to current resuscitation protocols (i.e. epinephrine) and the body's general autoregulation to raise blood perfusion back to nominal levels. This phenomenon is characterized by hypoperfusion (low blood perfusion). The onset of hypoperfusion can be from hours to days, which is the thought to be the primary cause of neuronal cell death and learning/memory deficits after ischemia. Additionally, the release of neuropeptide Y (NPY) during hypoperfusion induces potent vasoconstriction that is 100-fold more potent than neuronal-derived vasoactive substances to further incur more ischemic damage. We have discovered that the number of normal neurons was decreased in the CA1 region of the hippocampus 7 days after asphyxial cardiac arrest (ACA)(global cerebral ischemia) (527±19) (via H&E and fluoro-jade C staining), while inhibition of NPY release from pre-synaptic sympathetic nerves via peptide YY (PYY)3-36 (NPY2R agonist) inhibited neuronal degeneration (881.5±21.6) of normal neurons. Additionally, post-treatment with PYY3-36 enhanced cortical CBF (56.90±4.48%) (detected via two-photon) 24hrs after ACA as compared to ACA-treated rats (-33.20±2.65%). However, the regulation of NPY is multi-faceted and currently unknown. We propose that protein arginine methyltransferases (PRMTs, known methylators of molecular targets) can be possible modulators of NPY. Both NPY and PRMT 8 (specific PRMT in the brain) mRNA expression was decreased at days 3 and 7 after ACA as compared to control. These results suggest that NPY may be a modulator of PRMT8 activity, in turn, have cellular and/or physiological consequences during ischemia.

Disclosures: H. Possoit: None. **A. Couto E Silva:** None. **C.Y. Wu:** None. **C.T. Citadin:** None. **R.H. Lee:** None. **H. Lin:** None.

Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.10/J14

Topic: C.08. Ischemia

Support: NIH R01 NS085272

Title: Phytoestrogen isoflavone increases brain aspartate levels and protects against ischemic stroke injury

Authors: S. KHANNA[1], S. C. GNYAWALI[2], H. HARRIS[2], M. BALCH[2], S. NIMJEE[2], ***C. L. RINK**[2];
[1]Indiana Ctr. for Regenerative Med. and Engineerings and Dept. of Sur, Indiana Universty, Indianapolis, IN; [2]Neurolog. Surgery, The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Glutamate Oxaloacetate Transaminase (GOT) metabolizes glutamate by transferring the amino group from glutamate (Glu) to oxaloacetate, forming aspartate (Asp) and the 5-carbon TCA cycle intermediate alpha-ketoglutarate in the process. We previously reported that transamination of Glu by GOT protects against ischemic stroke-induced injury by (1) clearing neurotoxic extracellular Glu from the ischemic site and (2) generating TCA cycle intermediates that sustain cellular respiration for neural cells in the absence of glucose. Therapeutic strategies that up-regulate GOT expression for protection against ischemic stroke are therefore of interest. We recently identified that phytoestrogen isoflavone Biochanin A (BCA) induces expression of glutamate metabolizing GOT in stroke-affected brain. Against that background, here we sought to determine the effect of BCA treatment on brain metabolites during cerebral ischemia *in vivo* using ^{1}H magnetic resonance spectroscopy. C57BL/6 mice received daily intraperitoneal injections of placebo vehicle or BCA (5 and 10 mg/kg) for five weeks prior to experimental stroke. After 30 min stroke onset, and while ischemia persisted, brain metabolites in the stroke-affected hemisphere were measured *in vivo* by ^{1}H magnetic resonance spectroscopy using a high-field strength (9.4T) magnet. Compared to placebo controls, BCA treated mice had strikingly higher levels of aspartate, the product of GOT transamination in stroke-affected brain. The ratio of aspartate to glutamate was also significantly higher in BCA treated mice as compared to placebo controls. Furthermore, BCA treatment significantly improved post-stroke sensorimotor function and attenuated stroke-induced lesion volume as measured by MRI. Taken together, outcomes support evidence that BCA induces expression and activity of GOT in brain and protects against ischemic stroke-induced injury.

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Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.11/J15

Topic: C.08. Ischemia

Support: NIH grant R01HL139158

Title: Dendrimer-based n-acetyl-l-cysteine therapy improves long term outcome after cardiac arrest in rat model

Authors: *H. R. MODI[1], Q. WANG[1], E. S. SMITH[3], S. J. BERTRAND[4], S. KANNAN[2], R. KANNAN[1], N. V. THAKOR[5];
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Abstract: Cardiac arrest (CA) entails significant risks of coma resulting in poor neurological and behavioral outcomes after resuscitation. Significant subsequent morbidity and mortality in post-CA patients are due largely to the cerebral dysfunction that accompanies prolonged whole-body ischemia called as Post-CA syndrome (PCAS). PCAS involves strong inflammatory responses including neuroinflammation. Currently, there are no proven neuroprotective therapies to improve post-CA outcomes apart from therapeutic hypothermia. Furthermore, there are no acceptable approaches which can target neuroinflammation and lead to good neurological outcome post-CA. Moreover, delivering drugs across the blood-brain barrier to the target injured cells for treating diffuse brain injury is a major challenge. We hypothesize that dendrimer-based N-acetyl-L-cysteine (D-NAC) therapy ameliorates neuroinflammation and leads to a marked improvement in post-CA neurological outcome. We used an asphyxic CA rat model to determine the effects of D-NAC treatment. D-NAC was administer 30-minute post return of spontaneous circulation after cardiopulmonary resuscitation (ROSC). The behavioral neurologic deficit score (NDS score) was evaluated at 4, 24 and 48 hrs post-CA. Overall there was a dramatic decrease in NDS score following CA. D-NAC treatment increased NDS scores compared to the saline group. We also tested long term (7 days) behavior outcome (novel object and fear conditioning) post-CA. Seven days post-CA performance on novel object and fear conditioning tasks was evaluated. Administration of D-NAC decreases freezing behavior in a novel context suggesting a long-term cognitive effect of D-NAC.D-NAC also improve long term survival rate. In sum, acute administration of D-NAC following CA results in physiological and behavioral improvements.

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Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.12/J16

Topic: C.08. Ischemia

Support: Endowment from Drs. Chantal and Peritz Scheinberg (APR)
Florida Department of Heath#7JK01 funds (HMB & APR)

American Heart Association Grant-in-aid # 16GRNT31300011 (APR)

Title: Effect of endogenous estrogen fluctuations on the post-ischemic innate inflammation in the brain of female rats

Authors: *V. G. REDDY[1], C. FURONES[1], J. DE RIVERO VACCARI[2], A. P. RAVAL[1]; [1]Peritz Scheinberg Cerebral Vascular Dis. Res. Laboratory, Dept. of Neurol., Univ. of Miami, Miami, FL; [2]Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Miami, FL

Abstract: One out of five women suffer stroke after menopause in the United States. Menopause is characterized by decline in endogenous estradiol-17 beta (E2). Estrogen is neuroprotective against ischemia; however, concern regarding the safety of E2 therapy in menopausal women has prohibited translation of this phenomenon to the clinic. In spite of these findings, women appear to be naturally protected against ischemic neuronal damage during pre-menopausal life, suggesting some estrogen-influenced neuroprotective mechanism. Thus, a better understanding of the cellular and molecular mechanisms by which endogenous estrogen fluctuations govern the female brain is required. In a recent study, we presented data suggesting a key role of estrogen in regulation of inflammasome activation in the female rat brain. Inflammasome is a multiple protein complex and is the main component of innate immune response. The aim of current study is to test effects of higher and lower levels of endogenous E2 on post-stroke inflammasome proteins. We hypothesized that prevailing higher levels of endogenous E2 during estrus would protect the brain from ischemic injury by reducing inflammasome activation. The proposed hypothesis was tested using young female (4-6 months), retired breeder (9-13 months), and age-matched male Sprague-Dawley rats. Rats were randomly exposed to transient middle cerebral artery occlusion (tMCAO; 90 min) or sham surgery. Twenty-four hours after tMCAO, brains were removed rapidly and sectioned into 1 mm slices beginning from the rostral end, and the area of infarction was visualized by incubating the sections in 1.5% TTC (2,3,5-triphenyltetrazolium chloride; Sigma Aldrich) in PBS for 15mins at 37°C. The infarct volume was measured using ImageJ software. In a separated cohort of rats, brain tissue was collected for western blot analysis 24h after tMCAO/sham surgeries. We observed significantly ($p<0.05$) reduced infarct volume in young female rats, which underwent tMCAO during estrus stage (higher levels endogenous E2) as compared to diestrus (lower levels of circulating E2). Results also showed that the brain of young rats had significantly lower infarct volume and reduced inflammasome activation in the brain as compared to reproductively senescent female rats. Our study demonstrated that endogenous E2 regulates innate immune response in the brain of female rats.

Disclosures: V.G. Reddy: None. **C. Furones:** None. **J. de Rivero Vaccari:** None. **A.P. Raval:** None.

Poster

390. Ischemic Stroke III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 390.13/J17

Topic: C.08. Ischemia

Support: H2020-MSCA-IF-2016 (Marie Skłodowska-Curie Individual Fellowships)

Title: Microbiota immune cell interaction: The critical role of gut metabolites in stroke

Authors: *C. BENAKIS[1], R. SADLER[2], D. FINK[3], A. LIESZ[4];
[1]Inst. For Stroke and Dementia Res., Munich, Germany; [2]Inst. for Stroke and Dementia Res., Ludwig-maximilians-Universität München, Muenchen, Germany; [3]Inst. for Stroke and Dementia Res., Munich, Germany; [4]Inst. for Stroke and Dementia Res., Univ. Med. Ctr. Munich, Munich, Germany

Abstract: Stroke is characterized by the recruitment of circulating immune cells to the injured brain. We have shown that immune cell function is modulated by the gut microbiota and migrate from the gut to the brain where they contribute to neuroinflammation and secondary brain injury [Benakis et al. *Nat. Med.* 2016, Singh et al. *J. Neurosci.* 2016]. There is accumulating evidence showing that metabolites derived from the gut microbiota influence brain diseases via the regulation of intestinal immune cell function. In particular, indole metabolites derived from the amino acid tryptophan play a crucial role in immune tolerance. Importantly, indoles are ligands of the transcription factor aryl hydrocarbon receptor (AhR), which is highly expressed in immune cells. Upon binding, AhR ligands induces T cell polarization directly or via dendritic cells (DCs).
Here, we seek to determine how the AhR pathway regulates peripheral immune homeostasis after stroke. Understanding how immune cells are modulated by gut metabolites may have strong therapeutic implications in stroke.
Cerebral ischemia was induced by the transient occlusion of the middle cerebral artery in wild-type C57Bl6 male mice leading to intestinal dysbiosis (Singh et al. *J. Neurosci.* 2016). Using 16S sequencing of the feces, we found that bacteria from the genus *Lactobacillus*—known to produce indoles—were significantly reduced in mice subjected to stroke in comparison to sham surgery. Immune cells were isolated from the intestine and analyzed by flow cytometry and by qPCR for AhR-related gene expression. We demonstrated that the AhR pathway is down-regulated after stroke in comparison to sham surgery, shown by a decreased expression of AhR-related genes (*Cyp1a1, Cyp1b1*) in DCs and T cells isolated from the gut. We tested the tolerogenic function of DCs in mice with AhR-deficient DCs ($CD11c^{cre}xAhr^{f/f}$). We identified that the intestinal frequency of the DC1 subpopulation of DCs was AhR-dependently upregulated after stroke, and correlated with an increased expression of regulatory (Foxp3+) T cells and a

concomitant decrease of Th17 cells in the mesenteric lymph nodes.
These results are the first to link stroke-induced dysbiosis and the AhR pathway as immunomodulators of DCs and T cells. Findings derived from this study may allow for the identification of microbial metabolites-based therapies to alleviate the neuroinflammatory response to stroke.

Disclosures: **C. Benakis:** None. **R. Sadler:** None. **D. Fink:** None. **A. Liesz:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.01/J18

Topic: C.09.Stroke

Support: NIH F31NS105486
NIH R01HD039343
NIH R01NS105759

Title: Altered sensorimotor pathways post unilateral subcortical stroke

Authors: *H. KARBASFOROUSHAN[1], J. P. DEWALD[2];
[2]Physical Therapy and Human Movement Sci., [1]Northwestern Univ., Chicago, IL

Abstract: Previous stroke brain imaging studies have frequently reported the damage to the corticospinal tract in individuals with subcortical stroke. However, less is known about other altered sensorimotor pathways. One reason for this lack of knowledge is that the previous studies have only investigated the morphological changes in the brain, where majority of descending and ascending brain pathways mostly overlap, while these pathways only delineate from each other in the brainstem. The goal of this study was therefore to use high resolution anatomical MRI and DTI of brainstem to identify the altered sensorimotor pathways post chronic unilateral stroke. Twenty individuals with chronic (>1yr) unilateral subcortical stroke and 20 age-matched controls were recruited to this study. The MRI scans were collected on a 3T Siemens Prisma magnetic resonance scanner. High resolution T1-weighted anatomical and diffusion weighed images were obtained from whole brain and brainstem. T1 weighted anatomical images were acquired with 0.8 x 0.8 x 0.8 isotropic resolution, TR = 2.3s, TE 1 = 1.86 ms, TE 2 = 3.78 ms, field of view (FOV) = 256 x 256 mm. Diffusion weighted images were acquired using spin-echo echo-planer imaging with the following parameters: TR = 3620 ms; TE = 68.40 ms; FOV = 222 x 222 mm^2; 8 B0 images and 68 diffusion directions at B = 1000 s/mm; flip angle = 90°; matrix size = 150 x 150; voxel size = 1.5 mm isotropic; number of slices = 108. Voxel-wise statistical analysis of the DTI data was carried out using Tract-Based Spatial Statistics (TBSS) in FSL. All subjects' FA images were aligned to the standard MNI template and the mean FA image was created and

thinned to create a mean FA skeleton representing the centers of all tracts. Each subject's aligned FA map was then projected onto this skeleton, resulting in each subject's brainstem skeletonised FA image. Randomise analysis with 50,000 permutations was then run on brainstem skeletonized FA between individuals with stroke compared to controls to identify the tracts with significant change in white matter integrity. The stroke patients compared to controls showed significant decrease in white matter integrity (FA) of lateral corticospinal tract, lateral reticulospinal tract and medial longitudinal fasciculus of lesioned hemisphere. Furthermore, our results indicated a significant increase in the white matter integrity of medial reticulospinal tract of non-lesioned hemisphere which projects ipsilaterally to the paretic side of body.

Disclosures: H. Karbasforoushan: None. **J.P. Dewald:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.02/J19

Topic: E.07. Rhythmic Motor Pattern Generation

Support: New Jersey Health Foundation Grant PC 5-18

Title: A pilot study to understand the differences in the cortical activation between healthy controls and individuals with brain injury

Authors: *K. K. KARUNAKARAN[1,2,3], N. EHRENBERG[1,3], K. J. NOLAN[1,3];
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Abstract: Brain injury is one of the leading causes of motor deficits in children and adults. It often results in lower extremity motor control and balance impairments due to cortical changes induced by injury. The objective of this study is to understand the differences in cortical activation in the cortical motor control network (bilateral supplementary motor area, secondary somatosensory cortex, premotor areas, and primary motor cortex) using Functional Near Infrared Spectroscopy (fNIRS) while performing lower extremity dynamic tasks (ex: ankle dorsiflexion and plantarflexion) in healthy controls (HC) and individuals with Brain Injury (BI). The outcome measures will include block averaged hemodynamic response (HDR) due to changes in oxygenated hemoglobin (O-Hb) and deoxygenated hemoglobin (DO-Hb) levels, and laterality index differences between the hemispheres' activation during the task in HC and BI. The results showed asymmetric activation of the cortical motor control network between the lesioned and non-lesioned hemispheres in BI compared to HC. Furthermore, increased neural activity was observed in the contralesional hemisphere in BI during the task compared to HC.

Disclosures: **K.K. Karunakaran:** None. **N. Ehrenberg:** None. **K.J. Nolan:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.03/J20

Topic: C.09.Stroke

Support: 1R21HD090453-01A1

Title: Biomechanical and electrophysiological assessment of the effect of botulinum neurotoxin injection in stroke survivors with spastic hypertonia

Authors: Y.-T. CHEN[1], C. ZHANG[2], E. MAGAT[3], M. VERDUZCO-GUTIERREZ[3], G. E. FRANCISCO[3], P. ZHOU[3], Y. ZHANG[2], ***S. LI**[3];
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Abstract: Spasticity is a common and disabling motor consequence after stroke. Botulinum toxin (BoNT), which acts on the neuromuscular junction, is considered as the first-line treatment for focal spasticity management. The study aimed to quantitatively assess biomechanical and electrophysiological effects of BoNT injection. 9 stroke subjects (Age: 52.4 ± 10.3 yrs; 3 women) received 100 units of ona- or inco-BoNTA to the biceps muscle. Elbow flexor spasticity was 1^+ ~ 2 on the Modified Ashworth Scale (MAS). Clinical and biomechanical assessment and electrophysiological assessment with maximum evoked action potential (M-wave) were measured before (Pre-injection), three weeks (3-wk) and three months (3-mon) after the injection. A computerized passive stretching was applied to the elbow flexors. Reflex torque (neural component of spasticity) of the elbow flexors was quantified as the difference between the baseline torque at 5°/second of stretching (indicative of passive muscle stiffness) and the peak torque at 100°/second of stretching. MAS decreased to 1~1^+ at the 3-wk visit and then returned to the pre-injection level at the 3-mon visit. The peak torque reduced at 3-wk (6.5 ± 1.0 Nm) from the pre-injection value (8.1 ± 1.1 Nm) and then returned at 3-mon (8.0 ± 1.3 N-m) after injection, ($p<0.05$). A similar pattern of reduction in the reflex torque was observed (pre-injection: 3.8 ± 0.7 Nm; 3-wk: 2.6 ± 0.5 Nm; 3-mon: 3.5 ± 0.7 Nm; $p = 0.052$). Similarly, the M-wave value reduced at 3-wk (6.3 ± 0.5 mV) and returned at 3-mon after injection (9.1 ± 1.1 mV) as compared to the pre-injection value (9.1 ± 0.8 mV) ($p<0.05$). Notably, both the M-wave and reflex torque reduced about 30% at the 3-wk visit. Nonetheless, the baseline torque (Pre-injection: 4.3 ± 0.6 Nm; 3-wk: 4.0 ± 0.5 Nm; 3-mon: 4.5 ± 0.9 Nm; $p = 0.4$) was not affect by BoNT injection. Interestingly, the baseline torque, as percent of total peak torque, inversely correlated with the reflex torque reduction at the 3-wk visit ($r = - 0.78$, $p = 0.01$), i.e., the higher the percent of passive muscle stiffness, the less the effect of BoNT. Our results provide

electrophysiological evidence of BoNT effects and its relation to the baseline passive muscle stiffness.

Disclosures: Y. Chen: None. **C. Zhang:** None. **E. Magat:** None. **M. Verduzco-Gutierrez:** None. **G.E. Francisco:** None. **P. zhou:** None. **Y. Zhang:** None. **S. Li:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.04/J21

Topic: C.09.Stroke

Support: Dixon Translation Research Grant, Northwestern Memorial Foundation
NIH R01HD039343
NIH R01NS105759

Title: Determine the usage of indirect motor pathways following a hemiparetic stroke: A pilot study

Authors: *Y. YANG[1], R. TIAN[1], M. CUMMINGS[1], C. H. E. Y. HOGENHUIS[1,2], J. DEOL[1], J. DROGOS[1], J. P. A. DEWALD[1];
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Abstract: A hallmark impairment after hemiparetic stroke is a loss of independent joint control resulting in abnormal co-activation of shoulder abductor and elbow/wrist/finger flexor muscles in the paretic arm, known as the flexion synergy. The flexion synergy appears while generating shoulder abduction (SABD) torques such as when lifting the paretic arm. This is thought to be caused by an increased reliance on contralesional indirect motor pathways following damage to direct corticospinal projections. The assessment of functional connectivity between brain and muscle signals, i.e., cortico-muscular connectivity (CMC), may provide insight into such changes regarding the usage of motor pathways.
However, previous studies of CMC focused on its linear portion, i.e., EEG-EMG coherence, which mainly reflects motor command transmission via the direct corticospinal tract. In contrast to that direct pathway, indirect motor pathways involve multiple intermediate synaptic connections. Our recent neural model simulation suggested that multi-synaptic interactions of indirect motor pathways can lead to the nonlinear distortion of motor command transmission, showing nonlinear CMC.
We hypothesize that increased usage of contralesional indirect motor pathways (as when increasing shoulder abduction load) will lead to an increase of nonlinear CMC from the contralesional hemisphere. To test this hypothesis, we measured scalp EEG, EMG from the

Intermediate Deltoid (IDL) and Bicep Branchii (BIC) muscles as well as flexion synergy-induced elbow torques when eight stroke participants generated 20% and 40% of the maximum SABD torque with their paretic arm.
We computed both linear and nonlinear CMC between EEG and IDL EMG. Additionally, EMG-EMG coherence was also computed between IDL and BIC muscles. We found the greatest nonlinear CMC over the contralesional/ipsilateral hemisphere for stroke, its magnitude at the peak channel increased with SABD load. The increased IDL-BIC coherence was also shown with the higher (40%) SABD load and is associated with flexion synergy elbow torques.
These results support our hypothesis, and indicate that nonlinear CMC does provide a novel quantitative indicator for determining the usage of indirect motor pathways following a hemiparetic stroke. As such, this study may permit a precision diagnostic tool for evaluation of motor recovery after a hemiparetic stroke.

Disclosures: Y. Yang: None. **R. Tian:** None. **M. Cummings:** None. **C.H.E.Y. Hogenhuis:** None. **J. Deol:** None. **J. Drogos:** None. **J.P.A. Dewald:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.05/J22

Topic: C.09.Stroke

Support: European Research Council Grant N. 291339

Title: Are resting state EEG measures longitudinally associated with spontaneous neurological recovery early post-stroke? A prospective cohort study

Authors: M. SAES[1], S. B. ZANDVLIET[1], A. S. ANDRINGA[1], *C. G. M. MESKERS[1,2], A. DAFFERTSHOFER[3], F. C. T. VAN DER HELM[4,2], E. E. H. VAN WEGEN[1], G. KWAKKEL[1,2]; [1]Dept. of Rehabil. Med., Amsterdam Univ. Med. Ctr., Amsterdam, Netherlands; [2]Dept. of Physical Therapy and Human Movement Sci., Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL; [3]Dept. of Human Movement Sciences, Fac. of Behavioural and Movement Sci., Vrije Univ. Amsterdam, Amsterdam, Netherlands; [4]BioMechanical Engin. Department, Fac. of Mechanical, Maritime and Materials Engin., Delft Univ. of Technol., Delft, Netherlands

Abstract: Stroke has been shown to result in increased low frequency oscillations, depending on stroke severity. The time course of cortical activation expressed by EEG-based power spectral density (PSD) measures and its relation with clinical measures during recovery after a first ever ischemic stroke is unclear and may reveal underlying mechanisms of spontaneous neurological recovery (SNR). First, we aimed to investigate the time course of cortical reorganization as revealed with PSD measures during awake rest in the first six months post stroke. Second, we

aimed to investigate development of PSD in relation to global neurological and motor recovery. Resting state 62-channel EEG was measured serially in 41 patients after a first-ever ischemic stroke within 3 and at 5, 12 and 26 weeks post-stroke. PSD measures Delta-Alpha Ratio (DAR), Brain Symmetry Index (BSI) and directional BSI (BSIdir) were derived. Additionally, DAR was calculated per hemisphere, and BSI was calculated per frequency band. At the same measurement moments the National Institutes of Health Stroke Scale (NIHSS) and Fugl-Meyer motor assessment of the upper extremity (FM-UE) were measured as reflection of SNR. The time course of PSD measures, as well as their longitudinal association with NIHSS and FM-UE within- and between-subjects were analysed using linear mixed models. All investigated PSD measures showed a gradual decrease over time post-stroke. They changed within and beyond the time window of SNR. A within- and between-subject association with NIHSS was found for DAR_{AH} (ß:0.16, p<0.01; ß:0.36, p<0.01) and $BSIdir_{Delta}$ (ß:0.67E-2, p<0.01; ß:0.02, p<0.01). This means that a decreasing DAR_{AH} or $BSIdir_{Delta}$ over time within a patient or a lower value compared to other patients is congruent with reduced neurological deficits. $BSIdir_{Delta}$ also demonstrated within- and between-subject associations with FM-UE (ß:-0.12E-2, p=0.01; ß:-0.18E-2, p=0.01). DAR_{AH} showed to be increased in patients who did not show recovery, compared to patients who did show recovery. DAR_{AH} and $BSIdir_{Delta}$ are suggested to reflect global severity of neurological deficits in the brain. Only $BSIdir_{Delta}$ probably reflects motor impairments. It was concluded that DAR_{AH} and $BSIdir_{Delta}$ potentially have added value in individual prediction of recovery, reflecting SNR over time within a subject and might have added value in distinguishing between patients with different recovery patterns.

Disclosures: M. Saes: None. **S.B. Zandvliet:** None. **A.S. Andringa:** None. **C.G.M. Meskers:** None. **A. Daffertshofer:** None. **F.C.T. van der Helm:** None. **E.E.H. van Wegen:** None. **G. Kwakkel:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.06/J23

Topic: C.09.Stroke

Support: NIH Grant R01NS093057

Title: Brain wide circuit dynamics of post-stroke recovery induced by optogenetic stimulation

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Abstract: Background: Post-stroke optogenetic pan-neural stimulation has been shown to promote functional recovery. However, the cellular and circuit mechanisms underlying such recovery remain unclear. Elucidating critical neural circuits involved in post-stroke recovery will be important for optimal translation of neuromodulation for stroke therapy. Here we used a technique called optogenetic functional magnetic response imaging (ofMRI) to examine brain-wide circuit reorganization induced by optogenetic stimulation treatment (OST) following ischemic stroke in mice.
Method: We performed ofMRI experiments targeting ipsilesional M1 (iM1) layer V excitatory neurons in mice by injecting AAV1-CamKIIa-ChR2-eYFP viral vector. Global brain circuit activation using ofMRI were performed on pre-stroke and post-stroke days (PD) 3 (prior to OST), 15 and 29. Sensorimotor rotating beam tests were conducted one day prior to ofMRI. Mice underwent transient middle cerebral artery occlusion (intraluminal suture model, 30 minutes). Mice were balanced into two groups based on their infarct: stim group (stroke mice received 10 consecutive days of 10Hz iM1 OST), and no stim group (stroke mice without OST). By comparing the areas of brain activation between the stim and no stim groups at different time points, we identified key brain circuits that recovered by the effect of OST. We also performed western blot to examine the expression of plasticity marker GAP43.
Result: Our results show that 1) Optogenetic excitatory neuronal stimulation in iM1 promotes motor performance after stroke (n=9 per group). 2) At pre-stroke, iM1 stimulation activates expected network including the ipsilateral M1, M2, S1, striatum, and thalamus, and the contralateral M1 and cerebellum. 3) On PD3, all mice exhibit a depressed response throughout the brain after iM1 stimulation. 4) On PD15, ipsilesional thalamus and S1 circuits are significantly recovered and strengthened by OST. Moreover, restoration of thalamic activation is correlated with behavioral recovery in the stim group. 5) On PD15, iM1 stimulated mice exhibited higher level of plasticity marker (GAP43) in the ipsilesional thalamus (n=5 per group). 6) On PD29, iS1 activation remains significantly higher in the stim compared to the no stim group.
Conclusion: Overall, our findings indicate that restoration of cortico-thalamic projections play an important role in stimulation-induced recovery at early phase post-stroke, while sustained strengthening of ipsilesional cortico-cortical connections may be critical in the later phase of recovery. Thus our findings revealed key circuits underlying stimulation-induced post-stroke recovery.

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Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.07/J24

Topic: F.03. Neuroendocrine Processes

Support: VIEP (QULU-NAT17-I).

Title: Effects of acetyl l-carnitine on the dendritic morphology of hippocampal pyramidal neurons of the CA1 and CA3 region of female pubic rats denervated sensorially at birth

Authors: *E. D. CASTILLO LÓPEZ[1,2], A. RODRÍGUEZ-RUBÍ[3], D. LÓPEZ-GUADALUPE[3], D. LÓPEZ-MORALES[3], R. A. VAZQUEZ-ROQUE, SR[4], G. FLORES[5], U. QUIRÓZ-LÓPEZ[2];
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Abstract: The onset of puberty and reproduction are processes that are regulated by the neuroendocrine axis hypothalamus-pituitary-gonads. In addition to this axis, there is a neural pathway between various structures of the CNS and the gonads, which contributes to fine regulation to these processes. Sensory innervation is one of the components of the neural pathway. We previously reported that in the newly born female rat sensory denervation induced by the administration of capsaicin (CAPS) causes a delay in the onset of puberty and decreased fertility. We have also shown that in the adult female rat in the estrus phase an increase of dendritic spines in pyramidal CA3 neurons of the left side of the hippocampus is observed. On the other hand, there are substances that can stimulate mechanisms that lead to neuroprotection, reinnervation and neuronal plasticity, such as acetyl l-carnitine (ALCAR) which has aroused great interest as an alternative in neurodegenerative diseases. The objective of this work is to analyze the effects of the administration of ALCAR on the dendritic morphology of pyramidal neurons of the CA1 and CA3 regions of the hippocampus of rats denervated at birth with capsaicin. Eighteen female rats newly born from strain CII-ZV were used, which were equally divided into three groups: one group received a dose of 50 mg / kg of capsaicin and from day 21 was administered saline solution i.p. (Group: CAPS + VH), another group was also administered the same dose of capsaicin and from day 21 of age and until their sacrifice they received a daily dose of 50 mg / kg of ALCAR i.p. (Group: CAPS + ALCAR). A third group received only ALCAR (Group: ALCAR). When the animals presented the first vaginal estrus, perfusion was performed and the brains were dissected for the Golgi Cox staining technique and the morphological analysis of CA1 and CA3 neurons of the hippocampus under the Sholl technique. The analysis of the dendritic morphology of the CA1 region of the hippocampus of the CAPS + ALCAR rats showed a significant increase in dendritic length and arborization and the number of order in comparison to the VH + ALCAR and CAPS + VH groups. On the other hand, in the CA3 region, there was only a significant increase in dendritic arborization in the CAPS + ALCAR group in contrast to the other two groups. Our results allow us to suggest that in the sensed denervated rat at birth, the systemic administration of acetyl L-carnitine induces changes in the dendritic morphology in pyramidal neurons of CA1 and CA3 of the hippocampus that probably regulates neuroendocrine and / or neural mechanisms that participate in the regulation of reproduction.

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Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.08/J25

Topic: C.09.Stroke

Support: K08 NS105914

Title: Persistent hydrocephalus and activated microglia drive the spatial memory decline after intraventricular hemorrhage in rodents

Authors: *K. KAMAL[1], T. BINYAMIN[1], J. '. KEITER[1], A. R. VERGARA[1], A. IZADI[1], K. ONDEK[1], R. F. BERMAN[1], F. R. SHARP[2], G. G. GURKOFF[1], B. WALDAU[1];
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Abstract: Intraventricular hemorrhage (IVH) can result in significant cognitive deficits and spatial memory impairments that can prevent over half of IVH patients from returning to work after recovery. The mechanism of this memory decline, however, is incompletely understood. To understand the role of mechanical trauma caused by rapid ventricular expansion versus the presence of blood components, such as thrombin, in IVH deficits, we developed a rodent model of IVH along with controls using 40 male Sprague-Dawley rats. IVH was induced by injecting 200 μL of autologous arterial blood into the animals' ventricles. The IVH animals' spatial memories were then assessed along with sham (no injection), vehicle control (200 μL aCSF), and intraventricular thrombin (IVT) (20 U thrombin in 5 μL aCSF) animals using Morris water maze. The IVH group performed worse on this spatial memory task compared to all other groups, and significantly worse than the sham group ($p=0.04$), indicating that ventricular expansion and presence of blood components both contribute to memory deficits. Based on MRI results, the IVH group was also the only group that developed persistent hydrocephalus. Contrary to our hypothesis that the cognitive deficits of IVH are due to decreased hippocampal neurogenesis, we did not find any statistical difference in the numbers of dentate gyrus progenitor cells (labeled with BrdU and DCX) nor neurons (labeled with NeuN) between the different groups. The number of microglia (labeled with Iba1) was also compared among the groups using stereology and found to be not statistically different. The microglia in IVH and vehicle control animals, however, were found to have significantly lower fractal dimension ($p<0.01$) and higher lacunarity ($p<0.01$) numbers compared to those of sham animals, indicating a more activated

state, likely caused by the mechanical trauma. Based on our results, microglial activation and hydrocephalus seem to be the drivers of spatial memory deficit after intraventricular hemorrhage.

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Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.09/J26

Topic: C.09.Stroke

Title: Stepwise regression modeling of impairments contributing to reaching function in chronic stroke

Authors: *G. C. BELLINGER[1], M. D. ELLIS[2];
[1]Interdepartmental Neurosci. (NUIN), [2]Dept. of Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL

Abstract: Chronic stroke survivors demonstrate a myriad of impairments impacting reaching function including flexion synergy, passive range of motion limitations, spasticity (i.e., hyperactive stretch reflexes), and weakness. Flexion synergy results in loss of independent joint control due to the abnormal coupling of shoulder abductors with elbow, wrist, and finger flexors. The relative and concurrent contributions of each of these underlying factors has not yet been quantitatively explored in depth. 34 individuals (23 males, 58.3 ± 10.8 years old) with chronic stroke (11.8 ± 8.3 years post-stroke) participated in the study. The upper extremity Fugl-Meyer Motor Assessment scores (26 ± 7) ranged from 15 to 49 indicating moderate to severe generalized levels of impairment. Reaching function was measured with a robotic device and defined as maximum reaching distance against gravity. The regressors were quantified using kinematics and electromyography (EMG). A stepwise regression analysis was implemented to investigate the constitutive elements of reaching function. The regressors included: 1) maximal shoulder abduction and elbow extension strength normalized to the unaffected side, 2) spasticity-related biceps activation measured as the increase in EMG occurring after elbow extension onset during reaching at a standardized load, 3) flexion synergy that was measured as the highest abduction load at which the participant could successfully lift the arm and reach two standardized targets, and 4) passive range of motion at the elbow. All regressors were z-scored and MATLAB was used to find an optimized regression model with an R-squared of 0.390 ($p < 0.001$). Interestingly, the model only included the flexion synergy emergence threshold with a regression coefficient of 0.217 and standard error of 0.057 ($p < 0.001$). Reaching function (mean ± SD; 0.608 ± 0.358) was significantly correlated with flexion synergy emergence threshold

(0.310 ± 0.244, r = 0.634, p = 0.001), shoulder abduction strength (0.557 ± 0.193, r = 0.390, p = 0.023), and elbow extension strength (0.437 ± 0.186, r = 0.407, p = 0.017). Passive range of motion, active flexor spasticity, and flexion synergy takeover threshold did not significantly correlate with reaching function. The results of the stepwise regression analysis indicate that impairments such as weakness, flexor spasticity, and passive range of motion limitations may not contribute to reaching dysfunction to the same extent as flexion synergy. The findings of this study suggest flexion synergy impairment may be the primary therapeutic target when attempting to restore reaching function in chronic moderate to severe stroke.

Disclosures: G.C. Bellinger: None. **M.D. Ellis:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.10/J27

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Support: NIH Grant R01NS094402
Shriners Hospitals for Children Grants 85600 PHI

Title: Coordinated control of distal axon integrity and responses to axonal damage by a specific palmitoyl acyltransferase

Authors: *J. NIU[1], S. S. SANDERS[3], H. JEONG[5], Y. SUN[6], S. HOLLAND[1], A. KETSCHEK[1], H. HUANG[8], M. R. HAYDEN[9], Y. HU[8], G. M. SMITH[4], G. GALLO[10], Y. JIN[7], G. THOMAS[2]; [2]Shriners Pediatric Res. Ctr., [1]Temple Univ. Sch. of Med., Philadelphia, PA; [3]Shriners Hosp. Pediatric Res. Ctr., [4]Dept of Neurosci., Temple Univ., Philadelphia, PA; [5]Shriners Hosp. Pediatric Res. Ctr., Philadelphia, PA; [6]UCSD, San Diego, CA; [7]Div. Biolog Sci, Neurobio. Section, UCSD, La Jolla, CA; [8]Dept. of Ophthalmology, Stanford Univ. Sch. of Med., Palo Alto, CA; [9]Univ. of British Columbia, Vancouver, BC, Canada; [10]Shriners Hosp. Pediatric Res. Ctr., Temple Sch. of Med., Philadelphia, PA

Abstract: Bidirectional protein trafficking is essential for neurons to exchange information regarding the health of their cell soma and distal axons and thus coordinate survival versus degeneration decisions for these two subcellular locations. Healthy neurons transport the survival factor NMNAT2 anterogradely to maintain distal axon integrity, but simultaneously supply DLK, a kinase that is critical for sensing distal axonal damage and subsequent retrograde axon-to-soma signaling. Here, we report that this ‘trust but verify’ system is controlled by a single palmitoyl acyltransferase, ZDHHC17, which directly binds and palmitoylates both DLK and NMNAT2. In the healthy optic nerve *in vivo*, ZDHHC17-dependent palmitoylation is essential for NMNAT-dependent distal axon integrity, while following optic nerve injury, ZDHHC17-

dependent palmitoylation of DLK is critical for retrograde injury signaling and subsequent somal degeneration. Similar ZDHHC17-dependent palmitoylation controls somal and axon integrity in cultured sensory neurons, and at the molecular level we identify specific motifs in DLK, NMNAT2 and ZDHHC17 that are essential for this mutual, coordinated regulation. This previously unappreciated mechanism enables neurons to monitor and respond to changes in axonal health and coordinate responses in both distal axons and neuronal cell bodies.

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Poster

391. Stroke II

Location: Hall A

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Program #/Poster #: 391.11/J28

Topic: C.09.Stroke

Support: AHA Grant 17POST3366019
AHA Grant 16SDG30980031

Title: Genetic deletion of fractalkine receptor promotes hematoma resolution in the acute-phase of intracerebral hemorrhage via Nrf2/HO1 signaling pathway

Authors: *X. LAN[1], X. HAN[2], A. QIAN[2], J. WANG[3];
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[3]Anesthesiology/Critical Care Med., Johns Hopkins Univ., Baltimore, MD

Abstract: Intracerebral hemorrhage (ICH) remains the most lethal type of all strokes. Exploring the endogenous mechanism of hematoma removal is critical in ICH study. Fractalkine receptor (Cx3cr1), expressed in microglia, participates in microglia migration and recruiting. Nuclear factor erythroid 2-related factor 2 (Nrf2)/ heme oxygenase-1 (HO-1) is an important pathway to protect against acute brain injuries by exerting antioxidant and regulating microglia phagocytosis. Our study is to investigate the role of Cx3cr1 after ICH and explore the mechanisms of Cx3cr1 in hematoma absorption. We subjected wild-type (WT), $Cx3cr1^{gfp/+}$ and $Cx3cr1^{gfp/gfp}$ [knockout (KO)] mice to ICH and assessed the short-term outcomes. To study the role of Cx3cr1 in microglia function, we labeled PKH26 dye to red blood cells (RBCs) from WT mice and then injected them into striatum. Microglia engulfing RBCs was observed under fluorescence microscope. We then studied the microglia activation and neuroinflammation after ICH. To further measure the relationship between Cx3cr1 and Nrf2/HO-1 signaling pathway, we examined the HO-1 level by immunostaining and western blotting. We then evaluated Nrf2 activation and nuclear translocation *in vivo* and in primary microglia. Lastly, we used a

phagocytosis PCR array to determine the key factor that regulates microglia phagocytosis after ICH. Our results showed that: **1)** Cx3cr1 deletion reduced injury volume and brain edema on day 3 post-ICH. It also decreased neurologic deficit score in 24-point evaluation test and increased the time mice were able to remain on a suspended wire. **2)** Cx3cr1 deletion suppressed the hemoglobin content after ICH and Cx3cr1-depleted microglia exhibited stronger phagocytosis ability in RBC-injection mouse model. **3)** Microglia were more activated in KO mice on day 3 post-ICH with a reduction of proinflammatory cytokines and upregulation of antiinflammatory markers. **4)** Cx3cr1 deletion increased nuclear Nrf2 expression both *in vivo* and *in vitro*. Moreover, Cx3cr1 deletion promoted more HO-1 to express in microglia than in astrocytes, indicating that Cx3cr1-mediated inhibition of the microglial HO-1 might be important in hematoma resolution. **5)** PCR array showed that the gene for the receptor for advanced glycation end products (RAGE) was suppressed in KO mice after ICH. In conclusion, we found that Cx3cr1 deletion contributes to accelerating hematoma clearance by enhancing microglial phagocytosis ability and promoting polarization of microglia antiinflammatory phenotype via Nrf2/HO-1 signaling pathway. The results suggest that targeting on Cx3cr1 might represent a new direction for developing methods of ICH treatment.

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Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.12/J29

Topic: C.07. Neurotoxicity/ Inflammation/ and Neuroprotection

Title: Characterizing smoldering inflammation in progressive MS by a multimodal approach combining CSF proteomics and quantification of slowly expanding/ evolving lesions

Authors: *I. LEAF, K. FERBER, D. P. BRADLEY, S. BELACHEW, E. CAHIR-MCFARLAND;
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Abstract: Development of successful treatments for progressive multiple sclerosis (MS) is extremely challenging, as molecular mechanisms driving pathophysiology at this stage are poorly understood partially due to the failure of current animal models to recapitulate relevant biology. Therefore, it is important to focus on **human biology** in understanding disease-associated mechanisms in progressive MS.
Here we apply a multimodal approach to investigate molecular mechanisms contributing to disease progression in MS by integrating CSF proteomics with radiological measurements of chronic active white matter lesions.
In MS patients, smoldering inflammation driving chronic white matter lesion activity in the

absence of ongoing active inflammation has been demonstrated to represent a pathological hallmark of progressive forms of MS and can be measured by conventional Magnetic Resonance Imaging (MRI) as slowly expanding/evolving lesions (SELs). The number and volume of SELs in progressive MS patients were shown to be significantly higher than in relapsing form of MS but biological mechanisms driving these processes are unclear. To elucidate the underlying biology, we applied an unbiased mass-spectrometry profiling approach and quantified 1,500 proteins in the Cerebrospinal Fluid (CSF) in SPMS patients (n = 34) with MRI measurements and SEL detection. To identify protein signatures and pathways associated with increased SEL burden and associated tissue loss, we performed a multi-variate analysis with disease progression and disability measurements (clinical: EDSS, 9PHT, T25FW) and SEL measurements (SEL number/volume and change in T1 signal intensity in SELs).
This work will help define protein signatures and pathways contributing to disease progression and allow selection of relevant *in vitro* and *in vivo* translational models that in turn will provide support for drug and biomarker discovery and development in progressive MS.

Disclosures: I. Leaf: None. **K. Ferber:** None. **D.P. Bradley:** None. **S. Belachew:** None. **E. Cahir-McFarland:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.13/J30

Topic: C.08. Ischemia

Support: NINDS R01NS104436

Title: Increased motoneuron excitability in a rabbit hypoxia ischemia model of cerebral palsy

Authors: P. STEELE[1], C. F. CAVARSAN[2], *K. A. Q. QUINLAN[2];
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Abstract: Cerebral palsy (CP) is caused by a variety of factors related to early brain damage, resulting in permanently impaired motor control, marked by muscle stiffness and spasticity. To study mechanisms of motor dysfunction, we use the rabbit hypoxia-ischemia (HI) model of developmental injury at 79% pregnancy (E25). After HI, kits are born with deficits including muscle stiffness and motor deficits, though there is a range in severity. At birth kits were classified as severely affected, mildly affected, or unaffected based on modified Ashworth scores. Whole cell patch clamp of motoneurons was performed on lumbar and cervical spinal cord slices. Properties of the motoneurons from kits severely affected by HI were significantly different from control and mildly affected kits. Specifically, using a multivariate analysis we

found severely affected motoneurons had depolarized resting membrane potential (-52.8 +/- 5.6mV vs -60.3 +/-6.6mV in control), higher frequencies of firing (129Hz +/- 23Hz instantaneous maximum firing rate in severe vs 98Hz +/-29Hz in control), more sustained firing on the downslope of current ramps (control motoneurons fire less on downslope: the difference in current between onset and offset of firing is 191pA +/- 160pA vs 84pA +/- 63pA in severe). While the most dramatic differences were observed in motoneurons from severely affected kits, mildly affected and unaffected motoneurons also showed some variation from the motoneurons from sham control kits (n=8 severe, n= 10 mild, n= 11 unaffected, n = 8 sham).

Disclosures: P. Steele: None. **C.F. Cavarsan:** None. **K.A.Q. Quinlan:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.14/J31

Topic: C.09.Stroke

Support: R01NS109361
R21NS113056
K25HL140153

Title: Longitudinal multimodal mapping of neural activity and blood flow reveals neurovascular dissociations in an awake mouse model of microinfarcts

Authors: *F. HE[1], C. SULLENDER[1], H. ZHU[1], T. A. JONES[2], C. XIE[1], A. DUNN[1], L. LUAN[1];
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Abstract: Brain functions and dysfunctions involve complex interactions between neuronal and other cellular and vascular activities at diverse spatiotemporal scales. This vast complexity demands novel technologies that are able to simultaneously resolve multifaceted brain activities at sufficient spatiotemporal resolutions and to longitudinally track their evolution over a long span of time. Here we demonstrate the development of a longitudinal multimodal neural platform that enables simultaneous mapping of neural activity and cerebral blood flow (CBF) in the same behaving brain. This multimodal neural platform combines intracortical neural recording using the ultra-flexible nanoelectronic threads (NETs) that permit exceptional tissue-compatibility, and a wide-field imaging system capable to quantifying both CBF and inducing targeted photothrombosis at controlled locations, size and onset time. We demonstrate the application of this multimodal neural interface in a mouse model of microinfarcts, for which the microscopic ischemic injuries are often associated with vascular dementia but are too small in size to be

detected using conventional techniques. Through the ability to simultaneously track the underlying neuronal and hemodynamic changes from the acute to the chronic phase, we are able to reveal their distant pathological evolutions. In the acute sections following the awake induction of microinfarcts, we observe that neurovascular coupling weakens with the level of ischemia and that short-lasting neuronal bursting precedes peri-infarct depolarizations. The neurovascular dissociation becomes the most severe at the sub-acute phases, during which reperfusion and hyperperfusion in the occluded arterioles and nearby tissue occur, but neural activity remains significantly suppressed. Furthermore, while restoration of CBF occurs promptly at a similar time, the deficits in neural activity are long-lasting, the magnitude and duration of which are injury dependent. These observed discrepancies in neural and hemodynamic responses alert the interpretation of neuroimaging that infer neural activity from hemodynamic responses and demonstrates the need for direct neurological measurements to evaluate brain impairment and recovery.

Disclosures: F. He: None. **C. Sullender:** None. **H. Zhu:** None. **T.A. Jones:** None. **C. Xie:** None. **A. Dunn:** None. **L. Luan:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.15/J32

Topic: C.08. Ischemia

Title: Hypothermic treatment for neonatal asphyxia in low-resource settings using phase changing material

Authors: H. T. T. TRAN[1], H. T. T. LE[4], H. T. P. TRAN[4], B. WINBLADH[2], L. HELLSTRÖM-WESTAS[5], D. M. TRAN[4], D. T. K. KHU[4], T. ALFVEN[1], *L. OLSON[3];
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Abstract: Aim: To evaluate if a matress made of phase changing material (PCM) can be used for transportation and during regular therapeutic hypothermia of asphyxiated newborns in need of cooling. And to evaluate if the cooling have neuro impact on child. Methods: A study to evaluate using rectal temperature (Tr) and measuring deviations from normal temperature range, by using the phase changing material mattress (PCM-melting point 32ºC). Hypothermia was induced with in the first 6h after birth and maintained for 72hrs by the PCM mattress then reheating according to international hypothermia guidelines. (target Tr:33-35 ºC). Results: A total of 52 babies were cooled using PCM between September 2014 and May 2016. The mean gestational age 39.25 ±

1.09 weeks. The mean temperature at initiation of cooling was 35.54 ± 1.14°C. The median time to reach the target temperature was 2±1.63 hours. The mean ± SD temperature during the cooling phase was 33.95 ± 0.2°C. Throughout the cooling phase, the target temperature range (33.5-34.5°C) was maintained in 80% of the time. Rate of rewarming is 0.5±0.14°C/hour. Conclusion: Phase changing maternial can be used as an effective cooling method. Though not a servo-controlled system, it is easy to induce hypothermia, maintain target temperature and rewarm babies in a slow and controlled manner using PCM without need for frequent changes, and minimum risk of skin injury. There is also a potential for PCM to be used for transport.

Disclosures: **H.T.T. Tran:** None. **H.T.T. Le:** None. **H.T.P. Tran:** None. **B. Winbladh:** None. **L. Hellström-Westas:** None. **D.M. Tran:** None. **D.T.K. Khu:** None. **T. Alfven:** None. **L. Olson:** None.

Poster

391. Stroke II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 391.16/J33

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

Support: NIH T32 Interdisciplinary Training Grant T32MH020068
Sidney Frank Fellowship
Brown BIBS/NPNI New Frontier Award
Office of Naval Research
Brown Presidential Fellowship

Title: Exploring the effects of oxygen-glucose deprivation in a 3D cortical microtissue model

Authors: *R. M. MCLAUGHLIN[1,2], J. SEVETSON[1,2,3], L. L. LIVI[4], D. HOFFMAN-KIM[2,3,4,5];
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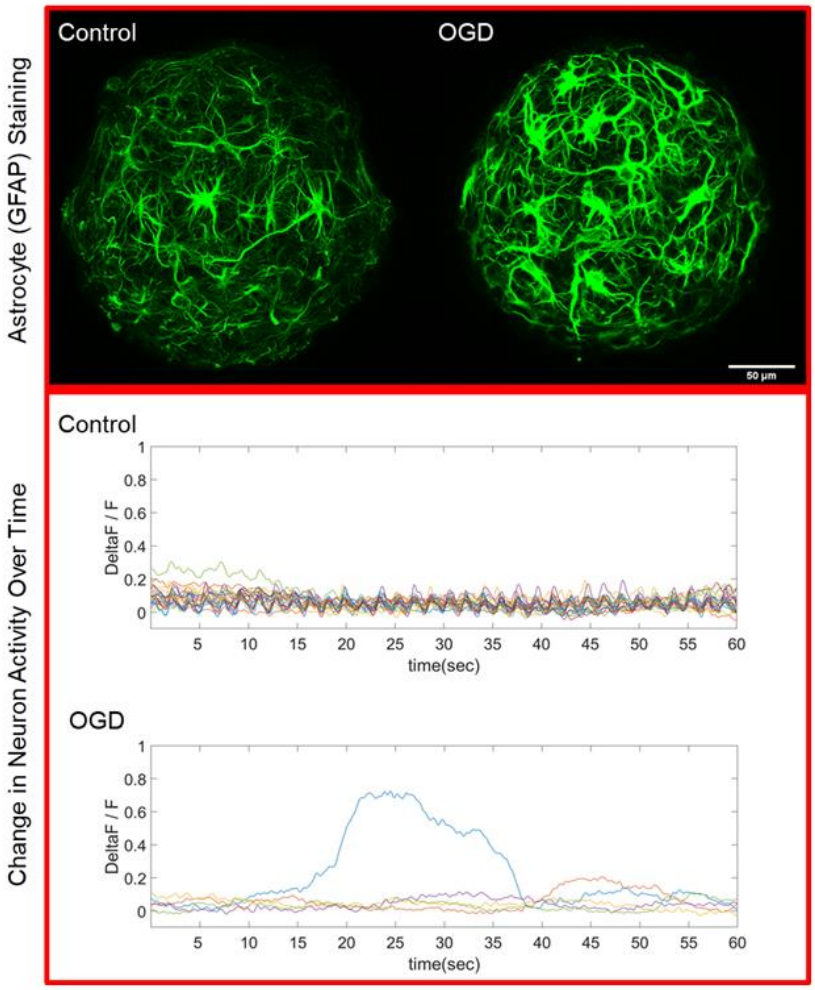
Abstract: Stroke is a leading cause of death and disability worldwide. The most common form of stroke is ischemic stroke, caused by a blockage of blood flow to a specific part of the brain. Treatments for ischemic stroke include clot removal and prevention of clot reformation, but there is a lack of pharmaceuticals to prevent secondary injury during reperfusion and/or promote regeneration. Here our goal was to develop an *in vitro* model of ischemic stroke. We explored the effects of oxygen-glucose deprivation (OGD) on morphology and function in our previously established, high-throughput compatible, 3D, *in vitro* cortical microtissue model (Dingle 2015). Additionally, we looked at oxygen (OD) and glucose (GD) deprivation separately to investigate

how these components contribute to ischemic injury. We used self-assembled, scaffold-free cellular spheroidal microtissues of 4000-8000 cells/microtissue, derived from postnatal (P0-P3) female rat primary cortical tissue and evaluated at culture day 10-14. These microtissues have cellular composition, tissue stiffness, and cell density similar to those found *in vivo*. Neurons in these microtissues are electrically active and form networks. For 24h deprivation assays (n = 6-10 microtissues), we replaced cortical media with glucose-free media and/or placed the microtissues in a chamber containing a BD GasPak EZ sachet which sequesters oxygen. Blinded analysis of immunohistochemistry showed that after OGD, OD, or GD, microtissues had increased reactive astrocytes and increased disruption of neurites. Microtissues exposed to OGD were also evaluated for neural activity using calcium imaging; they showed fewer active cells and no synchrony, as compared to controls. The deprivation assays we performed on our 3D cortical microtissue model have helped us characterize an *in vitro* model of ischemic stroke and start to parse out the separate effects oxygen and glucose deprivation have on our model. Future work will focus on characterizing this model quantitatively and using it to screen potential therapies to minimize the damage cause by ischemic injury.



Disclosures: **R.M. McLaughlin:** None. **J. Sevetson:** None. **L.L. Livi:** None. **D. Hoffman-Kim:** None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.01/J34

Topic: C.10. Brain Injury and Trauma

Support: VA RR&D SPiRE 1 I21 RX002232-01

Title: Biological signature of an immunomodulatory probiotic intervention for veterans with mild TBI and PTSD

Authors: *L. A. BRENNER[1,2,3,4,5], K. A. STEARNS-YODER[6,2,4,5], A. J. HOISINGTON[7,6,5], T. T. POSTOLACHE[8,6,5], C. A. LOWRY[9,6,2,5,10];
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Abstract: United States military Veterans from recent conflicts are coping with symptoms related to mild traumatic brain injury (mTBI), persistent post concussive (PPC) symptoms, and posttraumatic stress disorder (PTSD). One potential common underlying feature of both mTBI and PTSD is exaggerated inflammation, both peripherally and in the central nervous system, which is thought to play an important role in the vulnerability to, aggravation of, and perpetuation of adverse consequences of these co-occurring conditions. Therefore, a novel intervention strategy would be the use of immunoregulatory/anti-inflammatory probiotics to reduce inflammation. In this study, we investigated the effects of an immunoregulatory probiotic on both biological signatures of systemic inflammatory processes and proximal signatures of probiotic administration. Up to 40 Veterans ages 18 to 50 with a history of mTBI, PPC symptoms, and PTSD were enrolled in this feasibility and acceptability trial. Feasibility and safety were measured by the Adult AIDS Clinical Trials Group, Modified Morisky Medication-Taking Adherence Scale, and Probiotics Usage Log. Acceptability was measures using the Credibility and Expectancy Scales and Modified Treatment Satisfaction Questionnaire. Tolerability and safety were measured by the Generic Assessment of Side Effects, and Irritable Bowel Severity Scoring System. Final analyses are underway. Data regarding acceptability and feasibility will be presented along with results associated with inflammatory markers among those with TBI and PTSD. Results from this study are expected to provide key information

necessary to develop interventions aimed at reducing inflammation among those with co-occurring symptoms.

Disclosures: L.A. Brenner: None. **K.A. Stearns-Yoder:** None. **A.J. Hoisington:** None. **T.T. Postolache:** None. **C.A. Lowry:** None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.02/J35

Topic: C.10. Brain Injury and Trauma

Support: NIA 5P30AG013846-22
NIA RO1AG057902
VA National Center for PTSD

Title: Persistent astroglial pathology in repetitive head impacts blast injury and CTE

Authors: *B. R. HUBER[1], K. BABCOCK[2], J. D. CHERRY[2], V. ALVAREZ[2], T. D. STEIN[3], A. C. MCKEE[2];
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Abstract: Neurotrauma is associated with reactive astroglial changes and alterations in the expression of Aquaporin-4 (AQP4). Reactive astrocytosis is identified by increased expression of Glial Fibrillary Acidic Protein (GFAP) and is observed in many neuroinflammatory processes. In animal models of mild traumatic brain injury (mTBI) reactive astrocytosis appears soon after the initial injury, peaks after a few days, and then slowly resolves after weeks to months. The expression of the water channel AQP4 is also increased following neurotrauma and redistributed from the astroglial endfeet to the cell body (depolarization). Less is known about this process in humans. In our preliminary results using human brain tissue, with cumulative exposure to mTBI we have found that reactive astroglial changes persist long after the exposure stops and that the pattern of reactive glial changes shares features with Alzheimers Disease (AD) and aging. Recently, blast neurotrauma was shown to generate a distinct astroglial response referred to as interface astrocytosis. This pattern consists of astrocytosis at the glial externa (surface of the brain) and the gray-white matter junction. Using the Veterans Administration Brain Bank Biorepository (VABBB) and the Chronic Traumatic Encephalopathy (CTE) Brain Bank we have assembled cohorts of brains with different types of neurotrauma exposure. We examined the premise that interface astrogliosis was specific to blast neurotrauma. In our comparison, we examined four groups including: blast TBI with CTE, athlete TBI with CTE, repetitive head impacts (RHI) without CTE, and controls without a history of neurotrauma. Our results indicate

that blast neurotrauma is associated with a significant increase in gray/white matter interface astrocytosis compared with other forms of mTBI and with controls. Blast neurotrauma was also associated with a significant increase in glial limitans interface astrocytosis when compared with controls. The difference between blast neurotrauma and other forms of neurotrauma was not significant at the glial limitans externa, but had a similar trend to the gray-white matter interface. These findings indicate that blast neurotrauma results in more interface astrocytosis than other forms of neurotrauma, but that all forms of neurotrauma cause interface astrocytosis.

Disclosures: **B.R. Huber:** None. **K. Babcock:** None. **J.D. Cherry:** None. **V. Alvarez:** None. **T.D. Stein:** None. **A.C. McKee:** None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.03/J36

Topic: C.10. Brain Injury and Trauma

Support: Indiana CTSI 11917

Title: Mechanisms of secondary injury and auditory deficits following mild blast induced trauma

Authors: *J. FERNANDEZ[1], E. HAN[1], E. L. BARTLETT[1], R. SHI[2];
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Abstract: Blast-induced hearing difficulties affect thousands of veterans and civilians each year. The long-term impact of blast exposure on the central auditory system (CAS) can last months, even years, without major external injury, and is hypothesized to contribute to many behavioral complaints associated with mild blast traumatic brain injury (bTBI). Our group has previously documented the short-term (two-weeks) and long-term (2-months) effects of acute blast and non-blast acoustic impulse trauma on click/tone pip and sinusoidally amplitude modulated (AM) carriers in adult rats. However, the mechanisms that underlie these long-term impairments are still poorly understood. Examining the acute time course and pattern of neurophysiological impairment (within the first two weeks), as well as the underlying molecular and anatomical post injury environment is therefore critical to understanding the mechanisms that lead to long-term CAS impairments. Ultimately this research can inform improved diagnostic and therapeutic strategies for bTBI related deficits. Although initial mechanical injury likely plays a role in central auditory damage, a secondary molecular mechanism of damage likely results in the chronic auditory deficits following mild bTBI. Oxidative stress, along with inflammation, have been suggested as key players in secondary molecular damage in other models of CNS injury, including other TBIs, and may underlie functional auditory deficits in mild bTBI as well. Here, we recorded the changes in auditory brainstem response (ABR) and auditory evoked potential

(AEP) response to amplitude modulation (AM) and speech-like stimuli in blast-exposed and control rats over the course of two months. We compared these results to molecular and anatomical changes observed in immunohistochemistry staining. Taken together, our results suggest that a cascade of (axonal) membrane damage, oxidative stress, and excitatory/inhibitory imbalance contributes to blast-induced subcortical CAS impairments.

Disclosures: **J. Fernandez:** None. **E. Han:** None. **E.L. Bartlett:** None. **R. Shi:** None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.04/J37

Topic: C.10. Brain Injury and Trauma

Support: PhRMA Foundation
Brain and Behavior Research Foundation

Title: Alterations in CNS 5-HT_{2A} signaling and the generation of aberrant social behavior following blast-induced traumatic brain injury

Authors: *S. M. COLLINS[1], E. L. REEDER[1], K. LUNGANI[1], A. HUSSAIN[2], C. J. O'CONNELL[1], G. A. GUDELSKY[1], M. J. ROBSON[1];
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Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability in the United States, accounting for approximately 2.8 million emergency room visits and an economic burden of nearly $75 billion annually. The lack of any FDA approved medications to treat the acute or chronic effects of TBI contributes greatly to the societal and economic costs. Among the enduring comorbidities of mild to moderate TBI is the generation of neuropsychiatric disorders including altered social function and depression, which are strongly linked to altered serotonin (5-HT) signaling. The effects of neurotrauma on the normal, physiologic function of the 5-HT system are currently not well understood. We hypothesize that TBI alters 5-HT signaling networks, effects that impact mood and behavior states. To test this hypothesis, adult, wild type C57Bl/6J mice were subjected to either a single, moderate blast-induced TBI or sham treatment. Molecular and behavioral assays were performed 0-10 days post injury (dpi). High performance liquid chromatography (HPLC) revealed a significant increase (10 dpi) in total 5-HT and 5-HIAA levels within the raphe nucleus of TBI subjects compared to their sham counterparts, an effect not found in the prefrontal cortex (PFC) or somatosensory cortex (SSC). Administration of the 5-HT_{2A} agonist DOI (3 and 10 dpi) resulted in a significant potentiation of head twitch response (HTR) in TBI subjects as compared to their sham counterparts. This potentiation of DOI-induced HTR by TBI is dependent upon 5-HT_{2A} receptor activity, as HTR is attenuated by

pretreatment with the 5-HT_{2A} antagonist M100907. Western blot analysis of serotonin transporter (SERT) expression revealed a significant decrease within the dorsal raphe (3 dpi) in TBI subjects. As 5-HT signaling is a determinant of social behavior, we hypothesized that TBI-induced changes in homeostatic 5-HT signaling would impact social behavior. Using the Crawley Three Chamber Sociability Assay, significant social deficits were found in TBI subjects (10 dpi) as compared to their sham counterparts. Further corroborating these data, social deficits were observed (10 dpi) using the Tube Test Social Dominance Assay. Collectively, our studies suggest TBI alters 5-HT signaling within the CNS that drive TBI-induced changes in behavior. An understanding of how various forms of neurotrauma alter 5-HT signaling may lead to the discovery of pharmacotherapies aimed at ameliorating the neuropsychiatric complications of TBI.

Disclosures: S.M. Collins: None. **E.L. Reeder:** None. **K. Lungani:** None. **A. Hussain:** None. **C.J. O'Connell:** None. **G.A. Gudelsky:** None. **M.J. Robson:** None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.05/J38

Topic: C.10. Brain Injury and Trauma

Support: NIH R01DC012060 (HZ)
R21DC017293 (HZ)
R01DC014930 (WZ)
R21EY025550 (WZ)
CPN animal behavioral core (P30GM10332)

Title: Blast waves delivered into the ear canal induced traumatic brain injury (TBI) in rats

Authors: Y. OU[1], Y. PANG[2], T. CHEN[1], J. HUANG[1], J. ALLISON[1], D. SANDLIN[3], F. FAN[4], R. ROMAN[4], W. ZHOU[1], ***H. ZHU**[1];
[1]Otolaryngology, [2]Pediatric, [3]MD/PhD Program, [4]Pharmacol., Univ. MS Med. Ctr., Jackson, MS

Abstract: Exposures to blast shockwaves generated by explosions often cause traumatic brain injury (TBI) in military and civilian populations. How the primary blast waves affects the brain is not well understood. As air-filled structures and directly exposed to the surrounding air, the unprotected ears are among the most frequently damaged sites during blast exposure. Studies of blast victims reveal that there is a significant association between tympanic membrane perforation and loss of consciousness in blast exposure. In a previous study (Sandlin et al., 2018, ARO abstract), we showed that blast-exposure via the ear canal induced damage in the vestibular end organs and vestibulo-ocular reflexes (VOR) deficits. However, it is not known whether blast

exposure via the ear canal also causes damage to the brain. To address this issue, we developed a novel model that delivers precise blast overpressure waves solely into the ear canal. Adult female Long-Evans rats were anesthetized and exposed to a single blast of 0-40PSI to the left ear. Brain tissues were harvested for histology at 6 hours, 1, 3, 7, 14, 30, 60 and 500 days after blast exposure. A single blast through the ear caused substantial neuropathological changes in the brain, including increased expression of c-fos, chronic activation of microglia, macrophage infiltration, neuronal apoptosis and increased expression of beta amyloid precursor protein (APP). These changes occurred not only in the brain regions close to the internal auditory meatus, but also in distant areas of the brain related to cognitive functions (e.g. cortex and hippocampus). We further showed that the animals exposed to the blast exhibited signs of anxiety in an open-field test 2 months and 4 months after exposure and impairments of learning and memory in an 8-arm water maze test 13 months after the blast. These results provided evidence that the unprotected ear provides a vulnerable locus for blast waves to impact the brain and cause TBI. This study not only establishes a novel model of blast-induced TBI, but also highlights the necessity for wearing ear protection in blast-prone locations, such as battlefield, training or industrial environments, not only to protect hearing, but also to protect from blast-induced neurotrauma.

Disclosures: **Y. Ou:** None. **Y. Pang:** None. **T. Chen:** None. **J. Huang:** None. **J. Allison:** None. **D. Sandlin:** None. **F. Fan:** None. **R. Roman:** None. **W. Zhou:** None. **H. Zhu:** None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.06/J39

Topic: C.10. Brain Injury and Trauma

Title: High-rate overpressure influences on pro-oxidative potential in primary astrocytes

Authors: *F. GUILHAUME CORREA[1], N. HLAVAC[2], P. J. VANDEVORD[2,3];
[1]Translational Biology, Medicine, and Hlth., [2]Biomed. Engin. and Mechanics, Virginia Tech., Blacksburg, VA; [3]Salem Veterans Affairs Med. Ctr., Salem, VA

Abstract: Blast traumatic brain injury (bTBI) is a frequent injury within military personnel and Veterans due to their exposure to explosives with 80% of mild head injuries attributed to the blast exposure. Treatments for bTBI remain elusive and are limited to symptom management. bTBI can trigger secondary cellular responses, which include neuroinflammation, excitotoxicity and oxidative stress. There is a critical knowledge gap in understanding the cellular and molecular responses after blast. Astrocytes are central for homeostatic, repair, and signaling process mechanisms, critical for maintaining neural circuit post trauma and are important as therapeutically targeted cell for brain diseases and injury. In this study, we aimed to identify

unique injury mechanisms in astrocytes after exposure to blast. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and their elimination mechanisms. These pro-oxidative conditions caused by ROS are considered to play a role in neuronal toxicity and subsequent neurodegeneration. Cells respond to oxidative stress through different mechanisms such as epigenetic alterations. We hypothesize that astrocytes undergo oxidative stress related to cellular and epigenetic changes as a result of blast exposure. Using a 2D *in vitro* model of bTBI, primary rat astrocytes were exposed to an overpressure wave by using a high-rate fluid-filled device, which mimics transmission of a shock-like wave intracranial profile. The peak overpressure was 17psi to correlate with mild injury outcomes observed in animal models. Following insult, samples were collected for analyses at 4 or 24 hours. We measured protein levels of two oxidative stress markers, NOX4 and SOD2. The membrane-permeant JC-1 dye was used to estimate mitochondrial health and DHF-DA for intracellular ROS detection. Examination of gene expression for oxidative stress, as well as, epigenetic-related genes, thymine DNA glycosylase (TDG) and DNA methyltransferase enzymes (DNMT1 and DNMT3) was analyzed for an epigenetic link with oxidative stress. The results indicated a significant increase (p-value= 0.0079) in the expression of NOX4 between sham and blast at 24 hours. Changes of mitochondrial membrane potential was observed post exposure. Further, an oxidative stress gene array indicated multiple targets suggesting that astrocytes are reactive to oxidative stress mechanisms at 24 hours. Collectively, the results demonstrate that oxidative stress contributes to the astrocytes' reactivity post blast. We expect to identify potential pathways for intervention whereby a healthy astrocyte environment could be restored.

Disclosures: F. Guilhaume Correa: None. **N. Hlavac:** None. **P.J. Vandevord:** None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.07/J40

Topic: C.10. Brain Injury and Trauma

Title: Persistent neurobehavioral deficits and tauopathy in a co-morbid animal model of TBI and PTSD

Authors: *A. FESHARAKI[1], M. T. CHIASSEU[2], K. ST. LAURENT-ARRIOT[4], P. BERGOLD[4], S. M. STRITTMATTER[3];
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Abstract: The combination of post-traumatic stress disorder (PTSD) with traumatic brain injury (TBI) is known to produce a syndrome more severe than PTSD or TBI alone. Neuroinflammation produced by PTSD and TBI likely contribute to their chronic affective and

cognitive sequelae. We examined behavioral deficits and neuroinflammation in C57BL/6 mice that received; sham, closed head injury (CHI), a model for TBI 3) chronic variable stress (CVS), a model for PTSD; or combined CHI and CVS , CHI → CVS, or CVS → CHI. The CVS → CHI group had the largest cognitive deficits on a spatial task, Barnes Maze, and an Active Place Avoidance (APA) task (Barnes Maze, latency to target latency, $p<0.001$ & APA, $p<0.005$). The CVS→CHI group also had increased Iba-1 and Arginase-1 expression suggesting greater hippocampal inflammation (Iba1: Dentate; $p < 0.005$; CA1; $p < 0.01$; CA3, $p < 0.05$) Arginase 1: $p < 0.005$ in DG, CA1 and CA3 regions). Neuroinflammation in Dentate Gyrus (DG), and CA1 hippocampal area significantly correlated with the extent of behavioral deficits on Barnes Maze and Active Place Avoidance. (Barnes Maze: DG: $r =0.84$, $p<0.005$ APA: DG, $r =0.80$, $p<0.005$). These results suggest an importance of the insult order since the CVS→CHI group had larger deficits and greater inflammation than the CHI → CVS group and that neuroinflammation contributes to neurobehavioral deficits in co-morbid TBI and PTSD. Based on these results, a second model of co-morbid CHI and CVS was devised, consisting of 14 days of combined CVS and CHI (CVS/CHI). The mice underwent a modified CVS protocol in the morning session, followed by CHI injury in the afternoon session. The mice showed significant spatial memory deficits on Morris Water Maze (MWM) on Post Injury Day (PID) 30 and 90 (ANOVA, $p<0.0001$), as well as Novel Object Recognition Test (NORT) on PID 90 (ANOVA, $p< 0.0001$). They had increased entries to open arms in the elevated plus maze on PID 30 and PID 90 (t-test, $p<0.0001$) suggesting greater anxious behavior. Mice were sacrificed for further immunohistological analysis following behavioral testing. Levels of total Tau level and PHF-1 Tau significantly differed between the CVS/CHI group and sham group in total levels ($p=0.0079$). TDP-43 levels also differed significant between CVS/CHI group and sham group. These results suggest that the CVS/CHI model produces a persistent neurocognitive deficits accompanied by tauopathy, and alterations in TDP-43.

Disclosures: A. Fesharaki: None. **M.T. Chiasseu:** None. **K. St. Laurent-arriot:** None. **P. Bergold:** None. **S.M. Strittmatter:** None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.08/J41

Topic: C.10. Brain Injury and Trauma

Title: Structural network topology of military service members with PTSD, and PTSD-mTBI comorbidity

Authors: *F. PROESSL[1], S. D. FLANAGAN[1], A. J. STERCZALA[1], A. Z. BEETHE[1], C. DUNN-LEWIS[1], C. CONNABOY[1], B. C. NINDL[1], G. DESHPANDE[2], J. S. KATZ[2], T. S.

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Abstract: Introduction: The advancement from traditional voxel-based- to graph theory analyses provides a novel means to study complex brain networks in post-traumatic stress disorder (PTSD). Initial investigations demonstrate altered small-worldness neural networks in PTSD with functional data, but little is known about the structural network topology, or the common comorbidity of PTSD and mild traumatic brain injury (mTBI). **Purpose:** Compare structural brain network characteristics of active duty Service members (SMs) with PTSD, co-morbid PTSD and mTBI, compared to controls (CON). **Methods:**Sixty male SMs (mean age: 33.0±6.4 years) were split into three groups based on diagnosis: 28 CON, 18 PTSD+mTBI and 14 PTSD. All participants completed T1-weighted structural magnetic resonance (MRI) scans to determine cortical thickness on a voxel-basis and in 56 individual regions of interest (ROI). Further, cortical thickness values were used to compute a ROI×ROI adjacency matrix using Pearson correlations and setting negative correlations to zero. A weighted and binary undirected graph was created with each ROI representing a node and the connectivity between ROIs presenting the edges. Graph measures including average degree, characteristic path length, clustering, global efficiency and small-worldness were compared using nonparametric permutation tests repeated 1000 times. Two-tailed t-tests and ANCOVAs (covariate = age; α=.05) were used to test for between-group differences. **Results:** CON demonstrated significantly greater cortical thickness than PTSD in smaller temporal and parahippocampal voxels, while thinner cortex in the right temporal pole compared to PTSD+mTBI. No significant differences were observed for any of the global graph measures in neither the weighted, nor binary undirected graph. However, small-worldness trended towards a significant reduction in PTSD+mTBI relative to CON (0.93 vs. 0.88 respectively, p=.06, 95%CI [-0.05, 0.05]). **Discussion:** SMs with PTSD and PTSD+mTBI only demonstrated subtle cortical thickness, but no small-world topography differences, at least globally. Ongoing subnetwork and nodal analyses investigate whether structural network topology may change network- or node-specifically and if graph theory measures can be linked to behavioral outcomes.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.09/J42

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 5U01OH011314-03

Title: Neural correlates of severe cognitive impairment and posttraumatic stress disorder: Preliminary analysis of World Trade Center responders

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Abstract: World Trade Center (WTC) responders experienced multiple toxic and traumatic exposures during search, rescue, and clean-up efforts. A recent study of responders found that nearly 20% of them developed posttraumatic stress disorder (PTSD) and 13% had mild-severe cognitive impairment (CI). PTSD is increasingly recognized as a risk factor for CI and Alzheimer's disease and related dementias (ADRD). Little is known about mechanisms underlying this relationship between PTSD, CI and ADRD. The objective of this study was to analyze neural correlates of PTSD and CI in WTC responders. This report provides preliminary results comparing anatomical connectivity between CI cases, PTSD cases, CI and PTSD cases, and controls. The parent sample included 3,022 SBU WTC responders. A subset of 57 responders (13 with CI only, 18 with PTSD only, 9 with both CI and PTSD, and 17 with neither CI or PTSD) underwent a diffusion-weighted imaging (DWI) MRI scan acquired on a Siemens 3T PET/MRI scanner. Preprocessing was performed using FSL and whole brain voxel-wise group comparisons of fractional anisotropy (FA) were carried out in each clinical group vs the control group. All analyses were adjusted for age and sex, and are corrected for multiples comparisons. Preliminary results showed reduced FA in 3 clusters in CI compared to controls: the genu of the corpus callosum, posterior corona radiata and the superior longitudinal fasciculus ($p < 0.05$). When compared to control, PTSD cases presented reduced FA in the right genu of the corpus callosum ($p < 0.05$). PTSD+CI group showed reduced FA in the right superior longitudinal fasciculus and the right splenium of the corpus callosum compared to controls ($p < 0.05$). These preliminary results support anticipated trends observed in the literature suggesting that CI may be associated with decreased FA in the corpus callosum and the left superior longitudinal fasciculus. Corpus callosum white matter abnormalities have also been previously reported in PTSD. Results in the PTSD+CI group show some overlaps with the ones seen in the PTSD only and CI only groups. These results are important for completing the long-term clinical picture of WTC exposure effects and have implications for brain aging.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.10/J43

Topic: C.10. Brain Injury and Trauma

Support: DoD PRARP-CSRA; AZ140109
Electron Microscopy (EMC) Fellowship
Dept. of Pathology and Anatomical Sciences
I01BX003527
I21BX002215
I21BX003807

Title: Primary low-intensity blast exposure: Energy parameters inducing ultrastructural abnormalities and synaptic alterations in mice

Authors: *H. R. SIEDHOFF[1], L. M. KONAN[1], H. SONG[1], J. CUI[1,2], C. E. JOHNSON[3], B. RUTTER[3], D. GRANT[4], T. WHITE[4], M. CHEN[5], W. XIA[5], I. CERNAK[6], R. G. DEPALMA[7], Z. GU[1,2];
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Abstract: Service members during military actions or combat training are exposed to primary blast generated by explosive weaponry. The majority of military-related neurotraumas are mild TBI, often characterized as "*invisible*" and "*signature*" injuries of current military conflicts due to their prevalence. While prior studies have mostly focused on moderate to severe brain injuries, the mechanisms of low-intensity blast (LIB)-mediated pathobiology and subsequent neurological deficits require further definition. We have developed a militarily relevant, open-field LIB injury in mice, the "*Missouri Blast*" model, by detonating 350 grams of high-energy explosive C4. The open-field LIB formed incident and ground reflected shock waves generating a static peak overpressure of 46.7 kPa, where a primary shockwave velocity was 409 m/s and a secondary ground reflection 388 m/s, and the maximal impulse 60 kPa x ms measured at 3-m distance away 1-m above ground detonation. Using high-speed videography, we confirm the absence of visible impact / acceleration on the blast-exposed mice in prone position. Our previous studies have shown that LIB exposures resulted in nanoscale subcellular myelin and mitochondrial damages and subsequent behavioral disorders in the absence of gross or detectable cellular damage. In this study, we used transmission electron microscopy to delineate the LIB effects at the

ultrastructural level specifically focusing on the neuron perikaryon, axons, and synapses in the cortex and hippocampus of mice at 7- and 30-days post-injury (DPI). We found dysmorphic dark neuronal perikaryon and ‘‘cytoplasmic aeration’’ of dendritic processes, as well as increased microtubular fragmentation of the myelinated axons, along with biochemically measured elevated tau / phosphorylated tau / Aβ levels. The number of cortical excitatory synapses decreased along with a compensatory increase of the post-synaptic density (PSD) thickness both at seven and 30 DPI, while the amount of hippocampal CA1 synapses increased with the reduced PSD thickness. In addition, we observed a significant increase in protein levels of PSD95 and synaptophysin mainly at seven DPI indicating potential synaptic reorganization. These results demonstrated that a single LIB exposure can lead to ultrastructural brain injury with accompanying multi-focal neuronal organelle alterations. The *Missouri Blast* model provides an understanding of open field energy impacts including energy from shockwave velocity. This pre-clinical study provides key insights into disease pathogenesis related to primary blast exposure, and may link to dementia.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.11/J44

Topic: C.10. Brain Injury and Trauma

Support: VA Merit Review Award I01RX001144
VA Interprofessional Polytrauma and Traumatic Brain Injury Rehabilitation Research Fellowship

Title: Impact of mild traumatic brain injury and fear conditioning on dendritic plasticity in the basolateral amygdala and prefrontal cortex of mice

Authors: L. K. ALFILER[1], J. K. BABB[2], K. A. LEITE-MORRIS[3], S. C. HEINRICHS[1], ***G. B. KAPLAN**[3];
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Abstract: Mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) have emerged as the signature injuries suffered by US military personnel due to an increased probability of developing PTSD symptoms following a deployment-related mTBI. In an effort to

examine the behavioral and neurobiological consequences of these two conditions at a subacute phase of injury (3 weeks post-TBI), our project aims to develop a comprehensive model of comorbid mTBI and PTSD and examine impacts on structural plasticity in key brain regions. In order to explore whether mTBI alters fear learning in this study mTBI was administered by lateral fluid percussion at 1.7 atm force (LFP 1.7) after which fear conditioning (FC) and fear extinction (FE) was measured. Fear acquisition training generated significantly increased conditioned freezing responses over time in fear conditioned groups compared to naïve controls. Following fear extinction training in experimental groups, conditioned freezing decreased significantly over time. In addition, these results revealed significantly enhanced FE in groups previously exposed to fear and mTBI (fear/LFP 1.7) compared to the craniectomy control (fear/ LFP 0.0 atm) and surgically naïve controls that were fear conditioned. Consequently, our focus became to examine dendritic plasticity in the prelimbic (PL) and infralimbic (IL) regions of the prefrontal cortex, and the basolateral nucleus of the amygdala (BLA), which are critical nodes involved in FE learning. After the last day of testing, all mice were perfused and brains were sectioned and prepared for Golgi staining. Images of full neurons from the PL, IL, and BLA were analyzed using a microscope-based image analysis program. Fear conditioned mice that underwent extinction had significantly increased spine density in the IL compared to the non-fear conditioned group. Mice in the Fear/LFP 1.7 group had increased spine density in the BLA compared to fear-conditioned craniectomy controls (Fear/LFP 0.0) and unoperated controls. In summary, during subacute phase of injury, our model of coinciding TBI and fear interventions shows increases in FE learning that are accompanied by co-occurring increases in dendritic spines in the BLA in the fear/LFP 1.7 group. These findings suggest a potential treatment window post-injury in which FE processes are more robust after mTBI. Further research is critically needed to examine the converging neurobiological and pathophysiological mechanisms underlying the comorbid condition leading to more effective diagnostic and treatment approaches.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.12/J45

Topic: C.10. Brain Injury and Trauma

Support: The Miami Project to Cure Paralysis
The Buoniconti Fund

Title: Early life stress alters the threshold for cognitive impairments in mild traumatic brain injury

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Abstract: Mild traumatic brain injury (mTBI) accounts for more than 70% of all TBIs. Although the majority of these patients fully recover within two weeks, a subset of people suffer long-term neurocognitive dysfunction. Pre-injury comorbidities that contribute to long-term neurocognitive dysfunction after mTBI are poorly understood. One potential risk factor is early life stress. Early life stress includes child maltreatment such as neglect or abuse, and hampers the normal construction of cortical and hippocampal circuits. In the present study, we tested whether early life stress prior to mTBI in adulthood exacerbates neuroinflammatory sequelae and impairs hippocampal synaptic plasticity. Sprague Dawley pups were separated from their nursing mothers for 3 hours daily from postnatal days 2 to 14. At 2 months of age, the male rats received sham surgery or mild parasagittal fluid-percussion brain injury. At 24 hours after injury, inflammatory gene expression was measured in the hippocampus. PCR gene array analysis revealed that early life stress exposure upregulated the inflammatory genes toll-like receptor 4 (*Tlr4),* NOD-like receptor family, pyrin domain containing 3 (*Nlrp3),* caspase 1 *(Cas1)* and interleukin-1β (*Il-1β)* in mTBI animals. Further, we assessed whether this upregulation in neuroinflammation was associated with changes in synaptic dysfunction by evaluating long-term potentiation (LTP) in area CA1 of the hippocampus. At 2 weeks post-surgery, LTP was significantly impaired in mTBI animals exposed to early life stress as compared to non-stressed mTBI animals. These results indicate that pre-injury exposure such as early life stress may be a potential factor that worsens outcome after mTBI by exacerbating neuroinflammation and impairing hippocampal synaptic plasticity.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.13/J46

Topic: C.10. Brain Injury and Trauma

Support: Center for Neuroscience and Regenerative Medicine

Title: Detrimental effects of mild blast traumatic brain injury are augmented by chronic stress

Authors: *M. G. OYOLA[1], A. L. RUSSELL[2], S. E. SAPERSTEIN[1], T.-Y. J. WU[1];
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Abstract: Traumatic brain injury (TBI) affects 1.7 million people in the United States every year, resulting in increased morbidity and mortality. A significant portion of TBIs experienced by military personnel is induced by explosive blast devices. Active duty military personnel are especially vulnerable to mild blast-induced (mb)TBIs and their associated long-term effects, such as anxiety disorders, memory impairment, and mood disorders. Moreover, virtually every mbTBI on the battlefield is preceded and followed by a constant period of stress. However, how stress affects the brain to deal with an insult, like mbTBI, remains unknown. This work aims to identify the brain targets affected by mbTBI and to elucidate how chronic stress could prime the brain for worse outcomes after mbTBI. First, we examined the effect of mbTBI on restraint-induced limbic-HPA axis reactivity in mice 7-10 days post-injury. The study showed increased anxiety-like behaviors, marked by decreased entries into and time spent in open arms of the Elevated Zero Maze ($p<0.05$). We then extended our studies to interrogate the potential effects of mbTBI on the prefrontal cortex (PFC) and found that mbTBI blunted the restraint-induced neuronal activation of c-Fos immunoreactivity ($p<0.05$) in male and female infralimbic and prelimbic regions of the PFC. These data suggest that the PFC is a potential target of mbTBI in both sexes. In a second experiment, we used the Thy1-eGFP transgenic mouse model to elucidate the effects of mbTBI during a chronic variable stress (CVS) paradigm. Comprising this paradigm were two weeks of CVS prior to our single mbTBI model, followed by another week of CVS after the single blast. Animals exposed to mbTBI+CVS showed higher anxiety-like behaviors ($p<0.05$) and heavier adrenals compared to the Sham+CVS animals ($p<0.05$). These data suggest that CVS makes the system more vulnerable to the effects of mbTBI. Our preliminary data studying the effects of CVS and mbTBI on the dendritic spine architecture of the PFC indicates that the spine length in the prelimbic PFC is decreased. We detected significant gliosis (astroglia and microglia) in the corpus callosum region adjacent to the prelimbic PFC in the CVS treated mice. Interestingly, this response was dampened in the animals treated with mbTBI+CVS ($p<0.05$), suggesting that CVS compromises the brain's response to an insult. These data will help us understand the mbTBI effects observed in the PFC and whether the psychiatric disorders seen following mbTBI result from changes in the dendritic connectivity and architecture on the PFC.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.14/K1

Topic: C.10. Brain Injury and Trauma

Title: Social stress potentiates negative outcomes for male prairie voles (Microtus ochrogaster) in polytraumatic injury

Authors: G. A. PRATT, III[1], V. D. CHAPLIN[1], M. C. NORMANN[2], M. MCWATERS[2], O. I. AKINBO[2], W. WATANASRIYAKUL[2], A. J. GRIPPO[2], *N. MCNEAL[1];
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Abstract: Background: Psychological factors are known to influence long-term health; however, there is a dearth of evidence evaluating its impact upon biological and behavioral outcomes following injury. This experiment utilized prairie voles because they regulate cardiac function similar to humans and have been successfully used to model autonomic and cardiac dysfunction, compromised complement and innate immunity, and endothelial dysfunction. **Methods:** All subjects (N=36) were housed in male/female pairs for 5 days, then half of the pairs were isolated (for 7 days) resulting in 4 injury study groups: (1) Paired-Female; (2) Paired-Male; (3) Isolated-Female; (4) Isolated-Male and (5) Anesthesia-Shams. On injury day, electrocardiogram was monitored and all animals received a traumatic brain injury (TBI) impact then ~30% hemorrhage. Animals were held for 1 hour, then resuscitated with 2x Lactated Ringers bolus (with 1 hour wait), allowed to regain consciousness (20 minute stabilization period); procedure ended with an open field behavior test and tissue collection. **Results:** All isolated subjects displayed significantly higher mortality (p=0.050); Isolated-Males were more likely to die than Paired-Males (p=0.080). Electrocardiogram data indicates significantly elevated heart rate for injury animals over Anesthesia-Shams and impaired cardiac regulation (i.e. decreased heart rate) during resuscitation for Isolated-Males (p=0.003). Isolated-Males were unable to complete the open field, but Isolated-Females tended to display elevated anxiety-like behavior (p=0.129). Isolated-Males displayed significantly higher glucose (p=0.015), lower anion gap (p=0.011), and tended to have a higher iCa (p=0.125). Size issues limited other group blood comparisons. **Conclusions:** Results imply the polytrauma prairie vole injury model can be used to further investigate the psychological factors that impact traumatic injury recovery. This model provides insight into the protective influence of social bonds on recovery from a physical injury, potentially informing future interventions and treatment strategies.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.15/K2

Topic: C.10. Brain Injury and Trauma

Support: MOMRP

Title: Diet factors and psychological insults (TBI vs. psychological stress) co-perturb gut-brain axis

Authors: N. CHAKRABORTY[1], J. C. DEMAR, JR[3], A. GAUTAM[2], *J. L. MEYERHOFF[1], R. HAMMAMIEH[2], M. JETT[1], J. LONG[4];
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Abstract: Recent data suggest a significant role of alterations in the microbiome and gut microflora architecture in association with traumatic brain injury (TBI) and psychological comorbidities, such as anxiety. To determine how a shift in the microbiome population is coordinated with physical injury and stress responses, two animal models were examined. Rats were fed either a standard house chow diet (w-3:w-6 fatty acids (FA)=1:6, where w-3FA supplied by DHA, EPA, aLNA and w-6 FA supplied by ARA, LA) or a special diet simulating common US consumption (w-3:w-6FA=1:1, where w-3 FA supplied by aLNA, but no EPA, DHA, and w-6FA supplied by ARA, LA). At 6 w of age, a closed-head TBI model consisting of a blast overpressure wave exposure coupled with a weight drop concussion (named Blast) was used on a group of adult male rats. In parallel, an independent group of rats were subjected to an underwater trauma (UWT) psychological stressor, known to elicit “anxiety”. Rats were euthanized 14d post-stress. Dual-dye microarray analysis was performed using hemi-brain and blood samples, and the fecal bacterial populations were characterized by 16s ribosomal RNA. The special diet on its own activated neuroinflammation networks in the brain. Subsequent stress effects showed divergent trends in apoptotic pathways in brains. Notably, the PTEN-PPAR network was activated, but the P38-MAPK/ERK-MAPK/NFkb network was inhibited in the UWT cohort fed the special diet. Alternatively, the PTEN-PPAR network was inhibited, but the P38-MAPK/ERK-MAPK/NFkb network activated, in the Blast cohort provided the special diet. Principal coordinate analysis revealed the special diet as the dominant factor in separating the taxonomic phylogenetic profiles. Two-way ANOVA determined the specific phyla that could be the markers of neurological stress types influenced by the special diet. Predictive network analysis associated the gut microbial shift with bioenergetics perturbations. The special diet exclusively influenced host bioenergetics networks including the glycolysis, gluconeogenesis, and pentose phosphate pathways. In conclusion, the simulated US diet caused considerable disruption of the gut-brain axis; and additional neurological stress aggravated the condition.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.16/K3

Topic: C.10. Brain Injury and Trauma

Support: HD061963
Pennsylvania Department of Health SAP 4100079710
Pennsylvania Department of Health SAP4100077079

Title: Traumatic brain injury in the neonate rat impairs adaptation to stress in adolescence: Role of hippocampal glucocorticoid receptors

Authors: *D. LENGEL[1], J. W. HUH[2], R. RAGHUPATHI[3];
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Abstract: Each year, an estimated 250,000 children under the age of 4 sustain a traumatic brain injury (TBI). Children are at high risk for developing dysfunction of stress response systems following TBI, leading to abnormal stress reactivity later in life. Clinical studies have reported a stress-associated increase in new onset psychological disorders following childhood TBI, which may be indicative of increased vulnerability to stress. However, little is known about the relationship between neurological changes following TBI during childhood and long-term stress-related functional outcomes. Therefore, we subjected 11-day-old male and female Sprague-Dawley rats to closed-head injury or sham-injury and assessed changes in affective behavior at adolescence (postnatal day 35-45). In the Forced Swim Test (FST), there were no behavioral differences between sham an injured groups during the pretest. However, during the test phase when animals were re-exposed to the swim chamber 24 hours later, both male and female brain-injured rats showed a significant *decrease* in time spent immobile. This suggests that TBI may impair the ability of rats to adapt or habituate to stressful stimuli, leading to maladaptive responses to stress. Therefore, we hypothesized that brain-injured animals may exhibit greater stress coping deficits following exposure to repeated stress. In order to assess coping deficits, rats were exposed to three consecutive days of restraint stress and were subsequently tested in a shuttle box avoidance test. Brain-injured rats showed an increase in both escape latency and the number of escape failures relative to sham-injured rats exposed to the same stress paradigm, indicating a “learned helplessness” phenotype. Previous studies have demonstrated that downregulation or suppression of the glucocorticoid receptor (GR) within the hippocampus decreases both the retention of the immobility response in the FST and resilience to stress. Thus, in order to investigate the molecular mechanisms underlying our behavioral findings, we determined whether TBI affects the expression of hippocampal GRs using real-time quantitative

PCR (rt-qPCR). Our findings revealed that GR mRNA expression was significantly decreased within the hippocampus at 3 days after injury. Further experiments are needed to determine the role of the glucocorticoid system in these behavioral deficits, and whether transfection of GRs within the hippocampus following TBI can reverse stress coping deficits. These findings could provide support for targeting the glucocorticoid system to treat stress-related symptoms in children with TBI.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.17/K4

Topic: C.10. Brain Injury and Trauma

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Title: Early life stress moderates the effects of traumatic brain injury on hippocampal neurogenesis and increases neuroinflammation

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Abstract: Abuse is one of the leading causes of traumatic brain injury (TBI) in children. Animal models have shown that TBI affects cognitive performance, hippocampal neurogenesis, and increases inflammation. Although stress can occur early in life in cases of child abuse, studies on non-accidental TBI in pediatric populations do not consider the possible increase in vulnerability caused by stress. Hence, we sought to determine whether stress exposure during early life increases the effects of TBI on hippocampal neurogenesis and neuroinflammation. Male Sprague-Dawley rats were subjected to maternal separation (MS) or used as controls (CONT) during the first 21 postnatal days (PND). At PND 21 a controlled cortical impact (CCI) or sham injury was produced. At PND 32 they were injected with the cell proliferation marker

Bromodeoxyuridine (BrdU, 50mg/kg), evaluated for cognitive performance in a water maze, and sacrificed ten days after injection. BrdU, Ki67 and Iba1, immunostaining was performed as was immunofluorescent staining for GFAP, DCX, and NeuN to determine the cellular phenotype of the BrdU+ nuclei. CCI reduced the volume of the granular and subgranular layer of the dentate gyrus, as well as the number and density of BrdU+ cells. MS partially reversed the CCI-induced reduction on BrdU+ cell density. Stress increased the percentage of active microglia in the CA1 region of both hemispheres, as well as the density of Iba1+ cells in the ipsilateral hemisphere. Approximately 60% of the BrdU+ cells differentiated into neurons. The findings suggest that MS partially reverses the effects of TBI on hippocampal neurogenesis but increases hippocampal neuroinflammation.

Disclosures: **A. Díaz:** None. **M.A. Roque:** None. **C.O. Bondi:** None. **A.E. Kline:** None. **N. Lajud:** None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

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Program #/Poster #: 392.18/K5

Topic: C.10. Brain Injury and Trauma

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Title: Early life stress increases cytokine expression in the hippocampus after TBI in juvenile rats

Authors: *M. A. ROQUE[1,2], L. KUTASH[3], A. IOUCHMANOV[2], I. H. BLEIMEISTER[4], J. CHENG[2], C. O. BONDI[2], N. LAJUD[6,7], A. E. KLINE[5];
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Abstract: Early life stress (ELS) increases vulnerability for anxiety, depression, and impaired cognition in adulthood. Traumatic brain injury (TBI) is also associated with the development of psychiatric disorders and cognitive dysfunction. Moreover, repeated stress and TBI induce

molecular and cellular abnormalities in the hippocampus and prefrontal cortex, which correlate with memory disturbances. Hence, the aim of this study was to analyze the impact of ELS and TBI in juvenile rats on executive function, as well as hippocampal and prefrontal cortex cytokine expression. Male Sprague-Dawley pups were removed from the mother's nest once daily for 3 hr (i.e., 180 minutes) from postnatal day (PND) 1 to PND21 (maternal separation, MS180), while control rats were left undisturbed. At PND21, all litters were weaned and the rats were randomly assigned to four groups: Control + Sham, Control + TBI, MS180 + Sham, and MS180 + TBI. Rats received a controlled cortical impact (2.2 mm cortical deformation depth at 4 m/sec) or Sham injury and were allowed to recover for two weeks. From PND35 to PND40, all groups were evaluated for executive function using the well-established attentional set shifting task (AST). All groups were sacrificed at PND42 and the brains were dissected to analyze cytokine expression (IL-1b, IL-6, and TNF-a) by real time polymerase chain reaction (PCR). Trunk blood was collected to analyze corticosterone levels. The data showed that TBI, but not MS180, affected AST in the first reversal stage. The combination of MS180 and TBI increased IL-1b expression in the ipsilateral hippocampus, but not in the prefrontal cortex. Moreover, MS180 + TBI increased plasma corticosterone levels at sacrifice. In conclusion, early life stress and TBI affect cytokine expression in the hippocampus, but not prefrontal cortex, in juvenile rats sustaining a TBI of moderate severity.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

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Program #/Poster #: 392.19/K6

Topic: C.10. Brain Injury and Trauma

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PRCTRC U54MD007587
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UPR MSC Chancellor's Office
UPR MSC School of Medicine Deanship
Brain & Behavior Research Foundation (NARSAD)

Title: Effects of concussive-like head injury on fear-related behaviors in rats

Authors: *M. RIVERA LOPEZ[1], M. CÁCERES-CHACÓN[1], O. MARTÍNEZ-GUZMÁN[1], R. Y. RAMOS-SÁNCHEZ[2], D. M. OJEDA-MARTÍNEZ[2], P. ALVELO-FERNÁNDEZ[3], C. J. REYES-SEPÚLVEDA[3], D. SIERRA-MERCADO[1];

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Abstract: Each year 40 million people worldwide suffer from brain injury, and the most common type is concussion. Sustaining a concussion often leads to emotional deficits including fear-related disorders. Although human studies show a correlation between acquiring a concussion and enhanced fear, animal studies show conflicting results. Testing the effects of the injury too soon (one to two weeks post-injury) may be contributing to this conflict given that concussion-induced deficits worsen over time. We hypothesize that concussive-like injury will increase fear behaviors in rats 1 month post-injury. To test this hypothesis, we mimicked concussion in rodents using closed head injury (CHI) and sham injury. After 1 month, we examined fear behaviors using the elevated plus maze (EPM) and Pavlovian fear conditioning. In the EPM, time spent in the open arms was measured as an index of innate fear. In fear conditioning a tone was paired with a foot shock, followed by subsequent tests for memory. Here, freezing behavior was measured as an index of learned fear. Results showed no significant difference in time spent in open arms (Sham: 59s, n=8; CHI: 38s, n=8) or in percent freezing during conditioning (Sham: 76%, n=7; CHI: 53%, n=8). This suggest that emotional deficits induced by CHI may be detected at later time points where neurodegeneration takes place and behavioral deficits become more evident. Future studies will focus on examining longer time points and injury severity in the hopes of establishing how these contribute to anxiety behaviors following a concussion.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.20/K7

Topic: C.10. Brain Injury and Trauma

Title: Methylphenidate differentially alters risk-based decision-making in rats after TBI

Authors: *T. K. SHAVER, C. T. LAFFERTY, M. A. FRANKOT, J. E. OZGA-HESS, K. M. MARTENS, C. VONDER HAAR;
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Abstract: 2.8 million people in the United States suffer from traumatic brain injuries each year (Taylor, Bell, Breiding, & Xu, 2017). Brain injury often results in chronic, functional deficits that affect cognitive processes such as decision making and impulse control. Altered dopamine

signaling has been proposed to be a large driver of these cognitive deficits, however one of our previous studies found that a dopaminergic drug (methylphenidate) actually decreased optimal decision-making on the Rodent Gambling Task in both TBI and Sham groups. To better understand why this shift occurred, we continued investigating the effects of methylphenidate by assessing rats (N = 39) on the Risk Preference Task (RPT), a variation of the Rodent Gambling Task where rats chose among reinforcer-rate-matched options with varied probabilities of receiving reinforcement. The three options were 1 pellet (90% probability), 2 pellets (45% probability), or 3 pellets (30% probability). Motor impulsivity, or the inability to inhibit responses before trials was also concurrently assessed. Rats were given either a bilateral, frontal controlled cortical impact injury (AP/ML/DV: +3.0/0.0/-2.5 @ 3 m/s; n = 20) or sham procedures (n = 19) before training on the RPT. Half of rats were then treated daily with oral methylphenidate (5.0 mg/kg; n = 19) or saline (n = 20) fifteen minutes prior to behavioral assessment. TBI caused a significant increase in choice of the safest option. Methylphenidate caused differential effects on the medium and high risk options, with TBI-methylphenidate animals increasing choice of the medium-risk and sham-methylphenidate animals increasing choice of the high-risk choice. There was no significant difference in motor impulsivity between the various groups. These findings suggest that MPH may affect choice behavior through differential mechanisms in TBI versus intact animals. Further research will be needed to understand the mechanism by which these differences occur.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.21/K8

Topic: C.10. Brain Injury and Trauma

Title: Repeat closed-head injury selectively impairs attention in rats

Authors: *V. J. MILLESON[1], J. S. ADKINS[2], K. M. MARTENS[2], C. VONDER HAAR[2]; [2]Psychology, [1]West Virginia Univ., Morgantown, WV

Abstract: Traumatic brain injury (TBI) can result in significant cognitive and motor impairments, specifically deficits in attention and impulsivity. Notably, even mild TBIs can impair function, a problem that is compounded by multiple injuries. The current study investigated the effects of five closed-head injuries on impulsivity and attention in rats. Twenty-three Long-Evans rats were concurrently trained on two tasks, the five-choice serial reaction time task (5CSRT) and the delay discounting task (DDT). The 5CSRT measures motor impulsivity and attention, while the DDT measures choice impulsivity. Rats completed two

sessions daily, 5CSRT in the morning and DDT in the afternoon. Once trained to a stable baseline, injuries were delivered using the Closed Head Impact Model of Engineered Rotational Acceleration (CHIMERA) system, with a 100 g, 5-mm impact tip centered just in front of bregma, delivered at 8 m/s. Rats received TBI weekly (n = 14) for five weeks or sham procedures (n = 9). Testing continued for four weeks after the last injury was administered. Three deaths occurred as a result of the injuries. Attention was significantly impaired as a result of repeated injury, while choice and motor impulsivity were not affected by TBI. TBI rats also omitted more responses after injury. Rats showed no recovery during the four weeks of post-injury testing. These results stand in contrast to prior findings within our lab in which impulsive deficits were the largest change after injury. This suggests that the post-TBI phenotype may be more generalized than previously believed, particularly in light of the number of omitted responses. However, further study will be needed to understand why impulsive deficits emerge in some cases but not others.

Disclosures: V.J. Milleson: None.

Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

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Program #/Poster #: 392.22/K9

Topic: C.10. Brain Injury and Trauma

Support: NIHNS094950
NIH NS099683
Univ. Pitt. Rehab. Institute

Title: The effect of experimental brain trauma on sustained and flexible attention in female rats

Authors: *A. IOUCHMANOV, L. KUTASH, T. CRAINE, C. SUNLEAF, J. CHENG, A. KLINE, C. BONDI;
Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Impaired attention is central to the cognitive deficits associated with long-term sequelae for many traumatic brain injury (TBI) survivors. We previously demonstrated, for the first time, higher-order attentional impairments after moderate TBI, by using two frontal cortex-dependent ‘attentional tasks’, the operant 3-choice serial reaction time task (3-CSRT) and the digging attentional set-shifting (dAST) paradigms in male rats. Given that over 40% of TBIs occur in female patients, we hypothesized that adult female rats subjected to TBI will also exhibit attentional deficits seen as reduced accuracy and increased impulsivity. The 3-CSRT, a modified version of the five-choice serial reaction time task, requires the animal to orient toward and divide attention between three nose poke holes in an operant chamber, in which brief (300

ms) cues are randomly presented, to obtain a sucrose pellet reward. Upon reaching baseline of greater than 70% accuracy, which takes approximately 4-5 months using incrementally decreasing cue durations, adult female normal-cycling Sprague-Dawley rats were subjected to a controlled cortical impact (2.8 mm cortical tissue deformation depth at 4 m/sec) or sham injury over the parietal cortex in the right hemisphere. After two weeks of recovery, they were re-tested on 3-CSRT for ten days and then trained and tested on dAST, which involves a series of increasingly difficult stages, including simple and compound discriminations, stimulus reversals, and intra/extradimensional set-shifts, at approximately 28-29 days post-surgery. Dependent measures for the attention tasks include accuracy, omissions, trials to reach criterion, as well as total and perseverative errors. Results suggest that TBI females display reduced percent accuracy, particularly when the cue was presented on the Left side, suggesting a rodent analog of spatial hemi-neglect. Females alone exhibited partial recovery of function after ten days of re-testing, paralleling visual scanning therapy in the clinic. They also displayed increased omissions on 3-CSRT compared to Sham rats (n=3/group), which was similar in males. Ongoing analyses include dAST performance (total trials and errors), as well as within-subject correlations between attention test modalities. Statistical analyses will employ repeated-measures ANOVA followed by Newman-Keuls post hoc for individual test days when appropriate. Assessing attention post-TBI via multimodal testing is clinically-relevant and may provide reliable avenues towards developing therapeutic and rehabilitation targets in both males and females.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

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Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01 HD075760-01
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Title: Environmental enrichment enhances spatial learning in a pediatric model of asphyxial cardiac arrest

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Resuscitation Res., [4]Critical Care Med., [5]Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; [6]Univ. of Bath, Somerset, United Kingdom

Abstract: Every year, approximately 16,000 children suffer from a cardiac arrest (CA), with asphyxia accounting for most pediatric insults. After successful resuscitation from CA, hypoxic ischemic brain injury manifests as cognitive dysfunction and is present in most patients. Multiple studies have ascertained the role of environmental enrichment (EE) in reducing cognitive dysfunction after traumatic brain injury. To date, no studies have evaluated the effect of EE after CA. Hence, we sought to analyze the efficacy of EE in a pediatric model of asphyxia CA (ACA). It was hypothesized that EE would attenuate ACA-induced cognitive deficits in pediatric rats. Post-natal day16-18 rat pups received either a 9.5-minute ACA or sham injury. The rats were immediately placed in either standard housing (STD, 6-8 pups with the mother for 3 days, then weaned to single caging) or EE (6-8 pups with the mother for 3 days, then the mother was removed, and the pups remained). Four groups were studied: sham-STD, sham-EE, ACA-STD, and ACA-EE, n=8-10/group. Behavioral assessments included beam balance, days 1-5, novel object recognition (NOR) and open field, days 5-6, and Morris water maze, days 6-14. Both the ACA-STD and ACA-EE groups performed worse on the beam-balance task vs. sham controls ($p<0.05$) but did not differ from one another ($p>0.05$). Open field behavior did not differ among any of the groups ($p>0.05$), but there was a trend for the ACA-EE to perform better than the ACA-STD ($p = 0.06$). Spatial learning was improved in the ACA rats receiving EE vs. STD housing. ($p<0.05$). The EE-mediated improvement in the acquisition of spatial learning suggests that EE promotes beneficial effects after pediatric ACA and supports the hypothesis. The study supports the plethora of evidence showing that EE confers robust cognitive benefits after CNS injury and further validates it as a preclinical model of neurorehabilitation.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

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Program #/Poster #: 392.24/K11

Topic: C.10. Brain Injury and Trauma

Support: NIHNS094950
NIH NS099683
Univ. Pitt. Rehab. Institute

Title: Instrumental learning and behavioral flexibility in adolescent rats following pediatric brain trauma

Authors: *C. SUNLEAF, K. GROBENGIESER, A. TUROCY, A. PATEL, S. SHA, L. KUTASH, J. CHENG, A. KLINE, C. BONDI;
Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: With 500,000 yearly emergency room visits being attributed to childhood-acquired brain trauma (under 14 years of age), patients endure long-lasting cognitive, physical, or behavioral effects. Thus, it is vital to consider the effect brain trauma has on executive processes, such as learning, motivation, and behavioral flexibility, as one transitions from childhood to adolescence. We hypothesized that rats subjected to pediatric traumatic brain injury (TBI) will display task-dependent impairments in instrumental learning task (ILT) behavior and behavioral flexibility during adolescence. We employed a multimodal behavioral approach after moderate parietal lobe (2.2 mm tissue deformation depth at 4 m/sec) controlled cortical impact or sham injury to the right hemisphere in pediatric Sprague-Dawley male rats (postnatal day, PND 17). After ten days of recovery, they were trained on a fixed-ratio schedule of 1 for 12 consecutive days in operant chambers fitted with three nose-poke holes and a food trough, by learning to poke for sucrose pellet reinforcement in the center when illuminated. Outcome measures included the number of total trials completed, task-irrelevant pokes (left or right), and latency for pellet retrieval following instrumental nose-poking. Rats were then trained/tested on the attentional set-shifting test (AST) at PND 42-43, which involves simple and compound discriminations, stimulus reversals, and intra/extradimensional set-shifts. Dependent measures included the number of trials to reach criterion, as well as total and perseverative errors. Statistical analyses employed repeated-measures ANOVA followed by Newman-Keuls post hoc for individual test days when appropriate. ILT data (n=8/group) paradoxically demonstrate increased total trial rates and reduced task-irrelevant pokes, suggesting attenuated exploratory drive but also reduced impulsivity in adolescent rats after pediatric TBI ($p<0.05$). These effects were not due to motivation changes, as latency from poke to pellet was in fact shorter in TBI rats. Moreover, no group differences were detected in AST, possibly a result of higher baseline behavior in Sham adolescent rats, as previously reported. In summary, adolescent instrumental learning is affected by pediatric TBI in a divergent manner, reflecting reduced exploratory drive without affecting motivation. Executive function was not impaired, albeit adolescent rats naturally display enhanced cognitive rigidity compared to adults. These findings will advance our understanding of long-term higher-order cognitive and motivational deficits in adolescent survivors of childhood brain trauma.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

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Topic: C.10. Brain Injury and Trauma

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University of Pittsburgh Physicians /UPMC Academic Foundation, and the UMPC Rehabilitation Institute (COB)

Title: Amantadine as a bridge-therapy prior to neurorehabilitation after experimental traumatic brain injury

Authors: *K. GRIMM, C. LOMAHAN, D. BROOKS, I. BLEIMEISTER, M. HELKOWSKI, C. O. BONDI, A. E. KLINE;
Physical Med. & Rehabil. and Safar Ctr. for Resuscitation Res., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Traumatic brain injury (TBI) is a significant health care issue with limited treatment options. Environmental enrichment (EE), which consists of a complex living space and is vastly different from standard (STD) housing, is considered a preclinical model of neurorehabilitation because it improves cognition after experimental TBI. However, initiating EE immediately after TBI does not parallel the real world where patients will likely not receive rehabilitation until after critical care. Yet, treating TBI early is important for recovery. Hence, the goal of the current study was to evaluate amantadine (AMT) as a bridge therapy between critical care and rehabilitation. It was *hypothesized that AMT followed by EE would confer greater motor and cognitive recovery after TBI than either treatment alone.* Anesthetized adult male rats received a cortical impact of moderate severity (2.8 mm tissue deformation at 4 m/s) or sham injury and then were randomly assigned to enriched or STD housing where AMT (10 mg/kg) or vehicle (VEH; 1 mL/kg) were administered intraperitoneally beginning 24-hr after injury and daily for 7 days (AMT bridge) or every day for 26 days (daily treatments). Motor function (beam-balance/beam-walk) and spatial learning/memory (Morris water maze) were assessed on post-operative days 8-12 and 21-26, respectively. All TBI groups receiving EE performed significantly better than the AMT and VEH treated STD-housed groups on motor and cognition ($p<0.05$). No other comparisons were significant ($p>0.05$). The lack of an additive effect with AMT and EE does not support the hypothesis. Future studies should include abbreviated EE as neurorehabilitation as continuous EE may be too robust to afford additional benefits.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.26/K13

Topic: C.10. Brain Injury and Trauma

Support: George Mason University Psychology Department

Title: Intranasal zinc remediates behavioral deficits following stress and traumatic brain injury

Authors: *E. N. DOHERTY[1], W. R. KOCHEN[1], T. T. DIMOPOULOS[1], D. D. CERRI[1], J. M. FLINN[2];
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Abstract: Mild traumatic brain injury (mTBI) has become the "signature wound" of the military population in recent combat operations. Repeated mTBI (rmTBI) produce long-term cognitive and behavioral deficits, which can be exacerbated by the high-stress environment experienced by service members. This study employed a mouse model to examine the effects of chronic variable stress (CVS) on rmTBI, and how these may be ameliorated by intranasal zinc. Six-week old mice received two varied stressors (e.g., food deprivation, physical restraint, ice bath, predator urine) each day for seven days. A second week of CVS was administered concurrently with four closed-head mTBIs. Each injury was immediately followed by either intranasal zinc treatment or a vehicle control (water). A subset of mice was removed for analysis of acute protein changes in the brain and alterations in ionic zinc. The remaining mice were subjected to behavioral testing that assessed a) spatial memory via Morris water maze (MWM) and b) circadian rhythm via wheel-running activity. A significant interaction between stress and zinc was found in MWM testing; post-injury zinc treatment led to a significant *increase*, $p = .047$, in platform crossings for stressed mice when compared to the non-stressed controls. Circadian rhythm testing measured 24-hour performance and yielded a similar interaction. Stressed mice given vehicle treatment showed a significant deficit in activity at the beginning of the dark cycle, but when zinc was administered, the deficit was corrected and activity for stressed mice nearly doubled. These findings suggest that zinc had a therapeutic effect on behavioral deficits associated with rmTBI, with chronic stress increasing zinc efficacy. Western blots are currently being performed to investigate the biological mechanism underlying this interaction between stress and zinc on rmTBI.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

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Program #/Poster #: 392.27/K14

Topic: C.10. Brain Injury and Trauma

Support: USAMRMC / MOMRP Grant, Project #: 19250

Title: Omega-3 fatty acid deficiency increases neurological impairments in a rat model of combined blast-traumatic brain injury and traumatic stress

Authors: *J. C. DEMAR, J. G. ROSENBERGER, A. B. BATUURE, R. T. URIOSTE, O. D. EKEN, L. ZHILIN, D. M. WILDER, J. B. LONG;
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Abstract: BACKGROUND: US Soldiers suffer a high incidence of traumatic brain injuries from bomb explosions (blast-TBI). There is a high co-morbidity of blast-TBI with post-traumatic stress disorder (PTSD) development. Dietary long chain omega-3 polyunsaturated fatty acids (omega-3s) are building blocks of neuronal cell membranes and are converted to anti-inflammatory metabolites. Omega-3 deficiency is a risk factor for cognitive and psychiatric disorders. Providing dietary omega-3s improves outcomes in many models of brain trauma. Thus, we explored if an omega-3 poor diet increases vulnerability to blast-TBI and PTSD, and if combining these insults has an additive deleterious effect. **METHODS**: Adult male rats (n = 24 / group) were maintained for 6 weeks on diets devoid of omega-3s and increased in pro-inflammatory omega-6 content. Anesthetized animals were then exposed once to a blast over pressure wave (18 psi) in an advanced blast simulator, followed immediately by a weight drop skull concussion (500 g from 125 cm), to induce TBI symptoms. After 3 days of recovery, the rats were subjected once to a traumatic stressor, i.e., forced immersion underwater (30 sec), to induce “PTSD” behaviors. Shams were subjected to anesthesia, recovery, and free swimming. Diets were continued and the animal’s neurobehavioral function was evaluated out to 28 days, using rotarod, rotary pole, elevated plus maze, Y-maze, and open field tests. **RESULTS**: Our results showed that blast-TBI plus traumatic stress leads to minor increases in behavioral impairments, compared to each insult alone, e.g. coordination, “anxiety”, and hyperactivity disturbances. Omega-3 deprivation, however, markedly perturbed behaviors of both shams and injured rats to a similar degree. **CONCLUSIONS:** Our findings, in showing little impact on behavior of combining the brain insults, may indicate that their underlying pathophysiological processes can mask each other. Nevertheless, omega-3 deficiency caused a worsening of the outcomes. Future studies will examine the neuronal cell injury status of the animal’s brains, e.g. histopathology and cytokine assays. **DISCLAIMER:** Research was conducted under an institutionally approved animal care and use protocol in compliance with the Animal Welfare Act, and all other Federal requirements. Opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.28/K15

Topic: C.10. Brain Injury and Trauma

Support: NIH grants HD069620, HD069620-S1, NS060005, NS084967 (AEK), NS094950, NS099683 (COB)
University of Pittsburgh Physicians /UPMC Academic Foundation, and the UMPC Rehabilitation Institute (COB)

Title: Long-term cognitive benefits from abbreviated environmental enrichment after cortical impact injury

Authors: *C. LOMAHAN[1], K. GRIMM[2], M. ALVAREZ[2], M. WOLFF[2], H. RADABAUGH[2], C. O. BONDI[2], A. E. KLINE[2];
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Abstract: It is well established that a relatively brief exposure to environmental enrichment (EE) enhances motor and cognitive performance after experimental traumatic brain injury (TBI). It has also been shown that the EE-induced benefits can be sustained for up to six months. What is not known is whether rats that received EE and then transferred to standard (STD) housing would benefit further from "refresher rehabilitation." To address this rehabilitation-relevant concern, anesthetized rats received a cortical impact or sham injury, and for phase 1 of the experiment were randomly assigned to either 3 weeks of EE (6 hr/day) or STD housing. Neurobehavioral outcome was assessed by established motor and cognitive tests on postoperative days 1-5 and 14-18, respectively. Motor, spatial learning, and memory retention were facilitated in TBI+EE vs. TBI+STD ($p<0.05$). In phase 2, half of the EE rats were transferred to STD (TBI+EE+STD) and half of the STD went to EE (TBI+STD+EE). Cognition was re-assessed once per month for 6 months. TBI+EE performed better vs. TBI+STD continuous and TBI+STD+EE ($p<0.05$). Also, TBI+EE+STD performed better than TBI+STD+EE ($p<0.05$). In phase 3, the TBI+EE+STD group was provided an additional week of EE (i.e., refresher). The data showed continued improvement in the refresher rehabilitation group vs. the TBI+STD+EE group ($p<0.05$). These data replicate those of several studies from our laboratory showing that EE enhances recovery after CCI injury and extend those findings by demonstrating that the cognitive benefits are maintained for at least 6 months post-rehabilitation with abbreviated EE and that providing "refresher" EE further improves benefits. The persistent benefits shown with this paradigm provide further support for EE as a pre-clinical model of neurorehabilitation.

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Poster

392. Blast Injury, Traumatic Brain Injury, Stress, and PTSD

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 392.29/K16

Topic: C.10. Brain Injury and Trauma

Title: Inhibition of microglia using chemogenetics reduced proinflammatory cytokine levels after traumatic brain injury

Authors: *K. M. PECHACEK, K. M. MARTENS, B. ZHU, C. VONDER HAAR;
West Virginia Univ., Morgantown, WV

Abstract: Traumatic brain injury (TBI) often results in chronic neuroinflammation and is associated with cognitive deficits and psychiatric disorders. One of the major mediators of this process are microglia cells through the production of proinflammatory cytokines. The current study examined the effects of chemogenetic microglia inhibition on the neuroinflammatory response after TBI. Rats (N = 36) first underwent stereotaxic surgery to deliver the virus containing the Designer Receptor Exclusively Activated by Designer Drug (DREADD) construct pAAV CD68-hm4D-(Gi) to the four sites surrounding what would become the injury periphery (AP: -0.9/-3.9, ML:-1.0/-4.0, DV: -1.4). After allowing three weeks for protein expression, animals were trained for two sessions on the accelerating rotarod task, a measure of motor coordination. Rats then received a unilateral controlled cortical impact to the location of DREADD infusion (AP: -2.4, ML: -2.4, DV: -2.5) or sham procedures (craniotomy only). Subgroups were then given the DREADD-activating agent, clozapine N-oxide, starting at time points of 0, 4, 8, or 24 h after injury and every six hours thereafter. At 72 h post-injury, animals were re-tested on the rotarod and then sacrificed immediately after. Brain tissue was collected for multiplex ELISA of cytokine levels. Performance on rotarod revealed an effect of TBI that was not recovered by the DREADD in any groups. TBI increased proinflammatory cytokines IL-1β and IFN-γ but this response was attenuated in the perilesion by the DREADD at time points 0 and 4 h. The DREADD had no effect on inflammatory cytokines IL-6, TNF-α, and IL-10. Inhibition of microglia after unilateral TBI affected the proinflammatory response in the perilesion but yielded no functional improvements. Future work should consider the time sensitive nature of microglia inhibition as they development treatments for TBI.

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Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.01/K17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: PVA Grant PVA17_RF_0008

Title: Bioactive scaffolds of mobile molecules to repair the injured spinal cord

Authors: *Z. ALVAREZ PINTO, A. N. EDELBROCK, J. ORTEGA, I. R. SASSELLI, E. KISKINIS, S. I. STUPP;
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Abstract: Developing reliable therapeutic methods to treat spinal cord injuries (SCI) has been a major challenge due to the complex and dynamic cellular microenvironment during the disease progression. While several current therapeutic approaches have aimed to restore neural signaling, reduce neuroinflammation and prevent subsequent damage to the injured area, to date, there is no single biological intervention that can address all of the physiological events that damage the spinal cord. Self-assembling supramolecular systems have been identified as an important class of biomaterials given their ability to generate dynamic structures. Peptide amphiphiles (PAs) that self-assemble in water into high aspect ratio nanofibers and form hydrogel networks in the presence of physiological electrolytes have emerged as attractive supramolecular candidates for regenerative medicine. Inspired by the highly mobile environment at cell-extracellular matrix (ECM) interfaces, we investigate here supramolecular scaffolds formed by molecules with identical chemical bioactivity but drastically different mobility within the supramolecular fibers they form and their effect on neural regeneration, vascularization and glial scar formation. This was accomplished by designing and testing fibroblast growth factor 2 (FGF-2) mimetic molecules that self-assemble into nanostructures as an angiogenic and neurogenic biomaterial platform for treating SCI. The growth factor mimetic PA co-assembled with a known PA that presents the neuroactive pentapeptide epitope from laminin alpha-1, IKVAV, promoted vascularization, nerve regeneration, functional recovery, and limit the glial scar formation. Overall, our PA technology highlights the importance of the ECM in recapitulating in vivo conditions and offers a more physiological and translational platform to study the dysfunction of the CNS after injury.

Disclosures: **Z. Alvarez Pinto:** A. Employment/Salary (full or part-time):; Paralyzed Veterans of America Foundation. **A.N. Edelbrock:** None. **J. Ortega:** None. **I.R. Sasselli:** None. **S.I. Stupp:** None. **E. kiskinis:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.02/K18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NS088095
NS092616
NS099073
Welch I-1724

Title: Enhancing endogenous reprogramming for neurogenesis after spinal cord injury

Authors: *W. TAI, L.-L. WANG, H. NI, C.-L. ZHANG;
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Abstract: Spinal cord injury (SCI) induces pathological changes that frequently lead to functional impairments. Whereas endogenous neurogenesis is associated with innate cognitive recovery following brain injury, it is still unclear whether SCI induces neurogenesis. In the present study, we find that doublecortin-positive (DCX+) cells could be robustly detected surrounding the injury sites 7 days after a diverse SCI models including contusion, crush, hemi-section, and stab-wound injury. These cells co-express markers for NG2 glia but not astrocytes. However, the number of DCX+ cells declines rapidly with time and very few becomes neurons. Since our mouse genetics showed that SOX2 is essential for DCX-induction in NG2 glia, we then examined whether ectopic SOX2 in these cells could enhance their fate reprogramming. Indeed, ectopic SOX2 robustly induces DCX+ cells from NG2 glia. These NG2-derived DCX+ cells pass through an ASCL1+ progenitor stage and can become mature neurons. Rabies virus-mediated synaptic mapping showed that these NG2-derived neurons can integrate into the existing neural network. Together, our results indicate that SCI induces partial reprogramming of NG2 glia and ectopic SOX2 can enhance such reprogramming towards neurogenesis.

Disclosures: W. Tai: None. **L. Wang:** None. **H. Ni:** None. **C. Zhang:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.03/K19

Topic: C.11. Spinal Cord Injury and Plasticity

Support: FAPESP: 2015/26206-0
FAPESP: 2014/06892-3
Cnpq - 300552/2013-9

Title: Sensorimotor improvement following dorsal root repair with PRP and human embryonic stem cells

Authors: *M. V. DE CASTRO[1], M. SILVA[1], G. CHIAROTTO[1], S. KYRYLENKO[2], M. H. SANTANA[1], A. LUZO[1], A. L. OLIVEIRA[1];
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Abstract: Spinal dorsal roots can be affected by lesions to the brachial and lumbosacral plexuses, leading to loss of proprioceptive inputs to motoneurons, greatly hampering motor coordination. The loss of afferents greatly affects spinal circuits, and to date, there is no effective therapy to overcome such deficits. Reimplantation of roots, combined with stem cells therapy, have been proposed to mitigate the degenerative effects of dorsal rhizotomy. In this sense, the present study evaluated the motor and sensory improvement following dorsal root reimplantation with platelet-rich plasma (PRP) after dorsal rhizotomy. We also combined such scaffold with modified human embryonic stem cells (hESCs) overexpressing fibroblast growth factor 2 (FGF-2). hESC were used to enhance the repair process. For that, female adult Lewis rats (LEW/HsdUnib; n=5 per group) were subjected to unilateral dorsal rhizotomy (DRZ) of the L4-L6 roots and divided into the following groups: (1) Unlesioned/Control; (2) DRZ without reimplantation (DRZ); (3) DRZ followed by root reimplantation with PRP (DRZ+PRP); (4) RZ followed by root reimplantation with PRP associated with modified human embryonic stem cells (RZ+PRP+hESC). PRP was obtained from human blood subjected to centrifugation steps. It was characterized regarding platelet concentration, integrity, and viability. For cell therapy, hESCs were bioengineered to overexpress a human fibroblast growth factor 2 (FGF-2). qRT-PCR was used to assess gene expression of TNFα, TGFb, IL4, IL6 and IL13 in the spinal cord, and FGF2, BDNF, GDNF, VEGFA and IGF mRNA levels in hESC *in vitro*. We also evaluated glutamatergic pre-synaptic terminals (VGLUT1) and glial reaction (GFAP and Iba-1) immunolabeling in the spinal cord, eight weeks post-lesion. The results indicate that the combination of hESC overexpressing FGF2 with PRP induces regeneration of afferent fibers after dorsal rhizotomy, restoring the paw withdrawal reflex, significantly enhancing VGLUT1 immunoreactivity in deeper spinal cord laminae. Also, PRP+hESC therapy does not exacerbate glial reactivity. We hypothesize that local hESC+PRP immunomodulation at the site of injury overcomes the effects of inhibitory molecules that hamper the growth of regenerating primary afferent fibers within the spinal cord. Overall, the present data suggest that repair of dorsal roots with PRP application combined with cell therapy is efficient and may be considered as an approach to improve sensory-motor recovery following dorsal rhizotomy.

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Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.04/K20

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Modulation of microenvironment by iron oxide nanoparticles embedded in gelatin-genipin hydrogel along with magnetic field stimulation in contused spinal cord injury rats

Authors: *S. B. BHATTACHARYYA[1], S. JAIN[2], A. K. DINDA[3];
[2]Physiol., [3]Pathology, [1]AIIMS, New Delhi, New Delhi, India

Abstract: Traumatic spinal cord injury (SCI) is a great challenge for therapeutic management considering its high morbidity. It initiates cascade of events that creates an inhibitory milieu for axonal growth and repair. Thus to modulate this microenvironment there is a need to develop a biocompatible growth enhancing material which can fill the cysts, bridge the gap and act as conduit for axonal guidance. To formulate an effective therapeutic strategy with translational potential and understand its mechanism, we fabricated gelatin-genipin hydrogel (GGH) system which was impregnated with IONPs and injected at the lesion site in a clinically relevant contusion rat model of SCI. The rats were concomitantly exposed to electromagnetic fields 2h/day for 5 weeks. A significant improvement in behavioral, electrophysiological and morphological parameters was observed which was due to alteration in neurotrophin levels and reduction in activated microglia. The intervention thereby made the microenvironment conducive and facilitated neural repair and regeneration.

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Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.05/K21

Topic: C.11. Spinal Cord Injury and Plasticity

Title: A comparative study of human-derived UCMSC, ADMSC and iPSC-induced progenitor transplants in the treatment of spinal cord injury

Authors: *A. LIU, P. YU, L. SHI, K.-F. SO, L. ZHOU;
Jinan Univ., Guangzhou, China

Abstract: Spinal cord injury (SCI) is still a worldwide problem, and recent studies show stem cells transplantation provides a promising treatment in animal experiments and clinic trials. However, which type of stem cells should be considered for use remains unknown. In this study, we prepared human umbilical cord-derived mesenchymal stem cells (UCMSCs), human adipose-derived mesenchymal stem cells (ADMSCs) and human iPSC-induced neural progenitor cells (NPCs), and carried out cell transplantation 1 week after SCI using a T10 moderate contusion rat model. Most transplanted UCMSCs and ADMSCs survived up to 4 weeks and failed to further differentiate into neurons or glial cells, in contrast, about 30% transplanted NPCs could survive for 4 weeks and differentiate into a few neurons (7.9%) and astrocytes (1.8%). Both UCMSC and ADMSC transplants significantly improved motor and sensory function assessed by BBB scores, CatWalk tests, footslip tests, motor-evoked potential and sensation tests. However, NPC transplants were much less efficient for functional recovery. After SCI, UCMSC and ADMSC transplants indiscriminatingly contributed to spinal neuron survival and axonal regeneration, decreased the glial scar formation, lesion cavity and the number of active macrophages. However, NPC transplants displayed much less effect on improving spinal micro-environment after SCI compared to UCMSC and ADMSC transplants. Bioplex analysis with Spinal samples 3 and 7 days after cell transplantation showed IL-10, IL-13 were significantly increased and TNF-α were significantly decreased in the UCMSC and ADMSC groups. NPC transplants induced the decrease of MCP-1, IL-2 and IFN-γ. Seven days after cell transplantation, iTRAQ proteomics analysis of spinal samples showed there were 101 common proteins changed in the UCMSC and the ADMSC, 117 changed proteins particularly in the UCMSC group.Many common changed proteins with more than 1.5 fold increase were benificial for axon growth, including Fez1, Tnpo1, Dclk2, Cnpy2, Stat3, Ctss, Sort1 and Yes. However, in the NPC group, most changed proteins were different from two other groups. In conclusions, UCMSC and ADMSC transplants similarly contributes to motor and sensory function recovery after SCI probably via anti-inflammation, improving axonal growth and neurotrophic effect; transplanted NPCs can differentiate into some neurons, which number of neurons do not significantly improve functional recovery; short survival time of transplanted UCMSC and ADMSC suggests multiple transplantations should be considered in the clinic.
Key words : Spinal cord injury ; human-derived stem cells ; Cell transplantation ; Proteomics

Disclosures: **A. Liu:** None. **P. Yu:** None. **L. Shi:** None. **K. So:** None. **L. Zhou:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

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Program #/Poster #: 393.06/K22

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Efficacy of feeder-free hiPSC-OPC-enriched NS/PCS for transplantation in the subacute phase of spinal cord injury

Authors: *Y. KAMATA[1,2], M. ISODA[3], N. NAGOSHI[1], S. ITO[1], M. SHINOZAKI[2], O. TSUJI[1], M. MATSUMOTO[1], H. OKANO[2], J. KOHYAMA[2], M. NAKAMURA[1];
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Abstract: [Introduction]Cell-based therapy targeting spinal cord injury (SCI) is an attractive approach to promote functional recovery by replacing damaged tissue. We have previously shown effectiveness of transplantation of human-induced pluripotent stem cell-derived oligodendrocyte precursor cell-enriched neural stem/progenitor cells (hiPSC-OPC-enriched-NS/PCs) in SCI animal model, regarding robust remyelination to the demyelinated axons and motor functional recovery. For clinical application, the NS/PCs should be generated from feeder-free iPS cells cultured under xeno-free conditions. Here, the aim of this study is to examine therapeutic efficacy and safety of feeder-free hiPSC-OPC-enriched-NS/PCs (ff-hiPSC-OPC-enriched-NS/PCs).[Method]OPC-enriched NS/PCs were generated from feeder-free iPSCs. Contusive SCI was induced at the Th10 level in adult female NOD-SCID mice, and the ff-hiPSC-OPC-enriched NS/PCs were transplanted into the lesion epicenter 9 days after the injury. An equal volume of phosphate buffered saline was injected for the control groups. Motor function of injured mice was assessed by the Basso Mouse Scale score, Rota-rod test and DigiGait analysis. Histological analyses were performed to examine the survival and differentiation of the grafted cells.[Results]Histological analyses revealed that the transplanted cells well survived and migrated far into the host spinal cord without any tumor formation. The transplanted cells differentiated into three neural lineages including neurons, astrocytes and oligodendrocytes. In particular, the differentiation rate of oligodendrocytes evaluated by expression of APC was 36.6±2.8%. The myelinated positive area of the spinal cord was evaluated by Luxol fast blue staining at cross section, and it was significantly larger from the epicenter to +1.0 mm rostral and caudal sites in the transplanted group rather than in the vehicle control group. Furthermore, the active remyelination on damaged axon in injured spinal cord was observed in the transplanted group by immunoelectron microscopy. Importantly, hindlimb motor function was significantly improved in the transplanted group one week following transplantation and thereafter.In addition, both Digigait analysis and rotarod tests revealed a significant motor functional improvement in the transplanted group at 12 weeks after transplantation.[Conclusion]Feeder free-hiPS-OPC-enriched NS/PCs showed therapeutic potency similar to the conventional ones. These cells would be potential sources of therapeutic application for SCI patients in the clinical settings.

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Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.07/K23

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Electronics and Telecommunications Research Institute
NIH R01NS099872
Center for Neurotechnology (NSF EEC-1028725)

Title: A fully implantable, wireless neuroprosthetic device for delivery of stable and robust EMG-triggered spinal stimulation in rodents

Authors: *S. LEE[1,2], W. YOUM[2], K. MOON[3], R. L. MURPHY[1], R. ROBINSON[1], E. E. FETZ[1], S. I. PERLMUTTER[1];
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Abstract: Activity-dependent spinal stimulation has shown promise as a therapy following spinal cord injury (SCI). To improve volitional control of reaching and grasping movements in rats with cervical SCI, we facilitate spike-timing dependent plasticity (STDP) in descending pathways by triggering spinal stimulation on single motor unit action potentials from muscles of the impaired forelimb. To deliver this therapy more effectively, we have developed a small, fully implantable, wireless device that delivers robust activity-dependent stimulation with minimal stimulus artifacts, which can interfere with closed-loop performance. The device uses an Intan Technologies chip for recording, an ARM Cortex-M4 microprocessor for signal processing, and a custom-designed stimulation circuit. In this study, the device was implanted subcutaneously in injured rats and connected to electrodes implanted in 3 forelimb muscles and in spinal segments C6-C8. Electromyographic activity (EMG) was recorded differentially, sampled at 6.5 kHz, and transmitted wirelessly to a nearby laptop computer. The signals had low noise and high signal-to-noise ratios. Parameters to identify single motor unit action potentials with a dual time-amplitude window discriminator were determined offline and uploaded wirelessly to the implanted device. Motor unit action potentials from each muscle triggered delivery of a subthreshold, biphasic, electrical pulse to an intraspinal microwire from which contraction of that muscle could be evoked with higher currents. The delay between generation of a trigger and delivery of a stimulus was ~1.2 msec, well within the required window for STDP. Stimulus pulses of 150 usec width can be adjusted in 256 steps from -18 V to 18 V depending on therapy conditions. Recorded stimulus artifacts were brief and small in all EMG channels due to: a) a switching circuit that reduces the amplifier input gain during stimulation; b) reduction of charge build-up

on parasitic capacitances due to shielding of the system by the animal's body; c) low impedance EMG electrodes. These results demonstrate robust performance of a novel, fully implantable neuroprosthetic device that can deliver closed-loop electrical stimulation with dual time-amplitude window discrimination and wireless communication. The device enables implementation of real-time, activity-dependent stimulation for treatment of SCI in freely moving, untethered animals.

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Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

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Program #/Poster #: 393.08/K24

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation SCIRTS-296387

Title: Significant heterogeneity in neural stem cell populations for transplantation

Authors: T. N. G. ADAMS, S. TIWARI, C. RO, H. X. NGUYEN, R. NISHI, D. L. HAUS, B. J. CUMMINGS, A. J. ANDERSON, ***L. A. FLANAGAN**;
Neurol., Univ. of California, Irvine, Irvine, CA

Abstract: As clinical trials of human neural stem and progenitor cells (HuNSPCs) develop for neurological applications such as spinal cord injury (SCI), it will be imperative to ensure equivalent potency of each line of cells prior to transplantation. We hypothesized that multimodal profiling of HuNSPCs prior to transplant will enable quantitative detection of population composition and prediction of cell potency. We measured the following parameters: cell size, whole cell electrophysiological properties, cell proliferation, and marker expression. We discovered significant heterogeneity in HuNSPCs expanded for SCI transplant in all measures of the multimodal profile, creating problems for ensuring transplant reproducibility. We sorted HuNSPCs to reduce heterogeneity using markers (CD133+, CD34-). Sorted HuNSPCs differed from unsorted cells in cell size, electrophysiological properties, and proliferative ability, which is critical for cell expansion prior to transplant. Sorted cells also significantly differed from unsorted controls in expression of cell surface markers, which can indicate cell phenotype and predict ability of cells to interact with molecules in the transplantation niche. Thus, multimodal profiling identified several significant quantifiable differences between control and sorted HuNSPCs prior to transplantation.
Despite differences in the sorted and unsorted cells, randomized, blinded assessment of locomotor recovery using ladder beam showed that although animals receiving the sorted cells

had slightly higher performance values there was not significant improvement over controls. However, animals treated with sorted cells showed reduced heterogeneity in functional recovery, showing a tighter range of responses than those of animals treated with unsorted cells or vehicle controls. Importantly, animals treated with the sorted cells did not show evidence of increased allodynia, suggesting that enriching proliferative cells for transplant did not cause harm. Future studies of HuNSPCs that elicit a robust increase in locomotor recovery post-transplant will help to identify which quantifiable cellular characteristics most closely associate with the ability of HuNSPCs to repair the CNS.

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Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

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Program #/Poster #: 393.09/K25

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Department of Defense (W81XWH-18-1-0245)
Department of Veteran Affairs (I01BX007080)

Title: The relationship between frequency of acute intermittent hypoxia treatment, spinal plasticity, and functional improvements in chronic cervical spinal cord injury

Authors: *A. E. HAGGERTY[1], N. DE LA OLIVA[2], M. OUDEGA[3];
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Abstract: Spinal cord injury (SCI) results in nervous tissue loss and functional impairments. Acute intermittent hypoxia (AIH), a relative non-invasive exposure to cycles of normal and hypoxic oxygen levels, has been investigated as a potential therapy for spinal cord injury in both animal and human studies. It was shown that AIH treatment result in partial functional recovery in adult rats and in humans with SCI, especially when paired with exercise training. Currently, the mechanisms underlying these effects are largely unknown. One possibility is AIH leads to increased presence of growth factors that mediate axon sprouting in spared circuitries and/or damaged axons leading to the strengthening and/or formation of synaptic connections. The dose of AIH can be measured in terms of both frequency and duration. The previously defined “safe” range of hypoxia dose, i.e. one that has been shown to not elicit negative physiological consequences, is fairly wide (Navarrete-Opazo and Mitchell, 2014). Optimizing dose within this

“safe” range to maximize synaptic plasticity and improve functional recovery after SCI has not previously been reported. Here, we evaluated a range of frequencies of hypoxia/normoxia-cycles paired with neuronal tracing techniques and clinically relevant functional outcome measures including reach/grasp and grip strength using an adult rat model of chronic contusive cervical spinal cord injury. Optimizing AIH in our model may lead to a better understanding of the mechanisms of AIH and inform clinical implementations of AIH in studies involving SCI patients. Preliminary data suggests that the dose of AIH is correlated with synaptic plasticity at and around the injury and that spared connections within the chronic SCI environment can be engaged in new circuitry for improvements in function.
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Disclosures: **A.E. Haggerty:** None. **N. De La Oliva:** None. **M. Oudega:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.10/K26

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Assessing the effects of KCC2 and CLP290 in a mouse contusion model

Authors: *M. HE, B. CHEN, J. TANG, B. BROMMER, J. ZHU, Z. HE;
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Abstract: After spinal cord injury (SCI), most patients have anatomically incomplete injuries with spared axons around the lesion. How to maximally utilize the spared axonal connections surviving after SCI presents a new direction for the treatment of SCI. Our previous data showed that reactivating KCC2, via its agonist CLP290 or viral strategy-based KCC2 over-expression is sufficient to revive the dormant circuitry rendered by injury and restores stepping ability in staggered bilateral hemi-section mice. In order to test the translational potential of KCC2 related treatments, we used contusion injury which could model the wide spectrum of SCI patients. Our preliminary data showed that after contusion injury, KCC2 over-expression by tail vein injected AAV-PHP.B vectors led to significant functional recovery according to the score on the Basso mouse scale (BMS). Furthermore, kinematic analysis indicated that KCC2 treatment mice had significantly increased quality of hindlimbs standing (body weight support) and stepping (step height and step frequency). We are performing additional kinematics, electrophysiology, and histological analysis in contused mice with the treatment of AAV-KCC2 or CLP290. In conclusion, our data will indicate if KCC2 related treatments could support functional recovery in a clinically relevant, spinal cord contusion model.

Disclosures: **M. He:** None. **B. Chen:** None. **J. Tang:** None. **B. Brommer:** None. **J. Zhu:** None. **Z. He:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.11/K27

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NRF-2017R1D1A1B03032356
NRF-2017R1A2B4010100

Title: Adult human multipotent neural cells exerts neuroprotective paracrine effects against oxidative stress-induced apoptosis of spinal cord neurons

Authors: ***C. KIM**[1], A. NAM[1], Y. PARK[2], K. JOO[1], S.-H. LEE[3], K.-H. LEE[1];
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Abstract: Neural stem cell (NSC) is a promising therapeutic approach for many neurodegenerative and neurological disorders including spinal cord injury (SCI). NSCs replace and reestablish neuronal cells in damaged brain areas. We recently showed that adult human multipotent neural cells (ahMNCs) have significant therapeutic efficacy in animals with SCI, which is partly mediated by trophic and pro-angiogenic paracrine effects. In this study, we further investigated cytokines of ahMNCs that attenuate apoptosis of spinal cord neurons (SCNs). The treatment of ahMNC-conditioned media (CM) significantly reduced H_2O_2-induced cell death of primarily cultured SCNs. ahMNC-CM treatment significantly reduced expression of pro-apoptotic genes such as cleaved caspase3 and BAX in SCN cells. These data identified cytokines of ahMNCs that have significant neuroprotective effects in SCN cells, which could be utilized to enhance the paracrine effects of stem cell therapeutics for neurodegenerative diseases.

Disclosures: **C. Kim:** None. **A. Nam:** None. **Y. Park:** None. **K. Joo:** None. **S. Lee:** None. **K. Lee:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

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Program #/Poster #: 393.12/K28

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NJ Commission on Spinal Cord Research grant No. CSCR18ERG007
JFK Neuroscience Institute support package

Title: GDF10 promotes axonal regeneration and functional recovery: A novel gene therapy strategy for spinal cord injury

Authors: *P. M. ABDUL-MUNEER, S. BHOWMICK, V. D'MELLO;
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Abstract: Spinal cord injury (SCI) occurs when there is damage from trauma, loss of normal blood supply, or a mass effect due to compression from tumor or infection. Unlike other parts of the body, the regenerative ability of the spinal cord is relatively poor. The inability of axons to regenerate after SCI is attributable to a combination of effects of the non-permissive extrinsic factors including myelin proteins and chondroitin sulfate proteoglycans (CSPGs), and cell-autonomous intrinsic factors including cAMP, RhoA, Krüppel-like factors, mammalian target of rapamycin (mTOR) and phosphatase and tensin homolog (PTEN). However, the factor(s) that may be triggered to promote the initiation of a molecular growth program and axonal sprouting in SCI are largely unknown. In this project, we developed a novel therapeutic approach to treat SCI by exploiting the neuronal growth-promoting potential of growth differentiation factor 10 (GDF10), a potential gene belongs to the transforming growth factor beta (TGF-β) superfamily. GDF10 regulates several molecular signaling systems to induce a neuronal growth state. Our focus on GDF10 as a therapeutic target after SCI is based on the observation that GDF10 regulates major axonal regenerative cues including PTEN, phosphoinositide 3-kinase (PI3K) and suppressor of cytokine signaling 3 (SOCS3). Thus, we hypothesize that up-regulation of GDF10 mitigates PTEN-mediated inhibition of axonal regeneration. We examined the specific effects of GDF10 on other major regulatory signaling cascades of axonal regeneration, the PI3K, and SOCS3 pathways *in vitro* and *in vivo*. In order to up-regulate GDF10 in experimental animals, we delivered GDF10 gene via adeno-associated virus into the sensory-motor cortical area of the brain and into the spinal cord rostral to the SCI lesion, and evaluate the subsequent progress of axonal regeneration and functional recovery after SCI. To validate the role of GDF10 in axonal regeneration, we used the CRISPR/Cas9 gene deletion technology to remove GDF10 gene. Findings from this project would help to clarify the specific role of GDF10 in axonal regeneration and functional recovery after SCI and establish a basis for pursuing GDF10 as a therapeutic strategy for spinal cord injured patients.

Disclosures: **P.M. Abdul-Muneer:** None. **S. Bhowmick:** None. **V. D'Mello:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

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Program #/Poster #: 393.13/K29

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS NS101298
Craig H. Neilsen Foundation 460461

Title: The effects of 6-aminonicotinamide (6-AN) treatment on migration of Schwann cells transplanted in the contused spinal cord in adult rats

Authors: *T. MIHARA[1], A. HAGGERTY[1], K. YAMANE[1], Y. PRESSMAN[1], S. CERQUEIRA[1], M. B. BUNGE[1,2], M. OUDEGA[1,2,3,4];
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Abstract: In different models of spinal cord injury (SCI), transplantation of Schwann cells (SCs) into the injury elicits anatomical repair, which often is accompanied by some improvements in functional outcomes. Currently, SC transplantation for spinal cord repair is being tested in a clinical trial. One of the hallmark effects of SC transplantation is the promotion of axon regeneration. However, the responding axons remain 'trapped' within the SC environment; they do not grow beyond the transplant into the adjacent spinal cord nervous tissue. This failure has been a longstanding hurdle for achieving larger repair effects by intraspinal SC transplants. It is thought that the astrocytic scar in the spinal cord adjacent to the SC transplant arrests the growth of the axons. Treatment of SCs and reactive astrocytes in a confrontation assay with 6-aminonicotinamide (6-AN), an antimetabolite that interferes in the pentose phosphate metabolic pathway, promotes mingling of SCs within the astrocytic milieu. Moreover, in this confrontation assay, axons from dorsal root sensory neurons seeded on the SCs were growing across the SC-astrocyte border with 6-AN treatment but not in controls. We argued that 6-AN treatment could promote transplanted SC-astrocyte mingling in the injured spinal cord, thereby facilitating axon growth beyond the SC transplant further into the spinal cord. We tested different 6-AN concentrations and different treatment times and locations for eliciting SC-astrocyte mingling in the contused spinal cord in adult rats. SCs were transplanted at one week after impact into the contusion epicenter. We found that an injection of 6-AN at different concentrations into the spinal cord parenchyma caudal to the SC transplant caused damage. The degree of damage related to the amount of 6-AN and was especially visible in gray matter. An injection of 0.02 M

6-AN injected 1 mm caudal to the contusion epicenter, within the SC transplant did not affected SC survival and resulted in SC migration into the adjacent astrocyte-rich environment one week after injections. Long-term effects on SC migration using the latter treatment paradigm and associated axon growth responses will be discussed. Our data suggest that 6-AN treatment may support SC migration into astrocyte-rich terrain which modifies the axon growth obstructive astrocytic scar in the injured spinal cord.

Disclosures: **T. Mihara:** None. **A. Haggerty:** None. **K. Yamane:** None. **Y. Pressman:** None. **S. Cerqueira:** None. **M.B. Bunge:** None. **M. Oudega:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.14/K30

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Mayo Clinic Transform the Practice Award 2018
Regenerative Medicine Minnesota Award 2018
Neuro-Informatics Laboratory Support (PI: Dr. M Bydon)

Title: Preliminary results from CELLTOP trial: A phase I/II clinical trial of autologous adipose derived mesenchymal stem cells (AD-MSCs) for the treatment of paralysis due to traumatic SCI

Authors: M. BYDON[1], A. B. DIETZ[2], ***S. GONCALVES**[1], F. MOINUDDIN[1], M. A. ALVI[1], A. GOYAL[1], A. TERZIC[3], A. J. WINDEBANK[4], W. QU[5];
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Abstract: Clinical trials striving to improve neurological outcomes following spinal cord injury (SCI) have yet to identify an intervention that maintains the current medical standard of care while effectively improving neurological outcomes beyond initial surgical stabilization and comprehensive rehabilitation. Regenerative therapy with stem cells for SCI has recently attracted interest from scientists and clinicians alike for a variety of conditions, including neuronal degeneration and traumatic SCI. Adipose-derived MSCs (AD-MSCs) represent a readily accessible cell source with high proliferative capacity that overcome limitations of previously studied cell lines. We are currently enrolling patients in our CELLTOP trial which is a Phase I/II clinical trial investigating safety and efficacy of intrathecal autologous AD-MSCs in a patient with blunt, traumatic SCI. In the present report, we describe the outcome of the first subject, a 53 year old survivor from a surfing accident who sustained an ASIA A/B cervical SCI with subsequent neurologic improvement which plateaued at 7 months following injury. He was enrolled with ASIA C neurologic status, at 9 months post injury. An adipose biopsy was

performed and MSCs were isolated, expanded and cryopreserved in the Mayo Clinic Immune, Progenitor, and Cell Therapeutics (IMPACT) Lab. He received an intrathecal injection of 100 million AD-MSCs suspended in Lactated Ringer's solution and infused after a standard lumbar puncture at the L3-L4 level, 11 months after the injury. Significant improvement in International Standards for Neurologic Classification of Spinal Cord Injury (ISNCSCI) motor and sensory scores was recorded at 1 year following intrathecal administration. Significant improvement in Capability of Upper Extremity score (measured using Capabilities of Upper Extremity Instrument) and global health (measured using PROMIS-10) was also documented. AD-MSCs may be a promising candidate for improving, rather than stabilizing, neurologic impairment after SCI.

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Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.15/K31

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Allogeneic umbilical cord derived mesenchymal stem cells transplantation improves motor function in spinal cord injured rats

Authors: *F. MOINUDDIN[1], K. PETRUCCI[2], G. YEH[2], A. SIDDIQUI[1], Y. YOLCU[1], W. WAHOOD[1], A. GOYAL[1], M. ALI ALVI[1], S. GONCALVES[1], N. MADIGAN[1], A. WINDEBANK[1], B. MOHAMAD[1];
[1]Mayo Clin., Rochester, MN; [2]Animal Cell Therapies, Inc, San Diego, CA

Abstract: ***Introduction****:* Spinal cord injury (SCI) is one of the most devastating forms of trauma resulting in severe functional loss. The injury induces a cascade of secondary insults that limits spontaneous neural tissue regeneration. In recent years, stem cell treatment for SCI has been extensively investigated. Human umbilical cord derived mesenchymal stem cells (UCMSCs) are easier to isolate and expand, and exhibit greater proliferative activity. Using allogeneic UCMSC in spinal cord injured rats could be an appropriate preclinical representation of functional improvement. ***Methods****:* Twelve female Sprague-Dawley rats were randomly divided into two groups: control group (n=6), and rat UCMSC group (n=6). Both groups were subjected to T9 moderate spinal cord contusion injury using a Horizon Impactor machine with 150 kilodynes force. The control group received ringer lactate (RL) injections, whereas the UCMSC group were infused with cells through tail vein 7days after SCI. All rats were examined for motor function by Basso, Beattie, and Bresnahan (BBB) open field locomotion score. The contents of

axonal regeneration, cavity volume and glial scar were also explored by immunohistochemistry. ***Results****:* Recovery of hind-limb locomotor function was significantly enhanced in the UCMSC infused animals from 2nd week to 14th week after injection. BBB scores in UCMSCs- infused animals were 11.1, 11.9, and 14.1 at 2, 3, and 14 weeks after infusion, respectively, which were significantly higher than those of the RL-infused group (8.5, 9.1, and 10.1). Damaged lesions cavity area were lesser in the UCMSCs treated rats compared to the control group. ***Conclusion****:* Our results demonstrated that treatment with allogeneic UCMSC can facilitate functional recovery after SCI in rats. This will support that intravenous human-UCMSCs will be a useful translational therapeutic strategy that could improve the functional capacity among patients with SCI.

Disclosures: F. Moinuddin: None. **K. Petrucci:** None. **G. Yeh:** None. **A. Siddiqui:** None. **Y. Yolcu:** None. **W. Wahood:** None. **A. Goyal:** None. **M. Ali Alvi:** None. **S. Goncalves:** None. **N. Madigan:** None. **A. Windebank:** None. **B. Mohamad:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: SHC-56200-PHI-16
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SHC-85112-PHI-18
R01 NS105961-01
1R01NS079432
1R01EY024575

Title: Tacc3 is a therapeutic target for axon regeneration in adult CNS

Authors: *S. WANG, X. JIANG, Y. OHTAKE, S. LI;
Lewis Katz SOM, Shriners Hosp. Pediatric Res. Ctr., Temple Univ., Philadelphia, PA

Abstract: Severed axons fail to regenerate in adult mammalian CNS and currently there are no treatments for patients with CNS injuries. Microtubules (MT) are critical cytoskeleton proteins during axon elongation and regulation of their dynamics may affect axon regeneration of mature neurons. Transforming Acidic Coiled-Coil Containing Protein 3 (TACC3) is a MT plus-end-tracking protein and is essential for regulating axon extension during development. Here, we study the role of TACC3 for regulating regenerative capacity of mature neurons in adult mammals by a viral vector approach. We designed adeno-associated virus (AAV) vectors for TACC3 using synapsin I promoter to target neuron populations specially. We delivered AAV2-

Syn-TACC3 intravitreally to adult mice with optic nerve crush injury and demonstrated that upregulation of TACC3 in retina ganglion cell (RGCs) promotes robust axon regeneration past the lesion site of optic nerve (> 2 mm in length), in contrast to no regrowth in controls treated with AAV2-synapsin-GFP. Most regenerated axons display a linear trajectory growth towards the optic chiasm. Currently, we are studying whether treatments with our viral vectors that target TACC3 increase survival of RGCs after optic nerve injury and whether upregulation of this gene by a systemic delivery approach achieve the similar outcomes. We conclude that overexpression of TACC3 promotes dramatic axon regeneration in adult CNS neurons and that TACC3 is an important therapeutic target for CNS lesions, including axon injury along the visual system.

Disclosures: S. Wang: None. **X. Jiang:** None. **Y. Ohtake:** None. **S. Li:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.17/K33

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NJ Commission on Spinal Cord Research CSCR16ERG014
NJ Commission on Spinal Cord Research CSCR18FEL006.

Title: The effect of a glycosaminoglycan mimetic on Schwann cell-mediated repair of a severely injured spinal cord in adult rats

Authors: *A. OKUDA[1], T. MIHARA[1], S. HASHEMI[2], T. L. ARINZEH[2], M. OUDEGA[1,3,4,5]; [1]The Miami Project To Cure Paralysis, Miami, FL; [2]Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ; [3]Dept. of Neurolog. Surgery, Univ. of Miami Miller Sch. of Med., Miami, FL; [4]Affiliated Cancer Hosp. & Inst. of Guangzhou Med. Univ., Guangzhou Med. Univ., Guangzhou, China; [5]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Spinal cord injury (SCI) results in nervous tissue damage and functional impairments. Current repair approaches have limited effect on functional recovery, especially in the severely damaged spinal cord. Schwann cells (SCs) are explored for SCI due to their ability to promote axon growth and myelination and elicit numerous other repair-supporting events, which often result in functional recovery. Glycosaminoglycans (GAGs) are sulfated polysaccharides that can elicit axon growth depending on the position and degree of sulfation. Fully sulfated cellulose (fCelS), with three sulfate groups per glucose monomer unit, is a GAG mimetic that elicits axon growth (Menezes et al., 2019) and SC myelination *in vitro*. We argued that combining SC transplantation with fCelS treatment would enhance the overall axon growth response and elicit repair and recovery after severely injured spinal cord. To address this idea, we first evaluated the design of the scaffold, which was prepared in a spiral form containing aligned fibers for axonal

growth. After implantation into the completely transected adult rat spinal cord, we found that axons were abundant and growing into the rostral-caudal direction in the scaffold. Then, we evaluated the fCelS-containing scaffold in the same SCI model with or without SCs. We investigated the effects on SC survival, axon growth and myelination, inflammation, and vascularization in the scaffold, and on tissue sparing and scar presence in the spinal cord adjacent to the scaffold. We also discuss the presence of axons that regenerated from the scaffold into the caudal spinal cord and on behavioral outcomes.

Disclosures: **A. Okuda:** None. **T. Mihara:** None. **S. Hashemi:** None. **T.L. Arinzeh:** None. **M. Oudega:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.18/K34

Topic: C.11. Spinal Cord Injury and Plasticity

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Coulter Foundation Translational Grant
Cohen Translational Engineering Fund
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Abell Foundation Translational Award
NINDS NS101298
Craig H. Neilsen Foundation 460461

Title: A nanofiber hydrogel composite for mesenchymal stem cell transplantation improves immunomodulatory actions and induces robust axon regeneration in subacute spinal cord injury

Authors: *A. E. HAGGERTY[1], Y. NITOBE[1], I. MALDONADO-LASUNCION[1,3], K. YAMANE[1], M. M. MARLOW[1], X. LI[4,5,6], B. CHO[4,8], M. SEU[4,8], H.-Q. MAO[4,5,6,7], *M. OUDEGA[1,2,9,10];
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Abstract: Spinal cord injury (SCI) results in nervous tissue loss and so far untreatable functional impairments. A transplant of bone marrow-derived mesenchymal stem cells (MSCs) can elicit anatomical repair resulting in partial functional recovery, likely through paracrine actions. Previously, we showed that repair by a MSC transplant is limited by their poor survival in damaged spinal cord nervous tissue (Ritfeld et al., 2014). Angiogenesis in the injured spinal cord influences cell transplant survival, neuroprotection, and functional outcome (Ritfeld et al., 2015). Hydrogels are used as a matrix for cell transplants. Here, we tested the utility of a nanofiber hydrogel composite (NHC) engineered to resemble spinal cord nervous tissue in stiffness while providing a porosity that enables cell infiltration as a transplant matrix for MSCs. The effects of MSCs on SCI repair is in large part due to their ability to improve angiogenesis, growth factor secretion, neuroprotection, and immunomodulation. MSC transplants have limited effects on axon regeneration. Our quantitative data indicate that the NHC-MSC combination modulates the immune response in the injured spinal cord segment and elicits a robust axon regeneration response. These effects were found at later, rather than earlier, time points after implementation of the combinatorial treatment.

Disclosures: A.E. Haggerty: None. **Y. Nitobe:** None. **I. Maldonado-Lasuncion:** None. **K. Yamane:** None. **M.M. marlow:** None. **X. Li:** None. **B. Cho:** None. **M. Seu:** None. **H. Mao:** None. **M. Oudega:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.19/K35

Topic: C.11. Spinal Cord Injury and Plasticity

Support: R01NS107807

Title: Retrograde AAV transduction and 3D microscopy enable rapid assessment of axon sparing and regeneration after spinal injury

Authors: *Z. WANG[1], P. TSOULFAS[2], M. G. BLACKMORE[1];
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Abstract: White matter in the spinal cord is comprised of discrete axon tracts that arise from diverse sets of supraspinal neurons. Injuries to the spinal cord are often quite variable, even in controlled research settings, resulting in subject-to-subject differences in the number of surviving axons in each population. In addition, as researchers test strategies to promote axon regeneration after injury, there is inevitable variability in response between subjects (i.e. “responders” and “non-responders”) and differences in the response from neuronal sub-types. Thus, to evaluate

pro-regenerative treatments, and to meaningfully interpret behavioral outcomes, there is a pressing need to quantify the number and location of neurons whose axons were initially spared by injury, and to survey the neuronal populations that subsequently regenerated. To do so we have developed a method that combines viral delivery of retrograde fluorophores with optical clearing of brain tissue. In initial experiments adult mice received of Retro-AAV-H2B-mScarlet or H2B-mNeongreen to C6 spinal cord, in the presence or absence of unilateral dorsal wireknife injury at C4. Two weeks later, various 3DISCO-based clearing methods and 3D imaging revealed extensive label in supraspinal populations throughout the brainstem, midbrain, and cortex. Bright fluorescent signal and nuclear localization facilitated semi-automated quantification. As expected, the dorsal injury reduced cortical labeling by more than 99% while largely maintaining brainstem labeling, reflecting the dorsal versus ventral trajectory of axons. Thus, quantification of labeled nuclei throughout the brain can compare the degree of axon sparing between individual animals and between various injury models. In ongoing work, we are combining immediate H2B-mNeongreen injections with delayed H2B-mScarlet injections, in order to distinguish axon sparing and subsequent regeneration. Our current data indicate that retrograde transduction and 3D imaging provides a practical tool to examine CNS-wide axon maintenance and regrowth after spinal injury.

Disclosures: Z. Wang: None. **P. Tsoulfas:** None. **M.G. Blackmore:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.20/K36

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NYS Spinal Cord Injury Research Board contract 31291GG

Title: Augmenting corticospinal system output in a pre-clinical large animal model of cervical contusion injury with combined brain and spinal cord stimulation

Authors: *P. T. WILLIAMS[1], D. Q. TRUONG[3], A. DE PAOLIS[4], D. F. RYAN[1], H. ALEXANDER[1], M. D. SMITH[2], J. WONG[2], S. AMBIA[2], L. CARDOSO[4], M. BIKSON[4], J. H. MARTIN[1];
[1]Molecular, Cell. and Biomed. Sci., [2]CUNY Sch. of Med., New York, NY; [3]Biomed. Engin., City Col. of New York, CUNY, New York, NY; [4]Biomed. Engin., City Col. of New York, New York, NY

Abstract: Hand function is the highest priority to regain for people with cervical SCI. We are developing translational therapeutic neuromodulation approaches using brain and spinal cord stimulation that activate key motor pathways to promote recovery of hand function. We use

patterned cortical electrical stimulation (intermittent theta burst stimulation; iTBS), shown in the rat to strongly activate the corticospinal system (CS), the principal pathway for hand control, to promote recovery of skill and dexterity. We combine this with transcutaneous spinal direct current stimulation (tsDCS) to further strengthen connections and improve function after SCI. We determined if combined stimulation augments the efficacy of CS activation of muscle. In separate sessions we compared the motor evoked potential (MEP) before, during, and after iTBS or tsDCS alone, and in combination. The dose of tsDCS was based on algorithms to steer the direct current to influence corticomuscular output. In line with the findings in the rat studies, iTBS alone, tsDCS alone, and the combination, augmented the period of post-stimulation MEP facilitation.

To evaluate stimulation therapy after SCI we developed a pre-clinical large animal model of contusion injury, informed by modeling the tissue strain to the C4 spinal segment and pilot experiments (3.5 mm spherical indentor at 800 kDyn force with a 1-15 s dwell). MR-scans and histopathology indicate the lesion was incomplete and asymmetric, analogous to most human cases. The chronic motor deficits were asymmetric and associated with injury size. We tailored the delivery of iTBS to primarily activate the impaired CS, with tsDCS (4 mA cathodal), and for 30 min/day on 10 consecutive days in the chronic phase 1-2 months, after spontaneous recovery had plateaued.

We observed improved reach control following stimulation therapy. We also found augmented MEP recruitment that suggests CS strengthening. The behavioral and physiological improvements persisted beyond several weeks after therapy.

These encouraging results show a strengthening of motor cortex-to-muscle activation, facilitation of motor cortex-mediated MEP potentiation, and an improvement in distal motor skill. These preliminary findings point to a neuromodulatory strengthening of the corticospinal system for improved motor control after cervical injury.

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Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.21/K37

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DOD CDMRP W81XWH-17-1-0260
NIH NS096514

Title: Effects of different single-bout AIH protocols on LUT function in adult naïve and SCI rats

Authors: *W. F. COLLINS, III[1], I. C. SOLOMON[2];
[1]Neurobio. & Behavior, [2]Physiol. & Biophysics, Stony Brook Univ., Stony Brook, NY

Abstract: We have previously demonstrated that a single bout of acute intermittent hypoxia (AIH) produces sustained increases in reflex bladder inter-contraction interval (ICI) and micturition volume in both naïve rats and rats with moderate spinal cord injury (SCI) as well a sustained decrease in the frequency of non-voiding contractions in SCI rats. In the present study, we extend these observations on lower urinary tract (LUT) function in naïve and SCI rats by comparing different single-bout AIH protocols that keep the total duration of hypoxia (15 min; 12% O_2, balance N_2) constant but differ in timing and number of hypoxic episodes. To this end, adult female Sprague-Dawley rats were randomly assigned to either the naïve or SCI (moderate contusion (200 kilodynes) mid-thoracic SCI, 4-weeks survival) groups. In each rat, bladder intravesical pressure and void volume were recorded under urethane anesthesia (1.4 g/kg, spontaneous breathing) during continuous infusion of saline (0.04 ml/min) into the bladder to elicit reflex micturition events. Following >60 min of baseline recording, rats were exposed to a single bout of AIH with one of the following three protocols: (1) three 5-min episodes of hypoxia each separated by 5-min exposures to room air, (2) five 3-min episodes of hypoxia each separated by 3-min exposures to room air, or (3) ten 1.5-min episodes of hypoxia each separated by 1.5-min exposures to room air. Following AIH exposure, data acquisition continued for up to 120 minutes while the rats continued to breathe room air. Each of the single-bout AIH protocols produced comparable sustained (up to 120 min post-AIH) increases in bladder inter-contraction interval and micturition volume both in naïve and SCI rats. Moreover, each of the protocols appeared to be equally effective in reducing the ratio of non-voiding/voiding bladder contractions in SCI rats. Single-bout AIH exposure also produced transient increases in both bladder contraction threshold pressure and minimum bladder pressure. Modulation of LUT behavior during individual exposures to hypoxia and immediately following each hypoxic episode ("off effect") were also observed, with the intensity of the "off effect" tending to be lower following short duration (e.g., 10 x 1.5-min) hypoxic exposures. Our observations suggest that each single-bout AIH protocol is effective in reducing non-voiding bladder contractions and increasing micturition in SCI rats albeit subtle differences in magnitude and/or timing were noted across the protocols.

Disclosures: W.F. Collins: None. **I.C. Solomon:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.22/K38

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Nielsen Foundation
Blusson Integrated Cure Partnership

Title: Effect of oral ketone esters use on motor function recovery alter cervical hemicontusion in rats

Authors: *O. SEIRA[1], K. KOLEHMAINEN[1], J. LIU[1], W. PLUNET[1], J. NICOLE[1], S. KAMAKARI[1], L. RAFFAELE[1], K. CLARKE[2], W. TETZLAFF[1];
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Abstract: Our laboratory has previously shown that treatment with ketogenic diet (KD) improves forelimb motor control after cervical spinal cord injury (SCI) in rodents. KD is a high fat, low carbohydrate diet and as such perceived as unpalatable, which could reduce compliance in acute SCI patients. Consumption of KD results in increased liver production and release of ketone bodies like β-hydroxybutyrate (βHB). Our objective in this study is to evaluate the effect of an alternative ketogenic dietary approach. Here, we administered exogenous βHB in form of an oral ketone ester (ΔG®) as an alternative treatment that can be easily administered as an oral supplement. Beginning 4 hours following a C5 hemi-contusion injury, male Sprague-Dawley rats were treated with ΔG® or water via oral gavage, and fed *ad libitum* with either a standard control diet (SD) or KD resulting in 3 treatment groups: Ctrl+SD; ΔG®+SD & ΔG®+KD. Animals were orally gavaged with ΔG® during the first 2 weeks, followed by a gradual addition of ΔG® into their drinking water until the end of the experiment. We observed significant improvements in fine control of the forelimbs and digits after cervical SCI, such as the Irvine, Beattie and Bresnahan (IBB) Forelimb recovery score, digital extension test and the Montoya staircase in the group treated with ΔG®+SD but not with the ΔG®+KD group. The latter treatment resulted in βHB levels exceeding 6-8 mM while βHB levels in the ΔG®+SD group fluctuated in the 1-4 mM range. The spinal cords were harvested at the 8 weeks endpoint for histological analysis Our data indicate that and excessive amount of ketone bodies ΔG®+KD no longer have beneficial effects on behavioural outcomes after SCI. Further analyses to determine the mechanisms behind these behavioural improvements (and their losses) are needed. The oral administration of ΔG® might be a realistic translatable alternative to the KD. Supported by the Craig H Nielsen Foundation

Disclosures: O. Seira: None. **K. Kolehmainen:** None. **J. Liu:** None. **W. Plunet:** None. **J. Nicole:** None. **S. Kamakari:** None. **L. Raffaele:** None. **K. Clarke:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Oxford. **W. Tetzlaff:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.23/K39

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DOD CDMRP W81XWH-17-1-0260
NIH NS096514
NYS DOH SCIRB C32088GG

Title: Impact of severity and duration of acute hypoxic exposure on lower urinary tract (LUT) function in urethane-anesthetized adult female rats

Authors: *I. C. SOLOMON[1], W. F. COLLINS, III[2];
[1]Physiol. & Biophysics, [2]Neurobio. & Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Acute hypoxia is a common occurrence in cardiovascular and pulmonary diseases, and while hypoxia-induced changes in respiratory drive/function are well documented, the effects of acute hypoxia on non-respiratory somatic motor systems are less well understood. Ongoing work in our laboratory has been examining the effects acute hypoxia on lower urinary tract (LUT) function, and these experiments have revealed that exposure to an acute episode of hypoxia modifies LUT function with the hypoxia-induced modulation being present both during hypoxia and transiently after the hypoxic stimulus is removed in both intact and SCI rats. To better understand the influence of acute hypoxia on LUT function, the present study evaluated the impact of the severity and duration of an acute hypoxic episode on LUT behaviors in urethane-anesthetized, spontaneously breathing, intact adult female Sprague-Dawley rats. For this study, reflex micturition events (rME) were elicited by continuous infusion of saline into the bladder, with the rate of infusion set to achieve a baseline (BL) bladder inter-contraction interval (ICI) of ~4.5 min. Following ~30 min of normoxic BL recording, a single episode of hypoxia (10% or 12% O_2) was initiated at approximately the midpoint of BL ICI and maintained for 90 s, 3 min, 5 min, or 15 min, after which the rat was returned to normoxia and allowed to recover. In a subset of rats, 2 hypoxic exposures (*e.g.*, 2x 90s; 2x 3 min; 90 s and 5 min; separated by at least 30 min) were used. Bladder intravesical pressure (BP) and void volume were recorded, and peak BP, minimum BP, threshold BP, ICI, and void volume of individual ME were determined. Consistent with our prior observations, exposure to hypoxia elicited a premature rME (reduced ICI), which exhibited reduced peak and threshold BP, decreased void volume, and variable effects on minimum BP. With return to normoxia, the initial rME was delayed (increased ICI) and it exhibited a marked increase in threshold BP, variable increase in both minimum BP and void volume, and negligible effects on peak BP. The hypoxia-induced LUT behaviors were reproducible in the individual rats that had 2 similar hypoxic exposures (*e.g.*, 2x 90s; 2x 3 min). The magnitude of hypoxia-induced effects was minimally affected by the severity of hypoxia, but strongly influenced by exposure duration, with longer exposures (*i.e.*, 5 and 15 min) producing progressively greater or sustained magnitude changes during hypoxia and more robust effects during the initial stages of recovery. These data demonstrate that the duration of acute hypoxic exposure can impact the magnitude and duration of hypoxia-induced alterations in LUT function.

Disclosures: I.C. Solomon: None. **W.F. Collins:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.24/K40

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation
Blusson Integrated Cure Partnership

Title: Oral ketone esters induce widespread proteome changes after spinal cord injury

Authors: *K. L. KOLEHMAINEN[1], O. SEIRA[1], W. T. PLUNET[1], J. LIU[2], G. STACEY[3], L. FOSTER[3], K. CLARKE[4], W. TETZLAFF[1];
[1]Univ. of British Columbia, ICORD, Vancouver, BC, Canada; [2]ICORD, Vancouver, BC, Canada; [3]Univ. of British Columbia, Vancouver, BC, Canada; [4]Univ. of Oxford, Oxford, United Kingdom

Abstract: Spinal cord injury (SCI) affects over 80 000 people in Canada and significantly impacts quality of life. Previous findings in our lab demonstrated that the ketogenic diet (KD) may be a promising SCI treatment as rats fed with KD acutely after cervical SCI showed behavioural improvement in forelimb function. KD is a high fat, low carbohydrate diet and is clinically used in drug-resistant epilepsy in children. A hallmark of KD is the increased levels of ketone bodies, particularly β-hydroxybutyrate (BHB), which is formed through the metabolism of fatty acids. BHB can be used as an alternate energy source by cells but is also the only known endogenous ligand of the G protein-coupled receptor HCA2 (also known as the niacin receptor). Given our positive results with acute KD treatment, we were interested to see if administering exogenous BHB could provide similar benefits after SCI. To provide exogenous BHB we used the commercially available ketone ester, ΔG®, which can be administered as an oral treatment and produces a rapid and transient increase in plasma BHB. Sprague-Dawley rats were given a C5 hemi-contusion then gavaged with the ΔG® ketone ester (KE) starting 4 hours after injury. Gavage treatment continued for two weeks at which point rats were sacrificed and the spinal cord lesion site was prepared for mass spectrometry analysis. We performed a global proteomic analysis of the injury site. As expected we found multiple metabolic proteins upregulated by KE. Specifically, those involved in the TCA cycle and glycogen synthesis. As well we found upregulation of cytoskeletal components and proteins involved in neural outgrowth and differentiation. Overall, our results identify multiple pathways affected by BHB and we are following up on our proteomic analysis with specific *in vitro* and *in vivo* manipulation of candidate pathways.

Disclosures: K.L. Kolehmainen: None. **O. Seira:** None. **W.T. Plunet:** None. **J. Liu:** None. **G. Stacey:** None. **L. Foster:** None. **K. Clarke:** E. Ownership Interest (stock, stock options, royalty,

receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of Oxford. **W. Tetzlaff:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.25/L1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: W.M. Keck Foundation GR529005
NIH R21MH101525
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NSF CBET-1464686
NSF NeuroNex-1707352

Title: Non invasive optogenetic stimulation in a rat model of spinal cord injury

Authors: *G. KENDZIORSKI[1], E. D. PETERSEN[2], L. SHAFAU[2], M. PRAKASH[3], U. HOCHGESCHWENDER[1];
[1]Neurosci., Central Michigan Univ., Mt Pleasant, MI; [3]Neurosci., [2]Central Michigan Univ., Mount Pleasant, MI

Abstract: The ability to manipulate specific neuronal populations of the spinal cord following spinal cord injury (SCI) could potentially prove highly beneficial for rehabilitation in patients through maintaining and strengthening still existing neuronal connections and/or facilitating the formation of new connections. A non-invasive and highly specific approach to neuronal stimulation is bioluminescent-optogenetics, where genetically expressed light emitting luciferases are tethered to light sensitive channelrhodopsins (luminopsins, LMO); neurons are activated by the addition of the luciferase substrate coelenterazine (CTZ). This approach takes advantage of utilizing ion channels for current conduction while activating the channels through application of a small chemical compound, thus allowing non-invasive stimulation and recruitment of all targeted neurons. We previously showed the efficacy of this approach in improving locomotor recovery following severe spinal cord injury in rats expressing the excitatory LMO3 under control of a pan-neuronal and motor neuron specific promoter; CTZ was applied through a lateral ventricle cannula. Here we transduced spinal cord neurons with a synapsin-driven step function LMO, SFLMO(CS). In this construct the Gaussia luciferase variant sbGLuc is fused to the step function opsin ChR2(CS). As SFOs are significantly more light sensitive than other opsins, we stimulated transduced lumbar neurons by intraperitoneal application of CTZ, allowing for a less invasive treatment.

Disclosures: **G. Kendziorski:** None. **E.D. Petersen:** None. **L. Shafau:** None. **M. Prakash:** None. **U. Hochgeschwender:** None.

Poster

393. Treatment and Therapeutic Interventions for Spinal Cord Injury

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 393.26/L2

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Repeated transspinal stimulation reorganizes neural circuitries in spinal cord injured rat

Authors: *D. C. MALLOY, M.-P. CÔTÉ;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Electrical stimulation is commonly used to promote recovery of motor function in upper motoneuron lesions. After spinal cord injury (SCI), stimulation delivered over muscle(s), mixed or sensory nerves, primary motor cortex, and spinal cord transcutaneously or epidurally have all been employed with the aim to promote functional recovery. In this study, we probed spinal neuroplasticity after repeated cathodal transspinal stimulation via the plantar H-reflex, a well-established neurophysiological biomarker of hyperreflexia and spasticity in rodents. We also assessed changes in motor output via the transspinal stimulation induced muscle responses (transspinal evoked potentials; TEPs). Adult female Sprague Dawley rats underwent a severe T9 contusion SCI (250 kdyn) and were randomly assigned to sham or transspinal stimulation groups that was delivered three times per week for 5-6 weeks on awake animals, starting five days post-SCI. Animals laid prone with the cathode electrode placed over the lumbar enlargement and the anode electrodes placed over the abdomen. Stimulation was delivered at sub- (0.8T) and suprathreshold TEP intensity (1.2T) in alternating 3-minute bouts (1 ms pulses, 0.2 Hz) for 18 minutes. Terminal experiments were performed at 6-weeks post-injury to establish changes on the recruitment of flexor and extensor motoneurons, H-reflex frequency dependent depression, and H-reflex modulation profile following transspinal conditioning stimulation. We found that repeated transspinal stimulation increased the amplitude of TEPs recorded from hindlimb muscles in the experimental and not in the sham group. Lower recruitment threshold, steeper slope, and larger maximal responses were observed in the plantar muscle. In addition, the H-reflex excitability was decreased and frequency-dependent depression was potentiated. Lastly, repeated transspinal stimulation decreased the H-reflex when preceded by a TEP, a phenomenon that was not observed in the sham group. These results provide strong evidence that transspinal stimulation can be used as a neuromodulation tool to concomitantly decrease spinal reflex hyperexcitability and increase motor output after SCI.

Disclosures: **D.C. Malloy:** None. **M. Côté:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.01/L3

Topic: D.01. Sensory Disorders

Title: The co-occurrence of Alice in Wonderland syndrome and autonomous sensory meridian response

Authors: *S. BEDWELL[1], I. BUTCHER[2];
[1]Birmingham City Univ., Birmingham, United Kingdom; [2]Univ. of Manchester, Manchester, United Kingdom

Abstract: Alice in Wonderland Syndrome and Autonomous Sensory Meridian Response are both terms used to describe specific neurophysiological responses to external stimuli. Although different in their presentation and symptomatic descriptions, there are many commonalities in terms of symptoms of perception and of theories of aetiology. In the present study, we sought to establish the link between symptoms associated with Alice in Wonderland Syndrome and those described in Autonomous Sensory Meridian Response. In an online questionnaire study, participants known to experience Autonomous Sensory Meridian Response self-reported their specific experiences of the phenomena, along with experiences associated with symptoms of Alice in Wonderland Syndrome. Our findings show a striking high presence of Alice in Wonderland Syndrome symptoms amongst a population who are aware of experiencing Autonomous Sensory Meridian Response. Additionally, we observed a significant predictive relationship ($p = .036$) between the visual symptoms of Alice in Wonderland Syndrome in childhood and the age of onset of Autonomous Sensory Meridian Response. These findings provide a valuable insight into the possible underpinnings of two little understood neurophysiological phenomena and offer the foundation for gaining a further understanding of the possible therapeutic and neuroprotective effects of Autonomous Sensory Meridian Response.

Disclosures: S. Bedwell: None. **I. Butcher:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.02/L4

Topic: D.01. Sensory Disorders

Support: ANR-15-RHUS-000
ANR-10-LABX-65
BNP Paribas Foundation
FAUN Stiftung
LHW-Stiftung

Title: Components of the cochlear mechanoelectrical transduction machinery involved in auditory cortex development

Authors: M. D. MORAIS, M. GAGLIARDINI, O. POSTAL, P. JEAN, T. DUPONT, B. GOURÉVITCH, C. PETIT, *N. MICHALSKI;
Unite Genetique & Physiologie Audition, Paris Cedex 15, France

Abstract: The genetic approach, based on inherited forms of deafness, has proved particularly effective for deciphering the molecular physiology of the auditory sensory organ, the cochlea. Since the discovery of the first gene responsible for deafness in both humans and mice, the gene encoding myosin-VIIa, about 110 genes responsible for non-syndromic forms of deafness and about 300 genes responsible for syndromic forms have been reported in humans and/or mice. By contrast, the genetic approach has provided little information about the central auditory system, even though central auditory system dysfunctions are thought to affect ~5% of children and more than ~25% of elderly people. One possible explanation for this discrepancy is that intrinsic auditory central deficits may be concealed by peripheral deficits in genetic forms of deafness. Indeed, we have recently revealed that the genes encoding cdhr15 and cdhr23, the cadherin-related proteins forming the tip-link, the heart of the auditory transduction machinery in auditory sensory cells, are also expressed by precursors of many GABAergic inhibitory parvalbumin interneurons. We have shown that these interneuron precursors are specifically targeted to the auditory cortex in a process requiring these two cadherins. Therefore, these results have opened the possibility that the other proteins of the mechanoelectrical transduction machinery, in addition to cdhr15 and cdhr23, could be involved in the development of cortical interneurons and other cell types of the auditory cortex. To gain insight into other deafness genes that may have roles in the auditory cortex and more generally in the central auditory pathways, we have produced new cre genetic tools for the fluorescent tracing of Cdhr15/Cdhr23-expressing interneurons. Here, we will present these new genetic tools and the first insights they provided on the expression of other deafness genes in the auditory cortex. By characterizing cortical deficits in mouse models of previously identified forms of peripheral human deafness, our work should pave the way for future studies of intrinsic cortical defects in patients and improvements in auditory rehabilitation.

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Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.03/L5

Topic: D.01. Sensory Disorders

Support: NIH Intramural Grant
OSD Grant

Title: Long-term NAD^+ supplementation rescues high-frequency age-related hearing loss in mice

Authors: *R. KIMURA, M. N. OKUR, D. L. CROTEAU, V. A. BOHR;
Natl. Inst. on Aging, NIH, Baltimore, MD

Abstract: Hearing loss is the most common sensory impairment in elderly adults, affecting over one-third of adults beyond age 60. Age-related hearing loss (ARHL) is characterized by a progressive loss of sensitivity to sounds, starting with high-frequency stimuli. Our group previously showed that mouse models of Cockayne Syndrome (CS), a premature aging disease with a major symptom resembling an accelerated form of ARHL, display high metabolic activity and mitochondrial dysfunction. Given the importance of mitochondrial integrity in healthy hearing, interventions targeting mitochondrial homeostasis may prove beneficial for ARHL. Our current study shows that a short-term 4-week intervention of nicotinamide riboside (NR), a precursor of a key metabolite nicotinamide adenine dinucleotide (NAD+), in drinking water has preventative effects for high-frequency hearing loss in CS mice. Interestingly, our results show that NR also has a beneficial effect on wild type mice, which led to further inquiries assessing the efficacy of long-term NR treatment under non-diseased conditions. In our subsequent study, we subjected mice (N=48) to a prolonged 6-month NR treatment starting at 8 weeks of age. We used auditory brainstem response (ABR), a measure of auditory transduction and neural pathway integrity, and distortion product otoacoustic emissions (DPOAE), a measure of outer hair cell function, to characterize the hearing ability of our mice. Remarkably, our data shows that NR treatment dramatically reduces ($p<0.0001$) high-frequency hearing loss in aging mice, with 8-month-old treated cohorts displaying ABR thresholds statistically similar to that at 8 weeks. These results collectively support a model of metabolic deficit in ARHL and offer NAD+ supplementation as a promising therapeutic for this condition.
This work was supported in part by the National Institutes of Health, National Institute on Aging and ChromaDex.

Disclosures: R. Kimura: None. **M.N. Okur:** None. **D.L. Croteau:** None. **V.A. Bohr:** 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.; ChromaDex.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.04/L6

Topic: D.01. Sensory Disorders

Support: SOS Rétinite France

Title: Behavioral phenotyping of a novel Wolfram syndrome zebrafish model

Authors: *B. DELPRAT[1], L. CROUZIER[1], C. DELETTRE[2], T. MAURICE[1];
[1]MMDN, Univ. Montpellier, EPHE, INSERM, U1198, Montpellier, France; [2]Inserm U1051, Montpellier, France

Abstract: Wolfram syndrome (WS) is a rare multisystem neurodegenerative disorder also known as DIDMOAD (diabetes insipidus, insulin-deficient diabetes mellitus, optic atrophy and deafness). WS is an autosomal recessive disease causing degeneration of beta cells in the pancreas, ganglion cells in the retina and hair cells in the inner ear. The syndrome is due to mutations in *WFS1*, coding Wolframin, a MAM protein involved in regulation of ER Ca^{2+} homeostasis. We previously characterized a novel $Wfs1^{Exon8del}$ knock out mouse and analyzed their auditory and visual abilities. Mice developed a progressive optic atrophy at 3 months and a moderate hearing loss at 6 months. Since those mice do not recapitulate the human's symptoms, we used a zebrafish model system, which is a valuable animal model for studying development and function of the vertebrate inner ear and retina. In zebrafish, two Wfs1 gene were expressed, *Wfs1a* and *Wfs1b*. Wfs1a shares 45% identity with human WFS1 and *Wfs1b* shares 53% identify. Interestingly, several *Wfs1a* and *Wfs1b* mutations were identified and we chose $Wfs1a^{L692X}$ and $Wfs1b^{W493X}$ to reproduce WS in two zebrafish models. Preliminary results indicated that loss of *Wfs1b* decreases visual motor response and the number of eye's saccade/min in 5dpf zebrafish. In addition, acoustic startle response is also decreased in *Wfs1b* deficient zebrafish compared to controls. Therefore, this model will be useful to decipher the molecular mechanism leading to optic atrophy and hearing loss.

Disclosures: B. Delprat: None. **L. Crouzier:** None. **C. Delettre:** None. **T. Maurice:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.05/L7

Topic: D.01. Sensory Disorders

Support: International Retinal Research Foundation (IRRF)

Title: Drug screening utilizing the visual behavior of a night blindness zebrafish model

Authors: *L. GANZEN, Y. LEUNG;
Purdue Univ., West Lafayette, IN

Abstract: Retinitis Pigmentosa (RP) is an incurable retinal degeneration disease which affects approximately 1 in 4000 individuals globally, and the purpose of our work is to discover drugs for patients suffering from the disease. To accomplish this goal, we utilized a visual-motor response (VMR) assay to assess the diminished vision of a transgenic zebrafish RP model carrying a human Rhodopsin transgene with Q344X truncation mutation (Tg(rho:Hsa.RH1_Q344X)), and used this line to screen compound libraries. The Q344X larvae experiences significant rod degeneration by 7 days post-fertilization (dpf) and also carries a Tg(-3.7rho:EGFP) reporter for rod visualization (Nakao et al., 2012). To assess the extent of visual deficit in the Q344X zebrafish, larvae were acclimated to a scotopic light source (0.01 lux) for 1 hour. Their VMR was quantified at light-offset. Wildtype larvae exhibited an average distance travelled of 0.275 ± 0.043cm whereas Q344X larvae exhibited a significantly attenuated VMR only travelling 0.128 ± 0.038cm (Welch's Two-Sample T-test; p-value=2.124e-12, N=18 biological replicates of 48 larvae per group). This VMR assay was then utilized to screen drug compounds to identify novel drugs that can improve the visual behavior of the Q344X model. A pilot screen of SCREEN-WELL® REDOX library was performed to identify drugs that may alleviate excessive oxidative stress in the retina. The FDA-approved drug Carvedilol was identified from this screen due to its ameliorating effects on the Q344X VMR. To determine the cause of this improved behavior, the rod-containing area in the larval retina was quantified. Treated larvae had a larger average area of fluorescent signal near the marginal zone (3610±2841 μm ; N = 24)compared to the untreated larvae (1112±1305 μm ; N = 24) (Welch's Two-sample T-test, T=3.9; df=32.3; p-value= 0.0004). Treated larvae also had a larger average area of fluorescent signal in the ventral patch(2728±2468 μm ; N = 24) compared to untreated larvae (529±537 μm ; N = 24) (Welch's Two-sample T-test;T=4.2; df=25.2; p-value = 0.0002). We conclude that Carvedilol is a beneficial drug to the Q344X RP model. Fluorescent imaging indicates that rod-positive area near the marginal zone area and ventral patch were expanded by the drug treatment. This finding implies that the drug potentially increased rod numbers and in turn improved the vision of the Q344X RP model.

Disclosures: **L. Ganzen:** None. **Y. Leung:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.06/L8

Topic: D.01. Sensory Disorders

Support: University of Miami Provost Research Awards
University of Miami College of Arts and Sciences Gabelli Fellowship
NIH Grant R01DC009645
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Title: The role of GDF6A in the early development of hearing in zebrafish

Authors: M. RICHMOND[1], M. TEKIN[2], *Z. LU[1];
[1]Dept. of Biol., Univ. Miami, Coral Gables, FL; [2]Dr. John T. Macdonald Fndn. Dept. of Human Genet. and John P. Hussman Inst. for Huma, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Hearing loss is the most common congenital sensory disorder with 2-3 in every 1,000 individuals born with hearing defects. Myriad genes are responsible for establishing a proper environment for the development of the hearing system. Precise control of physical structures is needed for proper conduction of vibrations, and sensory epithelia must be properly established in order for an individual to hear. Bone morphogenetic proteins (BMPs) have been extensively studied for their role in the structuring of many tissue types important to the auditory system including bone, cartilaginous, and neural tissue. Mutations in Growth Differentiation Factor 6 (GDF6), otherwise known as Bone Morphogenetic Protein 13 (BMP13), cause several developmental disorders, such as Klippel-Feil Syndrome Type 1 and Multiple Syntosis Syndrome. Patients with these disorders display an increased likelihood of having hearing loss. However, there is little published clinical evidence relating the gene to sensorineural hearing loss directly. In this study, we used zebrafish as a model to investigate the role that *gdf6a*, a paralog of human *GDF6*, plays on the early development of the inner ear using electrophysiological and confocal imaging techniques. We used *radar*^{s327}mutants (*gdf*6a$^{-/-}$) created by the Baier lab with a C to A substitution in *gdf6a*, leading to a premature stop codon early in the ORF. The mutants have a truncated Gdf6a protein lacking the functional signaling domain. We observed that *gdf6a*$^{-/-}$ mutants display cellular and functional differences with their non-mutant siblings though there was no significant difference in gross anatomy of the inner ear. To assess the auditory function of the mutants, microphonic potential recordings were made from hair cells in the otic vesicle of 3- and 7-dpf zebrafish larvae. We found that the mutants had an increased hearing threshold and reduced microphonic amplitude compared to non-mutant larvae. Then we examined the sensory epithelium in the saccule, the major hearing organ in larval zebrafish, using *Et(krt4:GFP)*^{sqet4}

transgenics expressing cytosolic GFP in hair cells. Inspection of the saccular epithelium revealed that the overall hair cell count for the mutants is normal, but the orientation of hair cells in the anterior epithelium is obstructed, which likely results in a decrease in mechanotransduction efficiency. These results demonstrate that *gdf6a* most likely plays a novel role in the development of the zebrafish inner ear during the first-week post-fertilization.

Disclosures: M. Richmond: None. **M. Tekin:** None. **Z. Lu:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.07/L9

Topic: D.01. Sensory Disorders

Support: DoD Grant W81XWH-15-2-0032

Title: What white matter plasticity can tell us about the associations between hearing loss and tinnitus

Authors: *R. A. KHAN, S. SCHMIDT, Y. TAI, S. SHAHSAVARANI, F. HUSAIN;
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Abstract: Tinnitus, the perception of sound in the absence of an external source, has been attributed to changes in both the periphery (e.g. hearing loss) and the cortex. It is unclear, however, what specific neuroanatomical and functional changes co-occur with tinnitus. More confusing still is the nature of the association between hearing loss and tinnitus – most tinnitus patients have decreased hearing sensitivity, but half of those with hearing loss do not have tinnitus. The goal of the current study was to parse out the relationship between hearing loss and tinnitus by examining white matter (WM) tracts in the brain. Diffusion tensor imaging (DTI) data were collected from 76 participants across 4 groups – controls with normal hearing (n=17), controls with hearing loss (n=11), tinnitus subjects with normal hearing (n=11) and tinnitus subjects with hearing loss (n=37). Diffusion was measured along 60 directions using a 3T Siemens Prisma scanner. Fractional anisotropy (FA) analysis was conducted using FSL FMRIB and TBSS libraries. Whole brain analysis focusing on the two tinnitus groups revealed six major tracts that showed diminished integrity in the hearing loss group compared to the normal hearing group, using a FWE correction of $p<0.05$ - the left anterior thalamic radiation (ATR), bilateral anterior corona radiata (ACR), bilateral inferior longitudinal fasciculi (IF-OF), right uncincate fasciculus, right inferior longitudinal fasciculus, and bilateral superior longitudinal fasciculi (SLF). To parse out the association of observed changes with tinnitus and hearing loss, supplementary ROI analysis was conducted, with individual FA values being extracted from all 4 groups from each of the regions described above. FA values in the left ATR, ACR, IF-OF, and

SLF showed an inverse relationship with pure-tone average hearing loss (measured at 2,4,6,8 kHz frequencies), suggesting that these regions may be associated with the degree of hearing loss, regardless of tinnitus status. No strong associations were seen with the scores of Tinnitus Functional Index, which may be attributed to the relative homogeneity of tinnitus severity amongst our participants. With the collection of additional data, we hope to elucidate the neural correlates of these two associated disorders.

Disclosures: R.A. Khan: None. **S. Schmidt:** None. **Y. Tai:** None. **S. Shahsavarani:** None. **F. Husain:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.08/L10

Topic: D.01. Sensory Disorders

Support: NIH NEI 018608, 020647
Research to Prevent Blindness
Kentucky Lions Eye Research Endowed Chair
Jewish Heritage fund

Title: An allele independent gene therapy strategy induces rod function in a transgenic swine model of autosomal dominant retinitis pigmentosa

Authors: A. JALLIGAMPALA[1], G. PANGENI[1], M. H. JABBAR[1], W. WANG[1], F. EFRAT[1], A. RISING[3], W. HAUSWIRTH[4], A. LEWIN[5], M. A. MCCALL[2];
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Abstract: Introduction- Retinitis pigmentosa (RP) is an inherited neurodegenerative disease. The general phenotype is characterized by an initial loss of rod photoreceptors followed by the loss of cone photoreceptors, leading to irreversible vision loss. 30% of RP cases are autosomal dominant (adRP) and mutations in the rhodopsin (RHO) gene account for about 25% of these although there is significant allelic heterogeneity. Therefore an attractive approach is one that could be broadly applied across all rhodopsin mutations. In this study, we applied a gene therapy approach in a transgenic human P23H Rho swine model of adRP, using a dual rAAV vector that combines and short hairpin RNA (shRNA) to eliminate expression of both mutant human and WT pig rhodopsin protein and augment WT rhodopsin with a shRNA resistant Rho gene.
Methods- The rAAV dual vector contains both a short hairpin RNA (shRNA820) and a WT, shRNA-resistant, replacement *RHO* gene (*RHO820*). We injected 50ul of the rAAV dual vector

at titers between 5 x 10^{11} and 1 x 10^{12}vg/ml subretinally in Tg hP23H Rho piglets between postnatal day (P)3 and 7. The structural and functional integrity of the retinas were assessed non-invasively monthly through ~20 weeks post injection (wpi), using optical coherence tomography (OCT), and full-field electroretinograms (ffERG). After the terminal assessment, the animals were euthanized, their eyes enucleated and retinas processed for morphological analyses.
Results- The Tg hP23H pig retina has a large complement of rod photoreceptors in the first postnatal month, although a rod isolated retinal response is not evident. By P60, most rods have degenerated. In contrast, AAV shRNA820/RHO820 induced a scotopic ffERG b-wave in 4 out of 6 eyes that extended through 20 wpi. Consistent with the induction of rod function, these retinas retained rod photoreceptors that express normally localized rhodopsin and, the inner laminar integrity of the OCT. Additionally, the photopic ffERG of treated Tg hP23H pig responses remain unaltered over the period analyzed. Scotopic and photopic function weres not altered in control WT piglets treated with the same vector and retinal morphology also was not affected.
Conclusion- Our results show that the AAV shRNA820/RHO820 reduces rod degeneration and induces rod function in this adRP swine model. This suggests that this therapy should potentially be useful for patients with *RHO*-linked RP.

Disclosures: **A. Jalligampala:** Other; Precision Biosciences. **G. Pangeni:** Other; Precision Biosciences. **M.H. Jabbar:** Other; Precision Biosciences. **W. Wang:** None. **F. Efrat:** None. **A. Rising:** None. **W. Hauswirth:** None. **A. Lewin:** None. **M.A. McCall:** Other; Precision Biosciences.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.09/L11

Topic: D.01. Sensory Disorders

Title: Behavioral and mechanistic study of tinnitus induced by sustained optogenetic activation of inhibitory neurons in the mouse inferior colliculus

Authors: X. LIU, ***F. LI**, H. TANG, X. LIANG, G. BI, P. LAU, L. CHEN;
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Abstract: The inferior colliculus (IC) plays an important role in the transduction of auditory signals. In animal models of tinnitus, neural activity in the IC undergoes plastic changes, but it is still debated whether tinnitus can originate from a central source such as the IC without peripheral malfunction. To address this issue, we first designed a two-way choice paradigm for tinnitus detection and built an automated system for behavioral training and testing. The effectiveness of this approach was verified using established tinnitus model with induction by

sodium salicylate. We then directly manipulated the activity of the IC using optogenetic methods to chronically activate inhibitory neurons in the IC in VGAT-ChR2-EYFP transgenic mice. Interestingly, the transgenic mice exhibited strong tinnitus-like behavior within two hours after the optogenetic stimulation, and this behavior lasted for 1-2 days. Using multi-channel recordings, we found that after 2 days of optogenetic activation in VGAT-ChR2 animals, some IC neurons showed remarkable changes in their frequency tuning, indicating weakened lateral inhibition. Meanwhile, IC neurons showed increased synchrony in their spontaneous activity. These results suggest a neural plastic change in the IC induced by chronic optogenetic activation of the inhibitory neural circuits, which led to tinnitus-like behavior, thus supporting a central origin of tinnitus.

Disclosures: X. Liu: None. **F. Li:** None. **H. Tang:** None. **X. Liang:** None. **G. Bi:** None. **P. Lau:** None. **L. Chen:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.10/L12

Topic: D.01. Sensory Disorders

Support: ASHFoundation Grant

Title: Voxel-based morphometry in age-associated hearing loss: A preliminary study

Authors: *S. SHENDE, S. SHAHSAVARANI, Y. TAI, R. MUDAR, F. HUSAIN;
Univ. of Illinois at Urbana-Champaign, Champaign, IL

Abstract: Objective and rationale: Emerging evidence suggests gray matter (GM) volume alterations in auditory and a few non-auditory regions in individuals with age-associated hearing loss. Specifically, studies report that poorer peripheral hearing acuity is correlated with reduced GM volume in the auditory cortices and parahippocampal gyrus. However, a number of these studies have failed to include an older normal hearing control group, suggesting a need to further examine whether GM alterations observed in this population are due to age or hearing loss, or both. Methods: Data from seven older adults with mild to moderate age-associated hearing loss (age: 71.5 ± 1.29 years, better ear speech-frequency pure tone average [sp-PTA]: 29.69 ± 3.28 dB HL) and four older normal hearing controls (age: 63.5 ± 4.35 years, better ear sp-PTA: 15.53 ± 4.83 dB HL) were examined. Using magnetic resonance imaging, T1 weighted structural images were obtained from all participants. GM volume was assessed using whole-brain and region-of-interest (ROI) voxel-based morphometry in both groups using the Computational Anatomy Toolbox (CAT12) with Statistical Parametric Mapping (SPM12). The ROIs located in auditory regions including the inferior colliculus, medial geniculate nucleus, superior temporal

gyrus, and middle temporal gyrus; and in non-auditory regions including anterior cingulate cortex and parahippocampal gyrus were examined. The WFU Pick Atlas toolbox in SPM12 was used to generate bilateral 8-mm radius spheres for each ROI centered at MNI coordinates. Age, sex, and total intracranial volume were used as covariates of no interest. Results: Whole brain analysis using FWE corrected ($p<0.05$) at cluster level revealed reduced GM volume in the left and right cerebellum in older adults with age-associated hearing loss as compared to older normal hearing controls. Additionally, using a less stringent threshold, reduced GM volume in the right middle temporal gyrus was observed in the hearing loss group compared to the normal hearing group (uncorrected p-value; $p<0.001$ at voxel level). ROI analysis did not show any significant group differences. Conclusion: Our preliminary findings suggest GM alterations in auditory and non-auditory regions in older adults with age-associated hearing loss, echoing the results reported in younger and middle aged adults with hearing loss. However, given that our sample size was low, further examination is needed. We are currently collecting more data to better characterize GM changes in age-associated hearing loss.

Disclosures: S. Shende: None. **S. Shahsavarani:** None. **Y. Tai:** None. **R. Mudar:** None. **F. Husain:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.11/L13

Topic: D.01. Sensory Disorders

Support: NIH Grant R01-DC007905

Title: Synaptic zinc regulates cochlear resilience to noise-induced hearing loss

Authors: *B. B. BIZUP[1], A. THATHIAH[1], T. TZOUNOPOULOS[2];
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Abstract: Exposure to loud noise results in a degenerative cascade involving ribbon synapse loss, hair cell death, or even shearing of the basilar membrane. The capacity of the cochlea and spiral ganglion to be repaired following damage is limited and thus noise-induced hearing loss (NIHL) is often permanent. While some treatments, like growth factor application, have been shown to alleviate NIHL in certain circumstances, there are not any clinical strategies for curing NIHL, suggesting a need for novel insights into the mechanisms of NIHL.
Zinc is involved in neurodegeneration and is modulated by noise exposure within the central auditory system, but little is known regarding its contribution to NIHL in the cochlea. Vesicular (synaptic) zinc is involved in neurodegeneration following ischemic injury and nerve crush in the visual system. Synaptic zinc is dependent on ZnT3—the protein that loads zinc into synaptic

vesicles. ZnT3 is expressed in the cochlea, and while its precise location and roles in cochlear function are unknown, it may have a similar pathological role in NIHL.
To determine if ZnT3-dependent synaptic zinc is involved in NIHL, we measured auditory brain stem response (ABR) thresholds and wave 1 amplitudes—which represent auditory nerve activity--and performed cochlear whole mount histological analysis in wild-type (WT) and ZnT3-knockout (KO) mice following sham or noise exposure. After noise exposure, KO mice display better recovery of both ABR thresholds and ABR wave 1 amplitude relative to wild-type mice, indicating that KO mice have resilience to and better recovery of cochlear function from NIHL compared to WT mice. Furthermore, this protection in KO mice is associated with higher number of ribbon synapses than WT mice 1 day after NE. To evaluate whether synaptic zinc is involved in cochlear degeneration, we treated wild-type mice systemically with the zinc chelator, TPEN, prior to and following noise exposure. We found that chelating zinc results in improved cochlear physiological function and increased ribbon synapse density in noise-exposed mice. Together, these data suggest that ZnT3-dependent synaptic zinc contributes to vulnerability to NIHL, and that zinc may be a novel target in treating NIHL.

Disclosures: B.B. Bizup: None. **A. Thathiah:** None. **T. Tzounopoulos:** None.

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394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.12/L14

Topic: D.01. Sensory Disorders

Title: Inner ear exposure of SENS-401 is not altered by severe acoustic trauma in a rat model

Authors: C. ROMANET, C. TRAN VAN BA, P. LIAUDET, M. PETREMANN, ***J. DYHRFJELD-JOHNSEN**;
Sensorion SA, Montpellier, France

Abstract: The blood-labyrinth barrier (BLB) plays a role analogous to the blood-brain barrier, forming a selective protective barrier between the inner ear and the circulatory system. Damage to the inner ear has been demonstrated to increase BLB permeability for systemically administered macromolecules, however, the impact of this on systemically administered small molecules is unknown. Significant impact of BLB permeabilization on drug candidate local exposure of s in animal models would need to be accounted for in the translation of preclinically effective drug doses to the clinical setting for inner ear disease drug development.
SENS-401 is a small molecule otoprotectant currently in phase 2 clinical trials for the treatment of Sudden Sensorineural Hearing Loss (SSNHL). Preclinical otoprotectant efficacy with significant improvement of hearing outcomes by SENS-401 treatment vs placebo has been demonstrated in an animal model of SSNHL caused by acoustic trauma (Petremann et al., 2019),

one of many potential causes in the clinical setting.
The effect of acoustic trauma-induced damage to the BLB on SENS-401 local exposure was evaluated in a rat model. Male Wistar rats underwent severe acoustic trauma (120 dB SPL, 2 hours) or a sham equivalent exposure. A single oral dose of 13.2 mg/kg SENS-401 was administered orally either immediately after (n=4/group) or 24h after (n=16/group) end of acoustic trauma or sham trauma. In the 24h administration groups, hearing status was confirmed by auditory brainstem response (ABR) evaluations pre-trauma (all animals) and 24 hours post-trauma (acoustic trauma animals). Drug concentrations were measured in the blood and temporal bone (n=4/time-point) from 0.5 to 4 hours after administration using LC-MS/MS.
At 24h after acoustic trauma, ABR recordings confirmed an acute hearing loss with mean threshold shifts in the range of 53-58 dB across test frequencies of 8/16/25/32 kHz. Following administration immediately after severe acoustic trauma or with a 24h delay, no significant differences in SENS-401 concentration profiles of sham trauma versus acoustic trauma animals were found either locally or systemically.
In conclusion, while local exposure of macromolecular agents has been demonstrated to be significantly impacted following damage to the BLB for example by acoustic overexposure, a severe acoustic trauma had a minimal effect on local exposure of SENS-401 in the inner ear after oral administration. This supports the use of animal pharmacokinetics and local exposure data for clinical trial dose selection, without a potential impact of difference between etiologies of SSNHL in the preclinical model and the clinical setting.

Disclosures: **C. Romanet:** A. Employment/Salary (full or part-time):; Sensorion SA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sensorion SA. **C. Tran Van Ba:** A. Employment/Salary (full or part-time):; Sensorion SA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sensorion SA. **P. Liaudet:** A. Employment/Salary (full or part-time):; Sensorion SA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sensorion SA. **M. Petremann:** A. Employment/Salary (full or part-time):; Sensorion SA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sensorion SA. **J. Dyhrfjeld-Johnsen:** A. Employment/Salary (full or part-time):; Sensorion SA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sensorion SA.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.13/L15

Topic: D.01. Sensory Disorders

Support: MOMRP JPC-5 Program

Title: Neurosensory changes following low-level repeated blast exposure

Authors: *S. SAJJA[1], J. C. DEMAR, JR[2], L. HEYBURN[3], R. URIOSTE[4], A. BATUURE[4], D. WILDER[4], Y. WANG[3], A. PEETHAMBARAM[4], J. LONG[4];
[1]Walter Reed Army Insitute of Res., SilverSpring, MD; [2]Blast-Induced Neurotrauma Br., [3]Walter Reed Army Inst. of Res., Silver Spring, MD; [4]Walter Reed Army Inst. of Res., SilverSpring, MD

Abstract: Introduction: Multiple low-level blast exposures have been linked to impairment of neurosensory systems, prompting concern over the cumulative deleterious effects of blast and the need to define standards to mitigate this risk among Warfighters. There are no set guidelines establishing cumulative limits for number and intensity of blast exposures for training and operations of the Warfighter. Our objective in this study is to conduct a comprehensive assessment of auditory, visual and neurological outcomes using a rodent model of repeated low-level blast exposures.
Methods: Anesthetized rats (n=6/ group) were frontally positioned in an advanced blast simulator, which closely mimics "free-field" blast, and subjected to 1, 4 or 14 daily exposures at 4 or 6psi. Functional assessments were conducted with electroretinogram (ERG) for vision and distortion product otoacoustic emissions (DPOAE) and auditory brainstem response (ABR) for the auditory system. Assessments were made either 7 or 28 days following final exposure. Structural integrity of cochlea was evaluated using myosin VIIa and phalloidin markers and otoscopy determined the integrity of tympanic membrane.
Results: At 4psi (1, 4 or 14 exposures), significant decrements in DPOAE thresholds and impaired ABR response were observed at 7 and 28 days following blast, while significant reduction in ERG A- and B-waves were observed only in 6psi (14 exposure) groups at 28 days following blast. Outer hair cells were completely lost at the base and middle of cochlea but remained intact at the apex. Analyses for retinal damage are ongoing.
Discussion/conclusion: We identified in these preliminary findings that the ear is most susceptible to single or repeated blast exposures. Functional assessment of the visual system also shows vulnerability to low level repeated blast like that occurring in training and operations of Soldiers, which provides a first step towards identifying tolerable thresholds of blast exposure.

Disclosures: S. Sajja: None. **J.C. DeMar:** None. **L. Heyburn:** None. **R. Urioste:** None. **A. batuure:** None. **D. Wilder:** None. **Y. Wang:** None. **A. Peethambaram:** None. **J. Long:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

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Program #/Poster #: 394.14/L16

Topic: D.01. Sensory Disorders

Support: FFB Individual Investigator Award
NIH Grant MH113095

Title: Single-cell RNA-Seq development of AAV vectors

Authors: B. E. OZTURK[1], M. KLEYMAN[2], J. HE[1], S. TURUNC[1], A. PFENNING[2], W. R. STAUFFER[1], ***L. C. BYRNE**[1];
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Abstract: Efficient and targeted gene delivery is fundamental to experimental interrogation of the nervous system, as well as for the success of gene therapies. Sufficient levels of gene expression in the affected cell type are essential, while at the same time, off-target expression must be minimized for precisely targeted gene delivery. Here we create a complete atlas of viral vectors enabling efficient and specific expression of proteins across retinal cell types. Using single-cell RNA-Seq based methods, we have identified cell types by their transcriptome profiles and simultaneously quantified the performance of a library of viral vector variants, including naturally occurring serotypes and engineered variants selected for their ability to deliver transgenes across physical barriers to specific cell types. These methods are an unbiased and quantitative method by which viral variants can be compared across every cell type simultaneously, based on their ability to drive gene expression. This approach exponentially increases the rate by which new viral vectors can be developed. The experiments have been performed in primates, in order to create vectors with maximum potential to be translated for use in humans.

Disclosures: B.E. Ozturk: None. **M. Kleyman:** None. **J. He:** None. **S. Turunc:** None. **A. Pfenning:** None. **W.R. Stauffer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent applications on described methods. **L.C. Byrne:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder on viral vectors for gene therapy.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.15/L17

Topic: D.01. Sensory Disorders

Title: Analysis of viral vector targeting in cochleae after noise induced damage

Authors: *M. C. BAIRD[1,2], K. BEY[3], R. J. PINEDA[2], S. LIKHITE[2], R. LINDSAY[4], J. XIE[5], P. DISTEFANO[4], M.-A. COLLE[3], E. BIELEFELD[1], L. BIANCHI[6], K. MEYER[2];

[1]The Ohio State Univ., Columbus, OH; [2]The Res. Inst. at Nationwide Children's Hosp., Columbus, OH; [3]ONIRIS/INRA, Nantes Cedex 3, France; [4]Zebra Biologics, Concord, MA; [5]The Scripps Res. Inst., La Jolla, CA; [6]Oberlin Col., Oberlin, OH

Abstract: Noise-induced hearing loss (NIHL) is one of the most prevalent disabilities for which there is no effective therapeutic treatment. Auditory insult via noise exposure damages the sensory elements of the ear, leading to metabolic and/or mechanical damage to hair cells and degeneration of spiral ganglion neurons (SGN). To improve hearing in NIHL patients, the inner hair cell-SGN synapses must be maintained or regenerated. Though effective compounds for the treatment of NIHL have been suggested, challenges in delivery hinder the progress of these therapies to a clinical setting. In this study, we examined the efficacy of different AAV viral vectors and promoters, administered via different injection routes, in targeting the IHCs and SGNs. AAV vectors carrying a GFP transgene were delivered via different delivery routes. Vector transduction efficacy was compared in the presence or absence of noise injury. To induce auditory injury, mice were exposed to 100 dB sound pressure level (SPL) octave band noise for 2 hours. Five adult mice, both male and female, were used for each injection route and vector. Injections were performed at various time points pre and post noise exposure to determine whether noise exposure influenced vector transduction. Age-matched, uninjected mice were processed alongside experimental mice. Mice were sacrificed at varying time points post injection and cochleae were assigned a random number by a member of the lab to blind the processing and analysis of GFP expression in samples. Our results demonstrate that AAV vectors are effective in targeting cells in both healthy and noise damaged cochleae via multiple injection routes. Using gene therapy techniques, transgenes or therapeutic compounds can be efficiently delivered to the cochlea. Our findings open a novel avenue of therapeutic treatment for auditory injury and cochlear disorders.

Disclosures: M.C. Baird: None. **K. Bey:** None. **R.J. Pineda:** None. **S. Likhite:** None. **R. Lindsay:** None. **J. Xie:** None. **P. DiStefano:** None. **M. Colle:** None. **E. Bielefeld:** None. **L. Bianchi:** None. **K. Meyer:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 394.16/L18

Topic: D.01. Sensory Disorders

Support: Ohio Affiliate of Prevent Blindness

Title: Assessing the integrity of eye-brain communication in a mouse model of pediatric glaucoma

Authors: N. AMIRMOKHTARI[1], R. A. BOUHENNI[2], *M. A. SMITH[1];
[1]Northeast Ohio Med. Univ., Rootstown, OH; [2]Rebecca D. Considine Res. Inst., Akron Children's Hosp., Akron, OH

Abstract: Glaucoma is a group of eye conditions affecting both very young children and older adults where a buildup of eye fluid leads to an increase in eye pressure can stress/damage neurons in the retina of the eye-- causing them to die. New research in adult glaucoma suggests, retinal neurons lose their ability to transmit visual information via electrical signals to neurons in the brain long before retinal neurons themselves actually die. These findings suggest that eye-brain diseases like glaucoma may actually be preventable and/or treatable where restoring function to these “sick” retinal neurons may prevent/reverse vision loss, as opposed to replacing dead neurons with new ones---a more arduous task that has yet to be accomplished. Unfortunately, most glaucoma research is focused on adult rather than childhood manifestation of the disease, therefore, commonality of risk factors, timing, and etiologies of the disease between the developing and developed visual systems continues to be resolved. While elevated ocular pressure remains the primary modifiable risk factor for both childhood and adult glaucoma, the role of recessive pathogenic variants in the CYP1B1 gene continues to be a focus in pediatric glaucoma etiology. However, by what mechanisms and to what degree CYP1B1 variants contribute to disease onset and progression particularly regarding the relationship with elevated intraocular pressure remains to be determined. We set out to determine whether a predisposition to lack of CYP1B1 expression modulates timing, severity, and presentation of retinal ganglion cell structural and functional deficits when ocular pressure is artificially elevated. Using intracameral cannulation and microbead injection techniques we induced ocular hypertension in transgenic CYP1B1-knockout mice. Our data indicate additional alterations in CYP1B1KO RGC axons pertaining to the myelin sheath and nodes of Ranvier after induced ocular hypertension that are not seen in hypertensive RGC axons with normal CYP1B1 expression. Similar changes are seen to occur in naturally occurring glaucoma mouse models and have been suggested contribute to early changes in axon physiology. We conclude that CYP1B1 genetic variance may predispose developing RGC axons to factors that make them more susceptible for modification when ocular pressure is elevated.

Disclosures: N. Amirmokhtari: None. **R.A. Bouhenni:** None. **M.A. Smith:** None.

Poster

394. Discovery and Treatment Studies in Auditory and Visual Preclinical Neuroscience

Location: Hall A

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Program #/Poster #: 394.17/L19

Topic: D.01. Sensory Disorders

Support: American Foundation for Pharmaceutical Education Pre-doctoral Award in Pharmaceutical Sciences

Title: Ocular amyloid triggers signaling dysfunction in retinal ganglion cells

Authors: *E. S. PLYLER, M. A. SMITH, C. M. DENGLER-CRISH, S. D. CRISH; Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH

Abstract: Amyloid-beta (Aβ) is a major neuropathology of Alzheimer's disease (AD) that has also been implicated in age-related visual disorders such as glaucoma, macular degeneration, and diabetic retinopathy. In AD, retinal accumulation of Aβ appears prior to overt brain degeneration- likely contributing to visual defects seen in this disease. While early retinal amyloidosis shows promise as a biomarker, the effects of Aβ on visual function are not well understood. Previously, we found that ocular amyloidosis produces neuroinflammation in the primary visual pathway of mice, and caused retinal ganglion cell (RGC) death. However, the mechanisms of pathology remain unknown. Recently, our lab reported early neuronal signaling deficits between the eye and brain in glaucoma. These deficits occur in RGCs that remain connected to their postsynaptic targets in the brain and may drive later axon and cell body loss. As Aβ has also been implicated in glaucomatous neurodegeneration, we hypothesized that similar dysfunctions may precede RGC loss after retinal exposure to Aβ. We tested this hypothesis by assessing RGC activity in a validated model of induced ocular amyloidosis by measuring pattern electroretinogram (PERG) responses *in vivo* after visual stimulation. PERG is a minimally invasive approach that can be used to longitudinally screen mice for emerging functional deficits in the visual system, as well as to monitor disease progression. To induce ocular amyloidosis, we injected fibrillized $A\beta_{1\text{-}42}$ into the vitreal chamber of the left eyes of 6-8 mo. mixed-sex C57BL/6J mice. Control mice received comparable injections of saline vehicle. We measured PERG in these mice at post-induction time-points of 24 hours, 1 week, and 2 weeks. Our results indicated that Aβ-injected eyes had reduced RGC response to visual stimulation (i.e. reduced P1 amplitude) compared to baseline, and this signal worsened over the 2-week period. Control mice had reduced P1 amplitude at 24 hours post-injection, but recovered by 2-weeks post-injection. These results suggest that Aβ exposure may cause early RGC dysfunction, potentially contributing to visual deficits in glaucoma and AD. These functional deficits may provide a therapeutic window for rescuing cells by restoring function prior to overt RGC loss.

Disclosures: E.S. Plyler: None. **M.A. Smith:** None. **C.M. Dengler-Crish:** None. **S.D. Crish:** None.

Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 395.01/L20

Topic: D.02. Somatosensation

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Title: Primary-afferent-driven presynaptic inhibition of the C-fiber inputs to spinal lamina I projection and local-circuit neurons

Authors: E. FERNANDES[1], C. PECHINCHA[2], ***B. V. SAFRONOV**[2];
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Abstract: Spinal lamina I neurons play a key role in the nociceptive processing. They receive numerous inputs from unmyelinated C-afferents, which are processed and relayed to the specific areas of the brainstem and thalamus. Presynaptic inhibition of the primary afferents is critically important for the control of pain. Here we studied whether the afferent-driven presynaptic inhibition affects the C-fiber inputs to anatomically classified lamina I neurons. An isolated spinal cord preparation with attached dorsal roots was used for the whole-cell patch-clamp recording from biocytin- or retrogradely-labelled lamina I neurons. We have developed a novel method of the inverted current pulse stimulation to selectively evoke the pure C-afferent volley in the dorsal root. The degree of the presynaptic block was estimated by comparing the amplitudes of the monosynaptic C-fiber-mediated EPSCs evoked in a lamina I neuron by the pure C-fiber volley, and under conditions when arrival of the C-fiber volley was preceded by arrival of the Aβ-, Aδ- and C-volleys from the same or adjacent root. These results show that the homo- and/or heterosegmental Aβ-, Aδ- and C-afferents can mediate a complete or partial presynaptic block of the C-fiber inputs to the lamina I local-circuit and projection neurons. Thus, we present the first functional evidence for the primary afferent-driven GABAergic presynaptic inhibition of the direct C-fiber inputs to lamina I neurons. Our data suggest that, not only the low-threshold afferents, but also the high-threshold afferents mediate presynaptic inhibition of the primary nociceptors, and thus regulate the nociceptive information flow into the spinal cord.

Disclosures: E. Fernandes: None. **C. Pechincha:** None. **B.V. Safronov:** None.

Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 395.02/L21

Topic: D.02. Somatosensation

Support: NIH Grant NS100469

Title: Gucy2d is a novel and selective marker of a subset of dynorphin-expressing neurons in the spinal dorsal horn

Authors: *E. K. SERAFIN, J. LI, M. L. BACCEI;
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Abstract: Recent work has identified discrete subpopulations of inhibitory interneurons within the spinal dorsal horn (DH), defined by their expression of neurochemical markers such as parvalbumin, NPY and dynorphin, which modulate distinct modalities of somatosensory processing. However, none of the markers identified thus far are selectively expressed within DH neurons, instead showing widespread distribution across the peripheral and central nervous systems, thereby necessitating the use of complex intersectional genetic strategies to manipulate the function of a given neuronal population of interest. Here we identify the gene *Gucy2d*, encoding the membrane-bound guanylate cyclase GC-D, as a novel and highly selective marker of a subset of inhibitory DH interneurons derived from the dynorphin (*Pdyn*) lineage. An unbiased transcriptional analysis of dynorphin-lineage DH neurons revealed dramatic enrichment for *Gucy2d* within this population. *In situ* hybridization demonstrated that nearly all (~94%) of *Gucy2d*-expressing DH neurons also expressed *Pdyn*, although only ~54% of *Pdyn* neurons exhibited *Gucy2d* expression. The vast majority (~96%) of *Pdyn*-expressing neurons were located in laminae I-II with the remainder localized to lamina III. Furthermore, most *Gucy2d*-expressing neurons are inhibitory, as determined by co-expression of *Pax2* mRNA. Virtually all of these neurons (~99%) also co-express the transcription factor *Bhlhb5*, previously reported to be necessary for the development of dynorphin-lineage DH neurons which suppress chemical itch, as well as *Pde2a*, a phosphodiesterase previously used as a selective marker for *Gucy2d*-expressing neurons within the olfactory epithelium. The degree to which *Gucy2d* is expressed at the protein level in DH neurons remains unclear, as *in vitro* patch clamp recordings suggest that the application of the putative GC-D ligand uroguanylin (UG) fails to alter resting membrane potential or induce action potential firing in dynorphin-lineage spinal neurons. Importantly, within the brain, strong expression of *Gucy2d* mRNA was restricted to the olfactory bulb, with a very weak and diffuse signal visible in the hindbrain and an absence of detectable expression in all other brain regions. *Gucy2d* mRNA was also absent from the dorsal root ganglia (DRG), raising the possibility that utilization of the *Gucy2d* promoter could provide a novel means to

selectively manipulate dynorphin-expressing neurons at the level of the spinal dorsal horn. Future studies will investigate the role of the *Gucy2d*-expressing population in the regulation of pain and itch signaling within the DH.

Disclosures: E.K. Serafin: None. **J. Li:** None. **M.L. Baccei:** None.

Poster

395. Somatosensation: Spinal Circuits

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Topic: D.02. Somatosensation

Support: R01 NS 095162

Title: Movement gating of cutaneous signals in the cuneate nucleus

Authors: *Q. HE[1], A. K. SURESH[1], C. VERSTEEG[2], J. M. ROSENOW[3], L. E. MILLER[4], S. J. BENSMAIA[1];
[1]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [2]Biomed. Engin., [4]Dept. of Physiol., [3]Northwestern Univ., Chicago, IL

Abstract: The cuneate nucleus (CN), located in the medulla, is the first recipient of tactile signals along the medial lemniscal pathway. This neural structure is known to receive top down signals from cortex, and these signals have been implicated in modulating bottom up input. Indeed, sensitivity to cutaneous stimulation is reduced during movement or force generation, and this reduced sensitivity has been traced down to the CN. In the present study, we seek to characterize the spatial structure and temporal dynamics of this modulating. Specifically, we investigate whether the gating is spatially uniform, and affects the entire body surface equally, or sculpted to reduce sensitivity in body regions where cutaneous signals are expected to be behaviorally irrelevant. We also investigate the time course over which this gating occurs: Does it precede movement or force generation? If so, to what extent? To address these questions, we trained Rhesus macaques to perform a reach to grasp task while we measure the neural activity evoked by tactors placed over the receptive fields of CN neurons. Tactors are activated periodically before, during, and after movement and before, during, and after force generation. We then assess the degree to which the cutaneous response is modulated in the various task epochs.

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Poster

395. Somatosensation: Spinal Circuits

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Topic: D.02. Somatosensation

Support: NSERC
DMRF

Title: Comparison of dorsal and ventral sensory afferent involvement in crossed reflex

Authors: *O. D. LAFLAMME, M. IBRAHIM, T. AKAY;
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Abstract: Sensory afferent feedback is important for coordinating the movement of multiple joints within and between the legs during locomotion, as removal of sensory feedback severely impairs movement. It has been established in humans and cats that sensory information from one leg influences the motor neuron (MN) activity of the contralateral leg (crossed reflex) through spinal commissural interneurons (CINs). More recently, *in vivo* crossed reflexes in mice have been shown to modulate the contralateral motor responses through excitatory and inhibitory pathways (Laflamme and Akay, 2018; *J Neurophysiol*). In this previous study, crossed reflex responses were recorded by stimulating either the proprioceptive afferents from extensor muscles or the cutaneous afferents from the posterior surface of the leg. Crossed reflexes transduced by different sources of sensory afferents have not been shown. Our goal in this study is to determine if proprioceptive feedback from flexor muscles or cutaneous feedback from the anterior surface of the leg elicits similar crossed reflex responses. We describe crossed reflex responses elicited by the stimulation of the contralateral cutaneous afferents from the anterior surface of the leg, and from the proprioceptive afferents of flexor muscles (peroneal nerve stimulation). These results were compared to the stimulation of contralateral cutaneous afferents from the posterior surface and proprioceptive afferents from extensor muscles (tibial nerve stimulation). Our data show two findings. First, that peroneal nerve stimulation evokes crossed reflex motor responses, but no clear motor pattern could be detected, in contrast to tibial nerve stimulation. Second, that inhibitory crossed reflex effects are present when the peroneal nerve is stimulated; however, the inhibitory effect is only found in a subset of flexor and extensor muscles. This contrasts with our previous data involving tibial nerve stimulation where the inhibitory crossed reflex was observed in all recorded muscles. The patterned muscle activation resulting from crossed reflexes depends on the source of the afferent feedback. These outcomes provide the groundwork for our future aim to describe the spinal network that controls crossed reflexes in mice.

Disclosures: O. D. Laflamme: None. **M. Ibrahim:** None. **T. Akay:** None.

Poster

395. Somatosensation: Spinal Circuits

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Topic: D.02. Somatosensation

Support: CONACyT 50900

Title: Supraspinal and segmental contribution to the effects of systemic and local injections of picrotoxin on the patterns of functional connectivity between dorsal horn neurons

Authors: *L. MORENO[1], N. PLAMENOV DONCHEV[1], D. CHÁVEZ[1], E. HERNÁNDEZ[1], S. GLUSMAN[2], P. RUDOMIN[1,3];
[1]CINVESTAV, Mexico City, Mexico; [2]Stroger Cook County Hosp., Chicago, IL; [3]El Colegio Nacional, Mexico City, Mexico

Abstract: In the anesthetized cat, the patterns of functional connectivity between the dorsal horn neurons are not random but rather structured (Contreras et al, 2015). During low levels of neuronal synchronization this ensemble generates negative cord dorsum potentials (nCDPs) and there is a concurrent activation of the pathways mediating non-reciprocal postsynaptic inhibition. In contrast, during higher synchronization, the same neuronal network generates negative-positive cord dorsum potentials (npCDPs) as well as dorsal root potentials (DRPs), a sign of PAD. So far we assumed that this PAD was GABAergic. Yet, Hochman et al., (2010) suggested that a fraction of the DRPs could be of a non-GABAergic origin. The present studies were aimed to disclose the role played by $GABA_A$ mechanisms in the generation of the npCDPs and DRPs. This seemed a relevant issue because we have used different classes of CDPs selected according to their shape and amplitude to infer the changes in the global state of neuronal connectivity induced by nociception (Chávez et al., 2012; Martin et al., 2016). In pentobarbital anesthetized, paralyzed and artificially ventilated cats, we examined the changes produced by picrotoxin (PTX, a $GABA_A$ antagonist) on the ongoing correlation between CDPs recorded on the L4-L7 spinal segments. PTX was administered either systemically (0.5 mg/kg) or through a glass micropipette inserted in the deep L6 dorsal horn (4 μg/10 μL). In preparations with intact neuroaxis, systemic PTX induced slow synchronized “epileptic like” CDPs in the recorded segments. The participation of spinal and/or supraspinal mechanisms in the generation of this synchronized activity was elucidated by testing the effects of systemic PTX in a preparation with a spinal transection between L5 and L6. We found that PTX induced epileptiform activity and increased the occurrence of npCDPs only in those spinal segments that remained connected to supraspinal structures. In contrast, the segments below the lesion showed no epileptiform activity while there was a significant reduction in the occurrence of npCDPs. In another series of experiments the PTX microinjection decreased the number of npCDPs without inducing

epileptiform activity, that was however generated by a subsequent systemic injection of PTX, both in the rostral ventromedial medulla and in the dorsal horn. The present observations indicate that the PAD associated with the generation of the npCDPs and DRPs has a significant $GABA_A$ component that is blocked by PTX. They also provide evidence of a functional coupling between supraspinal and spinal neurons, a process of relevance in the shaping of sensory information (Plamenov et al., 2018).

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Poster

395. Somatosensation: Spinal Circuits

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QPRN

Title: Phox2a defines a developmental origin of the anterolateral system and is required for supraspinal nociception

Authors: *R. B. ROOME[1], S. RASTEGAR-POUYANI[1], C. SALESSE[1], B. MONA[2], K. LISTER[3], S. FERLAND[4], W. S. THOMPSON[1], S. SOTOCINAL[3], A. DUMOUCHEL[1], J. E. JOHNSON[2], M. KMITA[1], J. S. MOGIL[3], Y. DE KONINCK[4], A. KANIA[1];
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Abstract: The mammalian anterolateral system (ALT) is the main pathway relaying pain, itch and temperature information from the spinal cord to the brain. Classical anatomical and physiological experiments suggest a functional diversity of the spinal projection neuron (PN) subpopulations that give rise to this pathway. However, without molecular handles, their relative contribution to somatosensation remains unknown.
The Paired-like homeobox 2A (Phox2a) transcription factor is expressed in the dI5 spinal neuron lineage hypothesised to give rise to PNs. Using mouse genetic anterograde and retrograde labelling, we show that virtually all spinal neurons of the Phox2a lineage give rise to PNs that innervate ALT nociceptive targets, such as the parabrachial nucleus, peri-aqueductal grey, nucleus of the solitary tract and the thalamus. Nearly half of spinal PNs innervating the

parabrachial nucleus and the thalamus express Phox2a, revealing it as the first exclusive molecular handle of a major spinofugal PN population. Phoxa2 PNs are born concurrently with motor neurons, constituting the earliest spinal postmitotic population. The spinal dI lineage determinants Ascl1 and Ptf1a control the early aspects of Phox2a PNs specification, directing them to develop into all anterolateral system spinal PN types, first populating laminae I, then the deeper laminae II/III and V. A developmental loss of spinal Phox2a function does not affect nociceptive spinal reflexive behaviours. In contrast, behaviours that involve supraspinal relay of nociceptive information are impaired in these mutants, arguing that normal ALT development requires Phox2a.
Our experiments provide a molecular handle on a major constituent PN population of the ALT, allowing us to probe its precise adult function. Currently, we are determining whether Phox2a PNs encode noxious stimulus modality, location, intensity and/or its emotive dimension.

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Poster

395. Somatosensation: Spinal Circuits

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Topic: D.02. Somatosensation

Support: 1R01CA221363

Title: Feature extraction by muscle proprioceptors using machine learning

Authors: *S. N. HOUSLEY[1], T. C. COPE[2];
[2]Applied Physiol. and Engin., [1]Georgia Inst. of Technol., Atlanta, GA

Abstract: Spike train encoding of muscle stretch by muscle proprioceptors is altered in animals treated with chemotherapy. We find great variability in the magnitude of change expressed among the multiple measures used to paramaterize spike trains responding to dynamic and static mechanical stimuli. Interest in the behavioral consequences of spike train corruption led us to ask how neurotoxic effects of chemotherapy affect mechanical feature extraction by muscle proprioceptors. To answer this question, we applied modern machine learning techniques, including long short-term memory neural networks, to test what features of position and dynamics are preferentially decoded from spiking activity of physiologically identified proprioceptors. We first trained neural networks on populations of recorded mechanosensory responses from three classes of physiologically identified proprioceptors in healthy adult rats

anesthetized with isoflurane. Neurons were classified as muscle spindle group Ia or II or as tendon organ group Ib afferents. Trained models successfully predicted both stereotyped and pseudorandom movements with individual neuron classes preferentially contributing unique feature information during decoding. We then employed our trained models to test how feature extraction is impaired following a neurologic insult known to impair sensory encoding, chemotherapy.
Upstream consequences of these neural deficits are unknown but could provide valuable insight to understand and assist in predicting behavioral deficits in humans treated with chemotherapy. Population spiking activity of the three classes of proprioceptors recorded from animals treated with chemotherapy were used to generate predictions. Preliminary results indicate significantly corrupt feature extraction. We show both under and over estimation of stimulus features. For example, static positions are consistently under predicted. Our results suggest that unpredictable sensorimotor integration may contribute to lasting behavioral deficits documented in patients treated with chemotherapy.

Disclosures: **S.N. Housley:** None. **T.C. Cope:** None.

Poster

395. Somatosensation: Spinal Circuits

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Support: NIH 1R01NS092894-01
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Title: Reward and force modulation of neurons in the primate primary somatosensory cortex (S1)

Authors: *M. U. ATIQUE[1], J. T. FRANCIS[2];
[1]Biomed. Engin., [2]Univ. of Houston, Houston, TX

Abstract: Sensorimotor cortices play an important role during movement and action observation. Previous work has introduced the fact that reward level can modulate activity in S1 (McNiel et al 2016). In this study, we show that force related activity in S1 is also modulated by the cued level of possible reward. We analyzed data from two monkeys, one male (Macaca radiata, Monkey S) and one female (Macaca Mulatta, Monkey P). Trials consisted of a psychophysical task where the monkey had to apply an appropriate amount of grip force to a force transducer to enable a simulated robotic manipulator to grasp and transport an object to a targeted location. For each of these monkeys, we used two different blocks of manual trials. One

block consisted of trials where the monkeys were given a cue for the level of reward while the other block was uncued. We used the spiking activity of units from S1 for our analysis. We measured the significance (F-test, p-value < 0.05) between the spike rate of the units and applied grip force. Additionally, a separate analysis was performed to calculate the significance (t-test, a p-value < 0.05) for the reward level using the post reward spiking activity (500ms for each trial). The units that are common to both force and reward significance tests were collected. For Monkey S we have found 53 units that are significant for both force and reward. For monkey P the significant unit number was 22. The raster plots during the cue presentation and reward delivery periods showed the difference in activities of the same unit between rewarding and non-rewarding trials. The spiking activity of neurons during the grasping period represents a similar variation to the applied force. We used Multiple Linear Regression (MLR) on spike rate to predict force from manual cued and uncued blocks. The correlation coefficient (R-square) between the predicted force and the actual applied force for Monkey S was 0.86 and 0.83 for cued and uncued blocks respectively. For Monkey P the correlation coefficient was 0.83 and 0.82. We computed the linear tuning curves for the force during rewarding and non-rewarding trials and measured if they are significantly different for each individual units (F-test, p-value < 0.05). For Monkey S, in the cued manual block, there were 21 units that showed a significant difference between tuning curves of rewarding and non-rewarding trials whereas the number of significant units were 30 for Monkey P. The results we have presented here demonstrate that units from S1 have a representation of grip force and this representation is modulated by the reward expectation.

Disclosures: **M.U. Atique:** None. **J.T. Francis:** None.

Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 395.09/L28

Topic: D.02. Somatosensation

Title: Is this a vibration evoked potential? Preliminary study in healthy subjects

Authors: F. OVAK BITTAR[1], ***M. M. SABBAHI**[2];
[1]Texas Physical Therapy and Electrophysiology Services, Houston, TX; [2]Texas Electrophysiology and Physical Therapy, Houston, TX

Abstract: Vibration has long been known to be a strong and specific stimulus to muscle spindle afferents. Its effect on Ia afferents with its transmission to the cerebellum via spinocerebellar tracts has been a central neural information for most neurological testing. Its dysfunction in spinal cord injuries and other spinal disorders such as Tabes dorsalis, transvers myelitis and dorsal spinal column pathologies provide the clinical utility for a large array of neurological

disorders. Despite all these possibilities for clinical application, to our knowledge, vibration evokes potential has not been recorded or reported in the current literature. The purpose of this study is to present preliminary results to record Vibration Evoked potential in healthy subjects. Peroneal stimulation at the ankle level was electrically stimulated using 0.5 ms. Pulses at 0.5 pps at zero intensity. This was used to trigger the sweeps of a peroneal somatosensory evoked potential. The vibration potential was recorded using bipolar surface electrodes applied on T11-12 and C7 vertebral spinal levels with the ground electrode applied on the iliac crest. Subject stood on the surface of Whole-Body Vibration unit operated for 90 sec. at 50 Hz of low amplitude. Vibration electrical signal was averaged and recorded for the 90 sec. period. Three trials were recorded and analyzed. This is an exploratory descriptive study. Results showed a Vibration evoked potential after 11 msec. from the electric signal artifact. Signal for the T11-12 location, showed a peak to peak amplitude was 50 uv. Biphasic in shape. Vibration signal was smaller for the C7 vertebral location and was recorded at 14msec). Modulation of these Vibration Evoked potentials will be the focus of future studies. In a parallel study an attempt was carried out to record the current vibration potential via stimulation of the tibial nerve at the popliteal fossa. Vibration evoked potential could not be recorded using tibial nerve pathways. These vibration potentials can be useful testing method for patients with central spinal disorders and pathologies.

Disclosures: F. Ovak Bittar: None. **M.M. Sabbahi:** A. Employment/Salary (full or part-time):; Texas Woman's University School of Physical Therapy, Texas Physical Therapy and electrophysiology Services.

Poster

395. Somatosensation: Spinal Circuits

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Topic: D.02. Somatosensation

Support: Wellcome Trust (grant 102645)
Biotechnology and Biological Sciences Research Council (grant N006119/1)

Title: Electrophysiological and morphological characterisation of excitatory interneurons in the dorsal horn of the spinal cord

Authors: *A. C. DICKIE[1], A. M. BELL[1], N. IWAGAKI[1], E. POLGÁR[1], M. GUTIÉRREZ MECINAS[1], R. KELLY[1], H. LYON[1], K. TURNBULL[1], S. J. WEST[2], A. ETLIN[3], J. M. BRAZ[3], M. WATANABE[4], D. L. H. BENNETT[2], A. I. BASBAUM[3], J. S. RIDDELL[1], A. J. TODD[1]; [1]Spinal Cord Group, Univ. of Glasgow, Glasgow, United Kingdom; [2]The Nuffield Dept. of Clin. Neurosciences, Univ. of Oxford, Oxford, United Kingdom; [3]Dept. of Anat., Univ. of California San Francisco, San Francisco, CA; [4]Hokkaido Univ. Sch. of Med., Sapporo, Japan

Abstract: Excitatory interneurons (eINs) account for the majority of neurons in the superficial dorsal horn, but despite their presumed roles in pain and itch, our knowledge of their organisation and function is limited. We have shown that eINs can be assigned to several distinct populations based on the expression of neurochemical markers, and this broadly agrees with recent transcriptomic studies. We have used anatomical and electrophysiological techniques to characterise eINs that express Substance P (SP), Gastrin-releasing peptide (GRP), or the GRP receptor (GRPR), to determine whether they are functionally distinct. Transgenic mouse lines used to identify these eINs include; GRP::eGFP (GRP), $Tac1^{Cre}$ (SP) and $GRPR^{CreERT2}$;Ai9 (GRPR). $Tac1^{Cre}$ mice were intraspinally injected with AAV.Flex.tdTom or AAV.Flex.eGFP. In $Tac1^{Cre}$;GRP::eGFP mice intraspinally injected with AAV.Flex.tdTom there was minimal overlap between GFP+ (GRP) and tdTom+ (SP) cells, showing that they are distinct populations. This was confirmed with in situ hybridisation. Similarly, we found that GRPR cells are a distinct group. Patch-clamp electrophysiology in spinal cord slices, was used to study the eINs. Action potential firing patterns in GRP cells were mainly transient or single spike. This differed from SP cells, which mostly showed delayed firing, and GRPR cells, which showed delayed or single spike firing. EPSC frequency was higher in SP and GRPR cells, suggesting they have a greater excitatory drive than GRP cells. Almost all GRP cells responded to the MOR agonist, DAMGO, but were largely unresponsive to NA or 5-HT. In contrast most SP cells were sensitive to NA and 5-HT, but not DAMGO. Morphology was assessed in GRP cells filled with neurobiotin during patch-clamp recordings, and SP and GRPR cells in perfusion fixed tissue from mice injected with Brainbow AAVs. This demonstrated that these eINs were morphologically distinct. Although GRP cells were heterogeneous, some could be classified as central cells. In contrast, many SP cells resembled radial cells. GRPR cells exhibited vertical cell morphology. Our findings demonstrate that SP, GRP and GRPR eINs show major differences in their morphological, electrophysiological and pharmacological properties. Based on somatodendritic morphology and firing patterns, we propose that SP cells correspond to a population known as radial cells, while GRP cells are likely to overlap extensively with a population previously classified as transient central cells, and GRPR cells are a type of vertical cell. Our findings indicate that SP, GRP and GRPR cells are functionally distinct, and presumably have different roles in somatosensory processing.

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Poster

395. Somatosensation: Spinal Circuits

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Program #/Poster #: 395.11/L30

Topic: D.02. Somatosensation

Support: Wellcome Trust (grant 102645)
BBSRC (grant N006119/1)

Title: Connectivity and molecular identity of neurons expressing gastrin-releasing peptide in the superficial dorsal horn in a GRPeGFP transgenic mouse line

Authors: *A. BELL, M. GUTIERREZ-MECINAS, E. POLGAR, A. DICKIE, A. TODD;
Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Gastrin releasing-peptide (GRP) released in the superficial dorsal horn (SDH) of the spinal cord has an important role in itch sensation. This peptide is released by a distinct population of excitatory interneurons which have been shown to have roles as secondary pruritoceptors. In BAC transgenic mice that express enhanced green fluorescent protein (eGFP) under control of the GRP promotor, these cells have been shown to be functionally and morphologically homogeneous and show little neurochemical overlap with other defined classes of SDH interneuron. However recent transcriptomic studies report that Grp mRNA is widely distributed in the dorsal horn among several excitatory interneuron clusters.To resolve this apparent discrepancy, here we aim to define the molecular identity of specifically those GRP cells expressing eGFP. Additionally, the complex neuronal circuits that they engage have not been fully elucidated and using anatomical techniques we aim to identify both the source of input and synaptic targets of these cells.Multiplex *in situ* hybridisation was performed on spinal cord tissue using RNAScope with probes against both GRP and eGFP and other markers of interneuron populations. Virtually all eGFP mRNA+ cells in this mouse line contain GRP mRNA however eGFP expression was only evident in 20-30% of GRP mRNA+ cells. While GRP mRNA+ cells were present throughout lamina I and II, eGFP+ cells tended to form a tighter band in mid lamina II. GRP transcripts overlapped with markers for several excitatory interneuron clusters (Glut5-12 i.e Tac1, Tac2, Npff & Nmur2) but eGFP did not with the exception of Nmur2 where moderate overlap was evident.To investigate neuronal circuits involving GRP-eGFP cells, confocal microscopy was used to reveal excitatory synaptic inputs on dendritic trees using immunostaining for the postsynaptic protein Homer. This allows estimation of the amount of synaptic input to and from GRP-eGFP cells in an unbiased manner. Compared to SP-expressing excitatory interneurons, GRP-eGFP receive a higher proportion of their inputs (90% vs 40%) from primary afferent nerve fibres. These include non-peptidergic nociceptors and low threshold c-fibres. The axons of GRP-eGFP cells selectively innervate lamina II SP-expressing radial cells but do not target lamina I projection neurons directly.GRP-eGFP cells appear to represent a discrete functional population, even though GRP message is far more widely expressed. This discrete functional role is evident as GRP-eGFP cells form part of a neuronal circuit relaying convergent input from different classes of primary afferent to other specific interneuron populations with dendrites in lamina II.

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Poster

395. Somatosensation: Spinal Circuits

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Support: BBSRC grant BB/P007996/1

Title: Role of RorB-expressing lamina ii interneurons in gating nociceptive C-fibre input

Authors: *O. DAVIS[1], A. C. DICKIE[1], K. A. BOYLE[1], M. MUSTAPA[1], A. BELL[1], K. M. SMITH[2], B. A. GRAHAM[2], A. J. TODD[1], D. I. HUGHES[1];
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Abstract: Inhibitory interneurons in the spinal dorsal horn play a crucial role in controlling transmission of somatosensory information to the brain. Spinal inhibition is diminished in some chronic pain states, and inhibitory interneurons therefore represent a potential target for therapeutic intervention. Previous studies have identified a population of inhibitory interneurons in lamina II that express the calcium binding protein calretinin, and have shown that these are islet cells. We have identified a subpopulation of these cells that co-express the RAR-related orphan receptor beta (RorB), and the RorB-creERT2 mouse line provides a means of identifying and manipulating the function of these cells. We have used a combination of anatomical and electrophysiological approaches with genetically modified mice in which fluorescent proteins are expressed in RorB interneurons. We show that the dendritic trees of the RorB cells overlap extensively with central arbors of C-fibre mechano-nociceptors (defined by expression of Mas-related G-protein coupled receptor D; C-MrgD) and that they receive extensive synaptic input from these afferents. We also find that RorB-expressing axons form axo-axonic synapses onto central terminals of type I glomeruli, which originate from C-MrgD afferents. Peripheral stimulation under terminal anaesthesia revealed that RorB cells are preferentially activated by noxious mechanical stimulation, rather than noxious chemical (capsaicin) or noxious heat stimulation. A recent transcriptomic study assigned inhibitory calretinin cells to two subpopulations, Gaba8 and Gaba9, and reported that the Gaba9 population expressed mRNA for Tac1, the gene that encodes substance P. We had previously identified an inhibitory Tac1-expressing population and shown that these cells co-express calretinin, corresponding to the Gaba9 subpopulation. We find that the inhibitory Tac1 population includes RorB-expressing interneurons and accounts for approximately 40% of all inhibitory calretinin cells. The recent development of viral vectors coding for transgenes that depend on co-expression of two recombinases means that these cells can be targeted by crossing VGAT-Flpo mice with either calretinin-Cre or Tac1-Cre lines. This will allow studies of their role in pain behaviour.

Disclosures: **O. Davis:** None. **A.C. Dickie:** None. **K.A. Boyle:** None. **M. Mustapa:** None. **A. Bell:** None. **K.M. Smith:** None. **B.A. Graham:** None. **A.J. Todd:** None. **D.I. Hughes:** None.

Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 395.13/L32

Topic: D.02. Somatosensation

Support: MR/S002987/1

Title: Characterisation of gastrin-releasing peptide receptor-expressing neurons in the mouse spinal dorsal horn

Authors: *E. POLGAR, M. GUTIERREZ-MECINAS, A. BELL, A. C. DICKIE, R. CLARK, E. AB RASHID, A. J. TODD;
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Abstract: The spinal dorsal horn receives information from somatosensory afferents, including those that relay nociceptive and pruritic stimuli. Signals from these terminals are transmitted to a complex network of local interneurons. In lamina I-II of the spinal cord (SDH), excitatory interneurons account for ~75% of all neurons. Recently, among these cells we have identified five non-overlapping populations based on their neuropeptide content. In addition, transcriptomic studies identified a separate cluster of neurons, those that express the gastrin-releasing peptide (GRP) receptor (GRPR). While several lines of evidence implicate GRP-GRPR signalling in itch but not pain, our knowledge about the characteristics of GRPR expressing neurons remains elusive. We aimed to characterise the GRPR-expressing neurons in the mouse SDH. To study their morphology we performed intraspinal injection of Brainbow adeno-associated virus (AAV) in $GRPR^{CreERT2}$ mice. To determine whether GRPR cells form a distinct neuronal population we performed cluster analysis - based on morphometric dendritic parameters - and carried out co-localisation studies using various neuronal markers. We looked for phosphorylated ERK expression to test whether GRPR cells selectively respond to pruritic stimuli following intradermal injection of pruritogens and various noxious stimuli applied on the hind limb. In all of the above experiments bar the AAV-Brainbow injections, we used $GRPR^{CreERT2}$ mice crossed with the Ai9 reporter line, in which after tamoxifen treatment Cre^{+} cells are expected to be labelled with tdTomato. In order to confirm the recombination efficiency of the Cre we also injected AAV.flex.GFP into the spinal cord of $GRPR^{CreERT2}$;Ai9 mice. Our main findings are: (i) GRPR cells have specific morphological characteristics that correspond to vertical cells. (ii) The lack of co-localisation with other neuronal markers and cluster analysis data confirm that GRPR cells represent a distinct neuronal population within the superficial dorsal horn. (ii) Contrary to previous suggestions that these cells are required for mediation of itch sensation rather than pain,

we find that they also respond to various types of noxious stimuli. (iv) While there was a considerable overlap between viral labelled and tdTomato expressing cells, there were numerous GFP-only-labelled cells. This suggests that the intraspinal injection method is more efficient to capture GRPR-expressing interneurons. Since vertical cells have been shown to be presynaptic to lamina I projection neurons, our results suggest that GRPR cells may be an integral part of the itch circuit and are involved in spinal pain mechanisms.

Disclosures: E. Polgar: None. **M. Gutierrez-Mecinas:** None. **A. Bell:** None. **A.C. Dickie:** None. **R. Clark:** None. **E. ab Rashid:** None. **A.J. Todd:** None.

Poster

395. Somatosensation: Spinal Circuits

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Topic: D.02. Somatosensation

Support: BBSRC Grant BB/N006119/1

Title: Neuropeptide y-expressing dorsal horn inhibitory interneurons in spinal pain and itch circuits

Authors: *K. A. BOYLE, M. GUTIERREZ-MECINAS, A. C. DICKIE, E. POLGAR, A. M. BELL, D. I. HUGHES, A. J. TODD;
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Abstract: Previous work from our laboratory has identified neuropeptide Y (NPY)-expressing neurons of the dorsal horn as a population of inhibitory interneurons (INs) that are well placed to modulate spinal nociceptive circuits through connections with nociceptive projection neurons and other dorsal horn interneurons (Iwagaki et al. 2016). The aims of the current study were 1) to use intraspinal injection of adeno-associated viruses (AAVs) in adult RH26 NPY::Cre mice to allow targeted manipulation of the activity of dorsal horn INs that express NPY in the adult (NPY-INs), 2) to assess the effects of NPY-IN activity manipulation on dorsal horn nociceptive and pruriceptive circuit activity and associated behavioural output, and 3) to test the hypothesis that activation of NPY-INs will supress the pathological pain phenotypes observed in the plantar Complete Freund's Adjuvant (CFA) model of inflammatory pain and the spare nerve injury (SNI) model of neuropathic pain.
To achieve these aims, we unilaterally injected a Cre-dependent AAV construct that expresses the excitatory DREADD hM3Dq fused to mCherry (AAV.hM3Dq.mCherry), into the L3-5 dorsal horn of young adult RH26 mice. Systemic administration of the hM3Dq agonist CNO induced Fos expression (a marker of neuronal activation) in the vast majority of mCherry-expressing neurons in laminae I-III, and this was largely restricted to NPY-immunoreactive cells.

However the proportion of mCherry-negative neurons in laminae I-II that expressed Fos following noxious heat or a pruritic stimulus was significantly lower in CNO-treated animals compared to vehicle-treated controls, suggesting that NPY-IN activation can inhibit spinal neurons normally activated by these stimuli. Consistent with this conclusion, NPY-IN activation increased the noxious heat withdrawal threshold and markedly reduced chloroquine-induced itch behaviour, as well as increasing noxious mechanical and cold withdrawal thresholds. NPY-IN activation also reversed mechanical and thermal hypersensitivity in the plantar CFA inflammatory pain and SNI neuropathic pain models.
Based on these findings, we draw the following conclusions: 1) Intraspinal injections of AAV.DREADDs into adult RH26 mice allows the specific manipulation of dorsal horn NPY-IN activity, 2) NPY-IN activation supresses activity in dorsal horn circuits that process pain- and itch-related information, resulting in reductions in acute pain- and itch-related behaviours and 3) recruitment of NPY-INs reduces inflammatory and nerve injury-induced pain. Dorsal horn NPY-INs therefore represent a potential target for treatment of acute and/or pathological pain and itch.

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Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 395.15/L34

Topic: D.02. Somatosensation

Support: NIH Grant UH3NS100541

Title: Relationship of severity of sensory impairments and balance strategies in individuals with lower-limb amputation

Authors: *B. A. PETERSEN[1], P. J. SPARTO[2], L. E. FISHER[3];
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Abstract: People with lower-limb amputations often exhibit gait and balance impairments, with 52.4% reporting a recent fall. Amputees rely more heavily on vision than healthy controls, likely due to lack of somatosensory feedback. Additionally, most lower-limb amputations occur after chronic dysvascular disease, which can also impair sensation in the contralateral intact limb. However, there has been little research to date to correlate severity of sensory impairments and measures of postural control in this population. We evaluated sensation and postural control in eight people with lower-limb amputations (5 traumatic, 3 dysvascular). Somatosensory integrity of both the residual limb and intact limb was assessed by a physical therapist. Assessments

included monofilament testing, light touch sensation, protective (pinprick) sensation, two-point discrimination, and joint position sense. Participants' standing balance was assessed with the sensory organization test (SOT). In the SOT, participants stand on a force platform during altered visual (eyes closed, surround sway) or somatosensory feedback (platform rotation). Each of six feedback conditions consists of three 30-second trials. Standard measures of postural control (excursion, velocity, and 95% confidence interval ellipse) were collected in addition to equilibrium scores. Equilibrium scores indicate a participant's ability to stay within a normative 12° anteroposterior sway envelope. Sway area increased for all participants without vision. However, this decrease in performance was more substantial for those with lower-limb amputation compared with healthy controls. In the stable platform condition without vision, all subjects had lower equilibrium scores (76$\pm$16) than normative controls (92$\pm$ 3). Six subjects weight shifted toward the intact limb, however one subject lacking any sensation on the intact limb weight shifted to the prosthetic limb. Additionally, increased sway area without vision (indicating a higher reliance on visual feedback) was correlated with decreased sensation on the residual limb only. These results suggest that the degree of sensory impairment, primarily in the residual limb, may predict balance ability, particularly in cases where visual feedback is unreliable.

Disclosures: B.A. Petersen: None. **P.J. Sparto:** None. **L.E. Fisher:** None.

Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 395.16/L35

Topic: D.02. Somatosensation

Support: NIH Grant UH3NS100541

Title: Non-uniform recruitment of lower limb muscles by epidural stimulation of lumbosacral spinal cord in trans-tibial amputees

Authors: *D. SARMA[1], A. C. NANIVADEKAR[2], B. A. PETERSEN[2], M. LIU[2], E. R. HELM[1], M. L. BONINGER[1], L. E. FISHER[1], D. J. WEBER[2];
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Abstract: In the lower limbs, bilaterally coordinated spinal reflexes play an important role when responding to unexpected perturbations like slips and trips. These reflexes, responses to sensory inputs from the foot and ankle, also largely mediate the transitions between phases of gait. To restore proper ambulation to lower limb amputees, the ability to evoke and precisely control patterns of reflexive activity will be critical. Lumbar epidural spinal cord stimulation (eSCS) is an intervention that can modify dysregulated lower-limb sensorimotor functions and augment

residual motor capacity. In an ongoing study, we aim to improve balance and gait by electrically stimulating lateral structures in the lumbosacral spinal cord to generate sensations appearing to emanate from the missing limb, in turn evoking coordinated bilateral reflexive muscle activity. In addition to characterizing the conscious sensory percepts evoked by stimulation, a significant effort is being made to understand evoked reflex responses as stimulation engages spinal and subcortical reflex pathways. In one subject, epidural stimulation was delivered through percutaneously implanted (< 30 days) commercial SCS leads (Boston Scientific) to the L4-S1 spinal cord and dorsal roots. Stimulation was delivered at various pulse widths (0.2 to 1ms) at 1Hz across amplitudes ranging from 0.1mA to 6mA. Bipolar surface EMG was recorded bilaterally from 16 muscles, with reflex responses observed most robustly in the biceps femoris and semitendinosus in response to stimulation. The evoked reflexes (onset of 30±5ms) grew proportionally with stimulation amplitude, and were observed to be an order of magnitude larger during standing than sitting. Higher amplitude stimulation also recruited the residual gastrocnemius. These preliminary results suggest that laterally-targeted spinal cord stimulation can engage reflex pathways, which might be effective for restoring gait and balance adaptation after lower-limb amputation. Further studies will evaluate modulating and controlling these responses during active ambulation.

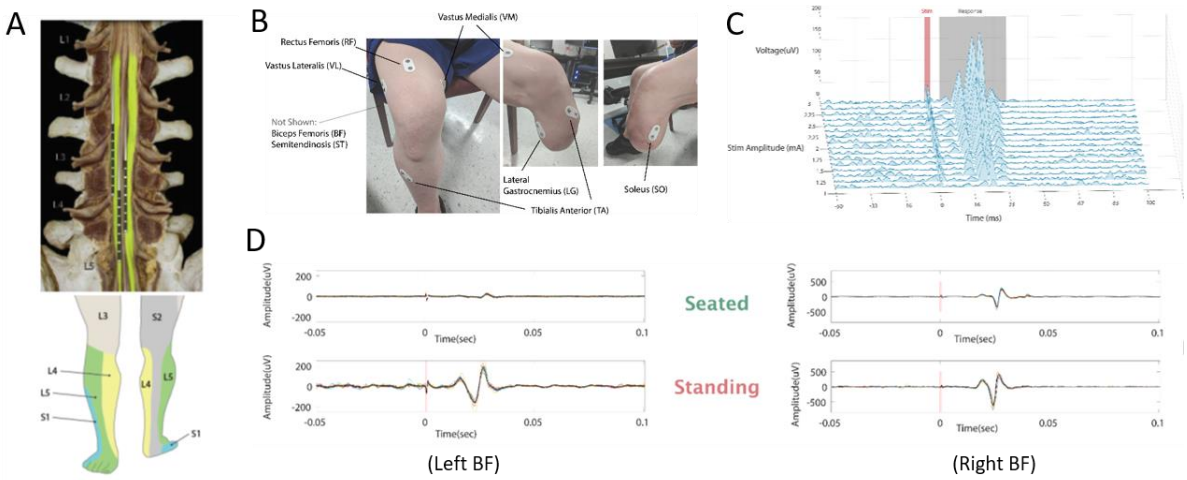

Figure 1 - Characterizing myoelectric responses to epidural spinal stimulation in a transtibial amputee. (A) Commercial spinal cord stimulation leads are implanted percutaneously to evoke sensory and myoelectric responses in the residual limb. (B) Bipolar sEMG recorded bilaterally. (C) Stim-evoked reflex responses grow with increased stimulation amplitude. (D) Reflex responses observed most robustly in hamstring muscles, bi- & uni- laterally, with responses during standing an order of magnitude larger than when sitting.

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Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 395.17/L36

Topic: D.02. Somatosensation

Support: NIH Grant UH3NS100541

Title: Effect of stimulus parameters on perceived sensation and phantom limb pain during stimulation of lumbosacral spinal cord in lower-limb amputees

Authors: *A. C. NANIVADEKAR[1], E. OKOROKOVA[4], B. PETERSEN[1], D. SARMA[1], S. J. BENSMAIA[5], E. HELM[1], M. BONINGER[1], D. J. WEBER[2], L. E. FISHER[3];
[2]Bioengineering, [3]Physical Med. and Rehabil., [1]Univ. of Pittsburgh, Pittsburgh, PA; [5]Dept. of Organismal Biol. and Anat., [4]Univ. of Chicago, Chicago, IL

Abstract: Numerous studies indicate that sensory feedback may enhance the embodiment and ease of use of prosthetic limbs and potentially alleviate phantom limb pain (PLP). Here, we present observations from human psychophysics experiments performed while stimulating the L4-S1 spinal cord to provide sensory feedback in an individual with trans-tibial amputation using FDA-approved spinal cord stimulation leads. Using percutaneous implantation techniques, we placed three 16-contact leads in the lateral epidural space near the lumbosacral spinal cord. Stimulation was delivered using a custom external stimulator during 15 in-lab sessions across 4 weeks, after which the electrodes were removed. Most evoked percepts (74%) were described as a ‘vibration’ or ‘buzz’ originating in the residual limb and in some instances extending down into the phantom limb. For all electrodes, the amplitude of stimulation had a strong positive correlation with the intensity of the reported sensation. As stimulation amplitude was increased the subject described the evoked percepts as feeling less natural. Stimulus frequency strongly affected the perceptual modality as well as the location of the evoked percept. For a subset of electrodes, stimulation frequencies above 200 Hz resulted in sensations exclusively in the phantom limb at the calf, ankle, toes and sole of the foot. At threshold, these sensations were described as faint ‘tickle’, ‘itch’ or ‘numbness’ that became more salient upon repeated stimulation. Additionally, a two-alternative forced choice task was administered to quantify the sensitivity to stimulation. The average detection thresholds across 4 electrodes was 1.33 ± 0.32 mA. Just-noticeable differences varied from 0.07 mA to 0.10 mA. To assess PLP, we administered the McGill Pain Questionnaire (MPQ) once before implantation, weekly during the 29-day study and one month after explant. We observed a 29-point decrease in MPQ score over the course of the implant period. These results suggest that stimulation of the dorsal spinal cord is a promising technique for providing sensory feedback and reducing PLP in individuals with lower-limb amputation.

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Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 395.18/L37

Topic: D.03. Somatosensation – Pain

Title: *In vivo* imaging: Dendritic spine dynamics on dorsal horn sensory neurons

Authors: *C. BENSON[1,3], K. K. FENRICH[4], M. HILL[2], K.-L. OLSON[3], S. PATWA[3], S. G. WAXMAN[1,3], A. M. TAN[1,3];
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Abstract: Neuropathic pain has been associated with maladaptive remodeling within the spinal cord pain circuitry. Specifically, our previous work has indicated a structure-function link between dendritic spine dysgenesis within the spinal cord and models of neuropathic pain. However, these prior studies relied on Golgi-stained post-mortem tissue, thereby limiting this analysis to static snap shots of a dynamic process.
To overcome this technical limitation, we have assessed the utility of implanting “glass windows” over the exposed spinal cord of living animals and applied the use multi-photon imaging technology to visualize neurons at the cellular level *in vivo.* Implanting a window over the lumbar spinal cord of thy1-YFP mice allows repeat imaging of dorsal horn neurons at a depth of 300-350um. At this depth we can visually document morphological (thin/mushroom) changes in dendritic spines within the superficial dorsal horn of the spinal cord. In uninjured animals, repeatedly imaging YFP expressing dorsal horn neurons reveals that fluctuations in dendritic spine morphology can be detected after 4 hours. Animals with implanted glass windows can survive for one month allowing us to track dendritic spines over an extended period.
To determine whether *in vivo* spinal cord imaging could detect neuropathic pain associated changes in dendritic spines, we repeatedly imaged animals after inducing a spared nerve injury (SNI). Seven days after injury, SNI animals displayed significant mechanical hypersensitivity that was associated with an increased number and stability of mushroom spines and increased turnover of thin shaped spines. Together, these results indicate that this technique can be used to monitor fluctuations in dendritic spines within the spinal cord pain circuitry. This provides a powerful tool to assess the efficacy of therapeutic interventions designed to modify structural and functional correlates of pain associated with injury.

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Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 395.19/L38

Topic: D.03. Somatosensation – Pain

Title: Prefrontal circuit dysfunction in a mouse model of neuropathic pain

Authors: *M. LI[1], S. TENG[2], G. YANG[1];
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Abstract: The prefrontal cortex is important for top-down regulation of sensory inputs. Recent *in vivo* electrophysiological studies in rodents suggest a decrease of neuronal firing rates in this region in chronic pain. However, the precise changes of prefrontal circuits underlying the development of chronic pain remain unclear. Here, using *in vivo* two-photon microscopy, we examined the Ca^{2+} activity of pyramidal neurons in different layers and regions of the medial prefrontal cortex (mPFC) in a spared nerve injury mouse model of neuropathic pain. In both prelimbic and secondary motor cortices, we observed a substantial reduction of pyramidal neuron activity across cortical layers. This reduction of prefrontal neuron activity occurred within days after peripheral neve injury and persisted for weeks. Using optogenetic manipulation in freely moving mice, we further showed that activation of prefrontal circuits in neuropathic pain ameliorated the animals' touch sensitivity and aversive quality of pain. Together, these results reveal prefrontal dysfunction in neuropathic pain and highlight mPFC as a potential therapeutic target for pain treatment.

Disclosures: M. Li: None. **S. Teng:** None. **G. Yang:** None.

Poster

395. Somatosensation: Spinal Circuits

Location: Hall A

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Program #/Poster #: 395.20/L39

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Conacyt CAO417840
Conacyt DCQL256990 CB 2015

Title: Organization of perineal muscle afferents on lumbosacral spinal cord in female rabbit

Authors: *C. ACOSTA ORTEGA[1], Z. FLORES-LOZADA[2], C. HERNÁNDEZ-BONILLA[3], I. JIMÉNEZ-ESTRADA[6], M. MARTÍNEZ-GÓMEZ[7], R. ZEMPOALTECA[4], D. L. CORONA-QUINTANILLA[5];
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Abstract: In mammalian females the pelvic floor provides a base to support the viscera of the pelvic cavity and allows reproductive and non-reproductive physiological functions. The complex innervation comes from different nerves such as the Levator ani and the pudendal. One branch of the sensory pudendal nerve innervates to bulbospongiosus muscles. Mainly, the afferents to travel to the dorsal horn of the spinal cord and are distributing transversely around the lateral and medial edge of the dorsal horn. The afferent and efferent information that carries bulbospongiosus nerve (Bsn) is important to regulate the contraction of its muscle and other functions of the urogenital system, as the micturition. Is very important to determine the distribution of Bsn cord dorsum potentials (Bsn CDPs) to identify the relevant structures, for instance pudendal afferents within dorsal sacral roots, which should be spared during rhizotomy procedures for treatment of spasticity or to establish treating lower urinary tract dysfunction by 'neuromodulation'. The aim of the study was determine the organization of these aferences across the Bsn CDPs activated by electrostimulation of the Bsn. We used young virgin rabbits (n = 8), the aged was between 6 ± 8 months and anesthetized with urethane, the Bsn was located and mounted in a silver electrodes. The organism in a spinal unit record was fixed in prone position and a laminectomy of the lumbosacral dorsal region (L5-S3) was performed. The dura mater was partially removed and the CDPs were recorded in response to the electrical stimulation of the Bsn. The signals were amplified with a Grass 7P511 amplifier and visualized with a Tektronix TDS2024C oscilloscope. Was analized the amplitude, latency, peak latency, duration and the number of components. Each registers obtained was average of 16 events. The Bsn CDPs showed differences in the number of components and their amplitude. The mayor CDPs amplitude was obtained in S1 lever and lowered to rostral and caudal spinal cord. This results showed the afferent information of perineal muscles is not limited to a single spinal segment, and could help to explain the regulation of micturition, deliver or other physiological processes in the pelvic floor components.

Disclosures: C. Acosta Ortega: None. **Z. Flores-Lozada:** None. **C. Hernández-Bonilla:** None. **I. Jiménez-Estrada:** None. **M. Martínez-Gómez:** None. **R. Zempoalteca:** None. **D.L. Corona-Quintanilla:** None.

Poster

396. Somatosensation: Itch Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 396.01/L40

Topic: D.02. Somatosensation

Support: National Natural Science Foundation of China (No. 31771158)
Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDBS01000000)

Title: Tac1-expressing neurons in the periaqueductal gray facilitate the itch-scratching cycle via descending regulation

Authors: *W.-Z. CHEN, Z.-R. GAO, M.-Z. LIU, X.-J. CHEN, X.-Y. ZHANG, L. YUAN, Y.-G. SUN;
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Abstract: Uncontrollable itch-scratching cycles lead to serious skin damage in patients with chronic itch. However, the neural mechanism promoting the itch-scratching cycle remains elusive. Here, we report that tachykinin 1 (Tac1)-expressing glutamatergic neurons in the lateral and ventrolateral periaqueductal gray (l/vlPAG) facilitate the itch-scratching cycle. We found that l/vlPAG neurons exhibited scratching-behaviorrelated neural activity and that itch-evoked scratching behavior was impaired after suppressing the activity of l/vlPAG neurons. Furthermore, we showed that the activity of Tac1-expressing glutamatergic neurons in the l/vlPAG was elevated during itchinduced scratching behavior and that ablating or suppressing the activity of these neurons decreased itch-induced scratching behavior. Importantly, activation of Tac1-expressing neurons induced robust spontaneous scratching and grooming behaviors. The scratching behavior evoked by Tac1-expressing neuron activation was suppressed by ablation of spinal neurons expressing gastrin-releasing peptide receptor (GRPR), the key relay neurons for itch. These results suggest that Tac1-expressing neurons in the l/vlPAG promote itch-scratching cycles.

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Poster

396. Somatosensation: Itch Mechanisms

Location: Hall A

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Program #/Poster #: 396.02/L41

Topic: D.02. Somatosensation

Support: JSPS KAKENHI Grant Number JP16K15337

Title: The direct and indirect activation of primary afferents by alpha melanocyte stimulating hormone involves in the induction of spontaneous itching in mice with atopy-like dermatitis

Authors: *T. ANDOH[1], C. AKASAKA[1], K. SHIMIZU[2], J.-B. LEE[3], Y. YOSHIHISA[2], T. SHIMIZU[2];
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Abstract: Atopic dermatitis is a chronic inflammatory skin disease with severe itching. In atopic dermatitis patients, itch control is very important for improving quality of life and treating atopic dermatitis. However, the underlying mechanisms of itching still unclear. Alpha melanocyte stimulating hormone (alpha MSH) is an endogenous peptide hormone involved in cutaneous pigmentation and our recent study showed that an intradermal injection of alpha MSH elicited itch related responses in mice. In this study, we therefore investigated whether alpha MSH was involved in itching in AD. In the skin of AD patients and mice with atopy like dermatitis (dermatitis mice), alpha MSH was distributed mainly in epidermis, especially keratinocytes. In the skin of mice with dermatitis, alpha MSH receptors (MC1R and MC5R) were expressed at the mRNA level and were distributed in the dermis. In the dorsal root ganglion (DRG) of mice with dermatitis, mRNAs encoding MC1 and MC3~5 were also expressed. MC1R antagonist agouti signaling protein inhibited spontaneous scratching in mice with dermatitis. Our previous study has shown that TXA_2 is an itch mediator and is involved in spontaneous scratching in mice with dermatitis. In healthy mice, intradermal alpha MSH elicited itch-associated responses, which were inhibited by TP thromboxane (TX) receptor antagonist. In mouse keratinocytes, alpha MSH increased the production of TXA_2. alpha MSH increased intracellular Ca^{2+} ion concentration in DRG neurons and keratinocytes. In addition, U46619, a TXA_2 stable analogue, also increased intracellular Ca^{2+} ion concentration in DRG neurons. These results suggest that alpha MSH is involved in itching in AD and elicits itching through the direct activation of primary sensory neurons and the indirect activation of the neurons through TXA_2, which is produced in keratinocytes.

Disclosures: T. Andoh: None. **C. Akasaka:** None. **K. Shimizu:** None. **J. Lee:** None. **Y. Yoshihisa:** None. **T. Shimizu:** None.

Poster

396. Somatosensation: Itch Mechanisms

Location: Hall A

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Program #/Poster #: 396.03/L42

Topic: D.02. Somatosensation

Support: NSFC 31825013
Shanghai Municipal Science and Technology Major Project 2018SHZDZX05

Title: Multimodal representation of itch, thermal, and mechanical sensation by the same S1 neuronal population

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Abstract: Much progress has been made in the neuronal mechanisms of somatosensory processing, particularly at the spinal and subcortical regions. Electrophysiological recording and macroscopic brain imaging studies have characterized neuronal responses in the primary somatosensory cortex (S1) activated by itch, thermal, and mechanical stimuli, but how somatosensory information of multiple modalities is processed by S1 at the level of single neurons remains largely unknown. Here, we report that itch, thermal, and mechanical stimuli activate largely overlapping neuronal population in S1, with modality-specific patterns but not spatial distinct clustering. Using and *in vivo* two-photon calcium imaging that simultaneously monitor hundreds of layer 2/3 neurons in S1 of awake mice, we observed neuronal responses reliably triggered by thermal and mechanical stimuli. To achieve precisely-timed itch sensation, we performed precisely timed optogenetic stimulation of spinal itch-selective neurons that elicited reliable responses in S1 neurons. We found that the same S1 neuronal population showed responses to all three modalities of stimuli, with the reliability and amplitude of responses variable among neurons within the population. Inter-neuronal distance analysis of neurons with similar modality preference revealed no evidence for spatial clustering. Further decoding analysis showed that the recorded population of a few hundred S1 neurons allowed discrimination of the modality and intensity of the somatosensory stimuli. These findings pave the way for further understanding the processing and integration of multimodal somatosensory information in the cortex.

Disclosures: **Y. Sun:** None. **X. Chen:** None. **Y. Liu:** None. **J. Deng:** None. **N. Xu:** None.

Poster

396. Somatosensation: Itch Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 396.04/L43

Topic: D.02. Somatosensation

Support: JSPS KAKENHI Grant Number JP17K10277 (Y.I.)
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Title: Effect of perampanel on acute itch behavior induced by chloroquine, serotonin or histamine in mice

Authors: A. HARUTA-TSUKAMOTO, Y. MIYAHARA, H. FUNAHASHI, K. EBIHARA, T. NISHIMORI, *Y. ISHIDA;
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Abstract: Glutamate is an excitatory amino acid in the central nervous system, and it plays crucial roles by binding to the NMDA receptor, AMPA/kainate receptor or metabotropic glutamate receptor. Recently, it has been reported that the pretreatment with CNQX, an AMPA/kainate receptor antagonist, attenuates acute itch induced by chloroquine, a pruritogen (Koga et al., 2011; Akiyama et al., 2014), indicating that the AMPA/kainate receptor is involved in pruriceptive processing. Interestingly, perampanel is an anticonvulsant that elicits its effect by attenuating the AMPA receptor activity (Zwart et al., 2014). Taken together with these previous data, it seems likely that perampanel may have the antipruritic effect; however, there is no available information on the antipruritic effect of perampanel. Thus, the objectives of the present study were determined to clarify whether perampanel exhibits the antipruritic effect on model animals with acute itch.
Pruritus elicits the desire to scratch. Therefore, pruritogen-induced scratching behavior was used to evaluate the effect of perampanel. Different concentrations of perampanel were administered into the subarachnoid space under isoflurane anesthesia, and then chloroquine (200 μg/ 50 μL), serotonin (10 μg/ 50 μL) and histamine (500 μg/ 50 μL) were subcutaneously injected into the nape of the neck. The numbers of scratching behavior after administration of chloroquine and serotonin were significantly attenuated by intrathecal administration of 5×10^{-4} μg/ 5 μL and 5 μg/ 5 μL perampanel, respectively, whereas there was little effect of perampanel on histamine-induced pruritus. These results indicate that the effects of perampanel on pruritogen-induced scratching behavior depend on pruritogens.
We thank Eisai Co., Ltd., Tokyo, Japan for their generous gift of perampanel.

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Poster

396. Somatosensation: Itch Mechanisms

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Topic: D.02. Somatosensation

Support: Pfizer grant (W1203521)
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Title: Role of keratinocyte STAT3 in itch

Authors: T. HASHIMOTO, K. SAKAI, G. YOSIPOVITCH, ***T. A. AKIYAMA**;
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Abstract: Recent findings suggest that not only the interaction between pruritogens and peripheral pruriceptor terminals, but also the interplay among peripheral pruriceptor terminals, immune cells, and epidermal keratinocytes play roles in itch induction. STAT3 plays critical roles in the regulation of keratinocytes. The aim of this study was to elucidate the involvement of keratinocyte STAT3 in itch sensation. To examine the role of STAT3 in keratinocytes in vivo, we crossed Stat3 flox mice with K5CreERT2 mice, and tamoxifen was injected to delete STAT3 in keratinocytes. We initially assessed the in vivo effect of keratinocyte-specific STAT3 depletion on acute itch in mice. The acute itch was evoked by intradermally injecting either histaminergic or non-histaminergic pruritogen (histamine, serotonin, or chloroquine). All three pruritogens evoked scratching responses in control mice. Histamine-evoked scratching behavior was significantly attenuated in STAT3 conditional knockout (cKO) mice while serotonin- and chloroquine-induced scratching responses did not change. To determine whether pruritogens elevate intracellular calcium in keratinocytes, we performed calcium imaging experiments on keratinocytes. In keratinocytes from control mice, intracellular calcium gradually increased and reached a maximum level 90-150 sec after application of histamine and serotonin. However, chloroquine did not increase intracellular calcium. The increased responses of keratinocytes to histamine and serotonin were significantly reduced by STAT3 knockout, or TRPV4 inhibitor reported to inhibit histamine-evoked calcium responses of keratinocytes. To investigate whether STAT3 is involved in the regulation of TRPV4 expression, we performed immunostaining experiments on epidermal sheets. In epidermal sheets from control mice, TRPV4 was detected on the cell surface of keratinocytes. This signal was considerably weaker in keratinocytes of STAT3 cKO mice, while TRPV4 mRNA expressions were detected in the epidermal sheets from both control and STAT3 cKO mice. STAT3 may be involved in the trafficking of TRPV4 to the plasma membrane. Overall, these results indicate that keratinocyte STAT3 plays a major role in

histaminergic itch, but not nonhistaminergic itch, possibly through regulation of TRPV4 expression.

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Poster

396. Somatosensation: Itch Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: D.02. Somatosensation

Support: NIH Grant AR057194

Title: Effects of rostral ventromedial medullary tachykinin 1 receptor (NK-1R) expressing neurons on the descending modulation of itch

Authors: *T. FOLLANSBEE[1], D. T. DOMOCOS[2], K. TAKANAMI[3], M. CARSTENS[1], E. E. CARSTENS[1];
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Abstract: The rostral ventromedial medulla (RVM) is a brainstem structure that has been known for decades to be important in descending modulation of spinal pain transmission. However, whether these descending pathways also modulate the spinal transmission of itch is unknown. Within the RVM, a relevant class of cells -the ON cells - are thought to facilitate spinal pain transmission and exhibit a significant increase in firing prior to the onset of a nocifensive withdrawal reflex. Furthermore, ON cells respond to intradermal injection of pruritogens. RVM neurons with descending spinal projections express the neurokinin-1 (NK-1) receptor, and ON cells were shown to be excited by local administration of substance P (SP). We hypothesized that ON cells express the NK-1 receptor, and that activation of NK-1 receptor- expressing ON cells may have an inhibitory effect ion itch opposite to their pronociceptive action. To test these hypotheses, chemogenetic and pharmacological approaches were used to activate ON cells and determine whether itch- related behaviors were reduced.
Using electrophysiological single-unit recording methods in lightly anesthetized mice, the magnitude of responses of RVM ON-cells elicited by hindpaw pinch were significantly potentiated (~50%) following local injection of SP (0.5 µl, 10 nmol) through a cannula adjacent

to the recording electrode. This effect was maintained throughout the 60 min recording period. Saline microinjection had no effect. In behavioral experiments, intracranial microinjection of SP (0.5µl, 10nmol), as compared with saline, through an implanted cannula significantly reduced the mean number of hindlimb scratch bouts directed to the site of intradermal injection of histamine (106 to 60, $p<0.05$) or chloroquine (157 to 24, $p<0.05$) as compared with saline microinjection. We also used a chemogenetic approach, injecting AAV5: DIO-hM3Dq-mCherry into the RVM of NK-1R-cre mice. Consistent with the pharmacological approach, chemogenetic activation of RVM NK-1 receptor-expressing neurons significantly reduced pruritogen-evoked scratching behavior. In each DREADDs mouse we compared the number of scratch bouts elicited by histamine or chloroquine following systemic administration of saline (control) vs. clozapine (0.01mg/kg). Compared to controls, clozapine administration significantly reduced the mean number of scratch bouts evoked by intradermal injection of histamine (6 vs. 76, $p<0.05$) or chloroquine (34 vs. 139, $p<0.05$).
We conclude that activation of NK-1 receptor-expressing ON cells in RVM significantly reduces the spinal transmission of itch.

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Poster

396. Somatosensation: Itch Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 396.07/L46

Topic: D.02. Somatosensation

Title: High-resolution analysis of the tickle response and of self-tickle suppression in humans

Authors: *S. PROELSS, S. ISHIYAMA, E. MAIER, M. BRECHT;
Bernstein Ctr. For Computat. Neurosci. Berlin, Humboldt Univ. of Berlin, Berlin, Germany

Abstract: Ticklishness is an all too familiar experience to most, yet the physiological underpinnings of this sensation remain poorly understood. Here, we address this issue by systematically mapping out physiological changes in humans during tickling in high definition recordings with the aim to 1) establish a time series of processes that accompany ticklishness responses from first onset of external touch to vocalisation at different body parts, and 2) assess the effects of self-generated motion and touch in the suppression of ticklish responses. In a within-participant design, we assessed vocalisation latencies, acoustic vocalisation properties, preparatory changes (i.e., breathing) and activation of facial action units (Facial Action Coding System; Ekman & Friesen, 1978) in conjunction with self-reported ticklishness scores in response to tickling and self-tickle suppression.
Overall results show that greater self-reported ticklishness co-occurs with significantly faster

vocalisation latencies, further characterised by significantly heightened pitch during laughter. The first point of external touch is followed by an inhale at short latencies (down to <200ms) and onset of mouth opening commences at <150ms before vocalisation. Whilst body parts differ across subjects in these parameters, the emergent pattern remains consistent across participants. Intriguingly, we further show that self-touch suppresses a ticklish response to tickling applied by second person. Such self-touch suppression requires the establishment of 'true self-touch' at the ipsilateral side to the external point of touch, resulting in significantly increased response latencies or even absence of vocalisation and greatly diminished self-reported ticklishness. In contrast, sole execution of a tickling-finger-motion without touch at the ipsilateral or contralateral side to the tickle does not alter response latencies or perceived ticklishness. Notably, the here reported necessity of direct bodily contact is indicative for a role for inhibitory mechanisms during self-touch. Our data suggest that self-tickle suppression might be mediated by an inhibitory tactile mechanism acting on sensations within a body hemisphere. This explanation is simpler than conventional models, which often evoke motor mechanisms (such as efference-copy) or invoke a neural computation that distinguishes between self- and allo-touch.

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Poster

396. Somatosensation: Itch Mechanisms

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Topic: D.02. Somatosensation

Support: NIH Grant F31AR075436-01

Title: Determining the function of TRPC3 in allergic contact dermatitis induced itch

Authors: *K. BEATTIE[1], H. JIANG[2], M. MACVITTIE[1], Q. LIU[2], W. LUO[1];
[1]Neurosci., Univ. of Pennsylvania Sch. of Med., Philadelphia, PA; [2]Anesthesiol., Washington Univ. at St. Louis Sch. of Med., Saint Louis, MO

Abstract: Itch is an irritating sensation that triggers the desire to scratch. It is a major symptom of dermatological diseases including Allergic Contact Dermatitis (ACD), a disease characterized by rash formation and intense itch sensation. Although the pathophysiology of the immune response in ACD is well-characterized, the molecular mechanisms underlying the sensation of itch in ACD are still being elucidated.
Transient receptor potential (TRP) channels comprise a superfamily of proteins that form nonselective cation channels and function in a variety of sensory pathways, including itch. In this study, we focus on one of the TRP channels, TRPC3, and investigate its role in mediating or modulating itch sensation in ACD. Our lab previously characterized *TrpC3* knock out (KO) mice

and found that they exhibited no phenotypic differences in a variety of behavioral tests for acute pain and itch sensation. In contrast, pilot studies our collaborator's lab demonstrated that *TrpC3* KO mice showed a marked increase in scratching behavior in a mouse model of ACD. To confirm this finding, we induced CHS in 3 to 4-month-old male and female C57/B6 (*n*=10) and *TrpC3* KO mice (*n*=8) and observed that *TrpC3* KO mice scratched significantly more than aged-matched C56/B6 mice (no sex-differences were found). These results suggest TRPC3 plays a critical role in dampening pathological itch triggered by CHS. *TrpC3* is known to be expressed in both the immune and nervous system. To understand the mechanism through which TRPC3 acts in CHS, we are generating conditional KO mice of *TrpC3* in either immune or nervous system. We have examined epidermal thickness and immune cell infiltration in the epidermis of C57/B6 and *TrpC3* KO mice with and without CHS (H & E staining, n=3 for each group). Both genotypes showed a significant increase in epidermal thickness and cellular infiltration with CHS compared to control skin, and there was no significant difference between the genotypes. These results demonstrate that the amount of skin inflammation is not correlated with itch behavior. In summary, our results suggest a novel role for TRPC3 in modulating chronic itch in ACD, and the exact mechanism is under investigation.

Disclosures: K. Beattie: None. **H. Jiang:** None. **M. Macvittie:** None. **Q. Liu:** None. **W. Luo:** None.

Poster

396. Somatosensation: Itch Mechanisms

Location: Hall A

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Program #/Poster #: 396.09/M2

Topic: D.02. Somatosensation

Support: DE026087

Title: Skin microbiome induced release of an itch-inducing cytokine

Authors: *S. PITAKE[1], L. FLOWERS[2], E. GRICE[3], I. ABDUS-SABOOR[1];
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Abstract: Atopic dermatitis (AD) is a chronic inflammatory skin disease that affects approximately 15-20% of children and 3% of adults overall. The exact pathogenesis of AD is poorly understood; however, studies have shown that the skin microbiome plays a crucial role in maintaining the barrier function and preventing dysbiosis. Loss of structural integrity and alterations in commensal bacteria of skin leads to increased colonization of harmful bacterium such as *Staphylococcus aureus*, thus contributing towards progression of AD. Early clinical studies have also shown that topical application of some commensal strains (*Staphylococcus hominis* or *Roseomonas mucosa*) reduce severity of AD in some cases; but the underlying

mechanism remains unclear. In this study, we collected skin biopsies from young and adult human donors and inoculated primary keratinocytes with up to 40 different commensal and AD associated bacterial strains in a time dependent (24 and 72 hours) manner. The supernatants from keratinocyte-bacteria co-culture were assessed for the itch-inducing cytokine Thymic stromal lymphopoietin (TSLP) content by ELISA and we found a time dependent increase in TSLP release. Interestingly, in addition to previously reported AD associated strains, we identified several new bacterial strains which could contribute towards AD directly via TSLP release.

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Poster

396. Somatosensation: Itch Mechanisms

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Topic: D.02. Somatosensation

Support: NIH Grant NS0850586
NIH Grant NS086372
Caterina Foundation Fellowship

Title: Modulation of touch and itch by neuropeptide Y signaling within the dorsal spinal cord

Authors: *D. ACTON, X. REN, S. DI COSTANZO, A. DALET, S. BOURANE, M. GOULDING;
Salk Inst., La Jolla, CA

Abstract: The neurons of the dorsal spinal cord express a multiplicity of neuropeptides and their receptors, and these function to shape the transmission and transformation of cutaneous sensory information. Peptidergic signaling has been shown to be important in the regulation of the central transmission pathway for chemically evoked itch sensation. In this pathway, fast synaptic transmission is complemented by the excitatory actions of neuropeptides including substance P, natriuretic polypeptide b and gastrin releasing peptide, and by the inhibitory actions of dynorphin. Recently, we demonstrated that a separate pathway exists for the transmission of itch sensation evoked by mechanical stimuli, such as the light touch of an insect on the skin. This pathway is subject to inhibitory gating by dorsal horn interneurons that express Neuropeptide Y::Cre (NPY::Cre INs) (Bourane et al. *Science*. 2015), such that ablation of these neurons causes compulsive scratching in mice. Previously it was unclear whether this inhibitory regulation was mediated by GABA/glycine or by NPY itself. We have used extensive genetic and pharmacological interventions to determine the role of NPY signaling in the dorsal horn transmission pathways for touch, pain, and mechanical and chemical itch. NPY receptors are expressed by multiple neuronal populations in the central and peripheral nervous systems, and

previous studies of the influence of NPY signaling on somatosensory processing have been unable to pinpoint its sites of action. We have now assessed the contribution that NPY signaling within the dorsal horn makes to the regulation of the mechanical itch transmission pathway. Given observations that some patients with debilitating chronic itch have reduced serum levels of NPY, and that NPY levels are inversely correlated with the intensity of itch (Reich et al. *Acta Derm.Venereol.* 2007), determining the role of NPY in the inhibitory regulation of this modality is of considerable interest.

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Poster

396. Somatosensation: Itch Mechanisms

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Title: MRGPRX4 is a novel bile acid receptor in cholestatic itch

Authors: H. YU[1,2,3], ***T. ZHAO**[1,2,3], S. LIU[1], Q. WU[4], O. JOHNSON[4], Z. WU[1,2], Z. ZHUANG[1], Y. SHI[5], R. HE[1,2], Y. YANG[6], J. SUN[7], X. WANG[8], H. XU[9], Z. ZENG[10], X. LEI[3,5], W. LUO[4], Y. LI[1,2,3,11];
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Abstract: Patients with liver diseases often suffer from chronic itch or pruritus, yet the itch-causing pruritogen(s) and their cognate receptor(s) remain largely elusive. Here, we identify a ligand-receptor pair, bile acids and orphan, primate specific MRGPRX4, underlying cholestatic itch. *In situ* hybridization and immunohistochemistry revealed that MRGPRX4 is expressed in ~7% of human dorsal root ganglion (hDRG) neurons and co-localizes with HRH1, a known itch-inducing GPCR. Bile acids elicited a robust Ca^{2+} response in a subset of cultured hDRG neurons. Intradermal injection of bile acids or a MRGPRX4 specific agonist induced significant itch in healthy human subjects. Surprisingly, application of a specific agonist for TGR5, a known sequence conserved bile acid receptor previously implicated in cholestatic itch in mice, failed to elicit Ca^{2+} response in cultured hDRG neurons, nor did it induce itch in human subjects. *In situ* hybridization and immunohistochemistry revealed that hTGR5 is selectively expressed in satellite glial cells, unlike mTGR5 (in mouse DRG neurons), likely accounting for the inter-species difference functionally. Finally, we found that plasma bile acids correlate well with cholestatic itch and the elevated bile acid level in itchy patients is sufficient to activate MRGPRX4. Taken together, our data strongly suggest that MRGPRX4 is a novel bile acid receptor that likely underlies cholestatic itch, providing a promising new drug target for anti-itch therapies.

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Poster

396. Somatosensation: Itch Mechanisms

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Program #/Poster #: 396.12/M5

Topic: D.03. Somatosensation – Pain

Support: NCSU startup

Title: Molecular pathway linked with chronic itch but no pain

Authors: *S. K. M. MISHRA[1], R.-R. JI[2], J. WHEELER[3], S. PITAKE[3], M.-C. KO[4], W. BAEUMER[3], T. OLIVRY[3];
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Abstract: Chronic itch associated with diseases like atopic dermatitis impair the lives of patients and their family members. Studying the neuronal mechanism (s) to cause allergic itch will lay the

foundation to shed light on signaling processes that become unregulated and sensitized during chronic allergic skin disorders. Recently, many painful conditions (inflammatory and neuropathic pain) have been associated with integrin signaling. Following engagement with extracellular matrix proteins, integrin signaling influences numerous cellular processes including migration, proliferation, and death. However, the unique role of the integrin receptor in chronic itch associated with the allergic itch is unknown. Here, we employ cellular, molecular, physiological, pharmacological, mouse genetics and mouse behavioral assays to illustrate a new pathway that is possibly linked with the chronic allergic itch. We identify a novel endogenous ligand that induces itch and is evolutionarily conserved among several animals. Consistently, we find a novel receptor that is expressed and is functional in the DRG sensory neurons that is involved in itch and not in pain. Finally, we show ligand activation of DRG neurons releases the neurotransmitter NPPB to further transmit the signal to spinal cord neurons in the CNS. Our current working model predicts that a localized allergic response in the skin generates and releases ligand which transmits a signal through DRG sensory neurons, which further targets transient receptor potential (TRP)-channels downstream in the signaling pathways. Overall, we identify a novel endogenous mediator and the mechanism that are fundamental to neuronal responses during chronic allergic skin diseases. The future targeting of ligand/receptor may provide innovative strategies for the development of new drugs to treat chronic itch.

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Poster

396. Somatosensation: Itch Mechanisms

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Topic: D.02. Somatosensation

Support: Start-up funds awarded to SKM.

Title: A meta-analysis of sex and animal models used in pre-clinical itch research

Authors: *J. J. WHEELER[1], K. N. ALLEN-MOYER[2], S. K. MISHRA[1];
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Abstract: Beginning in June 2015, the NIH has required researchers to include both sexes in preclinical research in biomedical sciences. Recent studies have indicated that neuroscience has historically been biased towards using male animals in preclinical research. Itch, a symptom of many diseases, is mediated by the nervous system; therefore, we were interested in whether itch research has the same sex biases as other neuroscience. However, there haven't been any studies to look at the use of both sexes in itch research or to determine if the itch sensation has any sex

bias. Therefore, we performed a meta-analysis on primary research articles indexed in the PubMed database that contained the terms “Itch” or “Pruritus” from August 2007 to December 2018. From these primary research articles, we collected the following information: the animal model used, type of itch (acute vs. chronic), the sex of the animals used, and whether sex was considered a variable. Further, we looked at the different strains of mice and rats used, species of non-human primate, and breed of dog used. We also looked at how the use of sex differed before and after the NIH’s 2015 mandate. We found that male animals were most likely to be used, regardless of the animal model used. Additionally, in research that used both male and female animals, researchers rarely tried to determine if their results were sex-dependent. We found that mice were the most frequently used animal model. We also determined that researchers using non-human primates were more likely to use both sexes and that researchers using rats were least likely to use females in their research. Overall, our report will provide a significant understanding of sex bias and its omission in itch research. Further, the information from this study will help guide researchers to consider this factor as an important variable in itch research.

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Poster

396. Somatosensation: Itch Mechanisms

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Program #/Poster #: 396.14/M7

Topic: D.03. Somatosensation – Pain

Support: NIH Grant AR057194

Title: Cinnamaldehyde-evoked scratching in mice involves activation of TRPV4- and TRPV1- but not TRPA1-expressing sensory neurons

Authors: A. NGUYEN[1], T. FOLLANSBEE[2], D. T. DOMOCOS[3], M. IODI CARSTENS[4], ***E. CARSTENS**[5];
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Abstract: Topical application of cinnamaldehyde (CA) elicits itch in humans. Intradermal cheek injection of CA in mice elicits dose-related scratching but little wiping, indicating itch. We previously reported that CA-evoked scratching behavior was reduced by ~50% in male and female knockout (KO) mice lacking TRPV1 or TRPV4, but not TRPA1. We presently used calcium imaging to investigate if dorsal root ganglion (DRG) cells of various genotypes are activated by CA and other itch and pain mediators. DRGs from wildtype (WT) and TRPV1KO, TRPA1KO or TRPV4KO mice were collected, acutely isolated, cultured and plated for

ratiometric calcium imaging using Fura-2. The TRPA1 agonists CA and allyl isothiocyanate (AITC; mustard oil) respectively activated 11.9% and 32.8% of DRG cells from WT mice. As expected, neither CA nor AITC activated any DRG cells from TRPA1KO mice. CA activated significantly fewer DRG cells from TRPV4KO mice (4%) compared to WT mice, consistent with the behavioral data in which CA elicited less scratching in TRPV4KO mice. However, compared to WTs, both CA and AITC activated significantly more DRG cells from TRPV1KO mice (17 and 38.7%, respectively), which is inconsistent with the behavioral data showing that CA evoked less scratching in TRPV1KOs. The higher percentage of CA- and AITC-sensitive DRG cells in TRPV1KOs may reflect a compensatory increase in TRPA1 expression in these mice. Since CA elicited normal scratching in TRPA1KOs, CA presumably acts independently of TRPA1-expressing sensory or epithelial cells. CA is a contact sensitizer that activates CD-1-restricted T cells. We therefore tested the type 2 cytokine, IL-4 (300 nM), which activated 10% of DRG cells from WT mice, 13.1% from TRPV1KOs, and 6.8% from TRPV4KOs. IL-4 appears to act via TRPA1, since IL-4 did not activate any neurons from TRPA1KOs. In conclusion, TRPA1 is not required for CA-evoked itch. CA may partly act via TRPV4 or a TRPV4-TRPV1 heterodimer in sensory neurons to elicit itch. CA may also have a TRPA1-independent immune effect leading to production of type 2 cytokines, although activation of DRG cells by IL-4 is TRPA1-dependent and thus does not appear to mediate CA-evoked itch.

Disclosures: **A. Nguyen:** None. **T. Follansbee:** None. **D.T. Domocos:** None. **M. Iodi Carstens:** None. **E. Carstens:** None.

Poster

396. Somatosensation: Itch Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 396.15/M8

Topic: D.02. Somatosensation

Support: JSPS KAKENHI Grant Numbers 23590721 and 26460705

Title: Characterization of responses to pain and itch stimuli in adult rats with neonatal dopamine depletion

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Abstract: The dopaminergic neural system plays a crucial role in motor regulation as well as modulation and processing of somatosensory information. Although rats with neonatal dopamine depletion exhibit motor hyperactivity and have been utilized as animal models of attention deficit hyperactivity disorder, characterization of their responses to pain and itch stimuli during

adulthood have rarely been investigated. In the present study, we performed open field tests to confirm motor hyperactivity, and then investigated behavioral responses to pain and itch stimuli in adult rats with neonatal dopamine depletion. The neural responses to these stimuli were also examined through immunohistochemical analysis of c-Fos protein and electrophysiological analysis of extracellular recording of neural activity in the spinal dorsal horn. The rats that received intra-ventricular injection of 6-hydroxydopamine (6-OHDA) 4 days after birth showed a significant increase in the distance traveled in the open field tests during adulthood compared to the vehicle-treated rats. The 6-OHDA-treated rats showed a significant increase in the nociceptive behavioral response in the formalin test. The von-Frey filament and tail flick tests failed to reveal significant differences in withdrawal thresholds between the vehicle-treated and 6-OHDA-treated rats. The 6-OHDA-treated rats showed a significant decrease in itch-related behavior evoked by injection of serotonin (5-HT) into the hindpaw compared to the vehicle-treated rats. Significantly increased number of c-Fos-immunoreactive neurons was observed in the ipsilateral spinal dorsal horn to the formalin injection site in the 6-OHDA-treated rats compared to the vehicle-treated rats. There was no significant difference in number of c-Fos-immunoreactive spinal dorsal horn neurons evoked by the 5-HT injection into the hindpaw between the two groups. No significant differences in numbers of neural activities in the spinal dorsal horn evoked by von-Frey filament and the 5-HT injection applied to the hindpaw were observed between the two groups. These results suggest that the dopamine system is a crucial role in the development of somatosensory system including pain and itch, and the neural circuit in the spinal cord for itch is different from that for pain. The effects of neonatal dopamine depletion on somatosensory system depend on the modality. Supraspinal mechanism may be implicated in the modulation of itch-related behavior induced by the neonatal dopamine depletion.

Disclosures: M. Ogata: None. **D. Uta:** None. **H. Akita:** None. **H. Ishibashi:** None.

Poster

396. Somatosensation: Itch Mechanisms

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 396.16/M9

Topic: D.04. Somatosensation – Touch

Support: P30 DA018310

Title: Quantitative assessment of peptides level changes related to murine atopic dermatitis and dry skin models in mice

Authors: *K. D. ANAPINDI[1], E. TILLMAAND[2], E. DELATOBA[3], J. GOU[4], A. A. PRADHAN[5], Q. LIU[6], J. V. SWEEDLER[7];
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Abstract: Chronic itch is a debilitating condition involving primary sensory neurons. Though several studies have been done in this field, the exact molecular mechanisms by which the itch signals are relayed from the peripheral nervous system to the central nervous system are not yet well understood. However, it is believed that the process of detecting the itch sensation and relaying it via the dorsal root ganglion (DRG) to the dorsal horn (DH) and other regions of the central nervous system involves cell to cell signaling molecules such as neuropeptides. In the current study, we employed a label-free mass spectrometry-based relative quantitation procedure to correlate the levels of various neuropeptides in the DH and DRG regions in mouse models of atopic dermatitis and dry skin. Using this approach, we were able to quantify over 400 peptides in the DRG and DH regions combined. Of these quantifiable peptides, 33 were found to be significantly changed ($p \leq 0.05$, adjusted for multiple testing corrections) between the treated and control models. Several peptides from neuropeptide precursor proteins such as proSAAS, proTachykinin-1 and proCGRP were among those that were significantly changed. Additionally, Substance P, a previously known peptide candidate for itch signal transmission, contributed towards differentiating between the treated vs. control replicates via principal component analysis (PCA) in the current study. Secondly, we compared the peptidomics results from the current study with a previous study performed on migraine model of mice. Though pain and itch are two distinct sensations, the exact mechanisms that differentiate these two are not completely understood. Here, we looked at the change in peptide profile levels from DH of mice treated for these two conditions to see if there are any peptide level differences associated with these two conditions. From a preliminary analysis, we found that 115 peptides were exclusively present in the pain model while 79 peptides were found only in the itch model. This suggests that a different set of peptides could potentially be involved in mediating these two disorders. Further investigation on the nature of these peptides and their relative levels of change should reveal greater details on the mechanism of itch.

Disclosures: K.D. Anapindi: None. **E. Tillmaand:** None. **E. Delatoba:** None. **J. Gou:** None. **A.A. Pradhan:** None. **Q. Liu:** None. **J.V. Sweedler:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 397.01/M10

Topic: D.03. Somatosensation – Pain

Support: NIH Grant GM115384

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NSF-CRCNS IIS-130764

Title: Closed loop brain machine interface modulation of acute pain in rats

Authors: *Q. ZHANG[1], R. TALAY[1], S. HU[3], Z. XIAO[4], B. CARAVAN[1], L. HU[1], Z. CHEN[5], J. WANG[2];
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Abstract: Acute pain is triggered by noxious stimuli that induce changes in the neural responses of specific circuits at both single neuron and population levels. However, current diagnosis of pain remains based on behavioral reports rather than cellular mechanisms, leading to the risk of under or over-treatment. Our previous studies have shown that certain brain regions, such as the prelimbic prefrontal cortex (PL-PFC), can be optogenetically or electrically stimulated to provide analgesia. Continuous stimulation, however, can result in unwanted nonspecific side effects. To address these challenges, we have developed a prototype brain machine interface (BMI) for acute pain onset detection and for on-demand therapeutic stimulation in freely moving rats. Silicon probes assembled with custom-designed drives were implanted in the anterior cingulate cortex (ACC). In addition, an optic fiber was implanted in the PFC after AAV-CamKII-hChR2 injection. Mechanical or thermal stimuli were delivered to the hind limbs contralateral to recording sites. Online-sorted ensemble spike activity was used to train and test a Poisson linear dynamical system (PLDS) to detect the onset of acute pain and trigger PL-PFC optogenetic stimulation. Our preliminary data show that we can achieve real-time decoding with a true positive rate of >80% and a detection latency shorter than rat limb withdrawal latency. At the same time, real-time pain detection can reliably trigger PFC stimulation to attenuate pain behavior. The paw withdrawal latency is significantly increased after BMI treatment. In addition, rats prefer to stay in the BMI-associated treatment chamber during conditioned place aversion (CPA) testing. These results suggest that a closed-loop BMI is able to relieve both sensory and affective acute pain behavior. Furthermore, this BMI model could be used to study mechanisms of cortical pain circuitry and relate neural codes to behavior.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 397.02/M11

Topic: D.03. Somatosensation – Pain

Support: NIH R01 NS094389
NIH R01 AA027214

Title: Hyperexcitability of mPFC-NAc neurons in a mouse model of incision pain

Authors: *S. ZHOU[1], K. C. JONES[2], B. K. ATWOOD[3], P. L. SHEETS[4];
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Abstract: Prescription opioid treatment of surgical procedure-related incisional pain has captured the national spotlight due to an alarming rise in opioid misuse and overdose. Before novel therapeutic pain strategies can be developed, cellular and circuit alterations to supraspinal pathways involved in both pain regulation and opioid reward must be resolved. The medial prefrontal cortex (mPFC) in rodents plays an important role in pain regulation. Using retrograde tracing strategies, we identified a major population of mPFC neurons that target the nucleus accumbens (NAc), a striatal region implicated in opioid reward. This corticoaccumbal pathway (i.e. mPFC-NAc) is involved in regulation of both pain and reward-driven behavior. Here we used the plantar incision model for surgical pain, which produced a significant reduction in 50% paw withdrawal threshold in response to von Frey fibers at post-operation days 1-4. Our preliminary whole-cell patch clamp data from acute brain slices show that retrogradely-labeled mPFC-NAc neurons from the incision group have higher frequency of current-evoked action potentials and lower voltage thresholds compared with the sham group. We are currently dissecting functional changes to mPFC-NAc neurons in surgical injury plus opioid-treated cohorts. Findings from this work will have important implications for how specific cortical circuits may underlie protective mechanisms against opioid addiction following surgical injury.

Disclosures: S. Zhou: None. **K.C. Jones:** None. **B.K. Atwood:** None. **P.L. Sheets:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Program #/Poster #: 397.03/M12

Topic: D.03. Somatosensation – Pain

Support: SFB1158
Grant of China Scholarship Council 201506140056

Title: Pain and brain oscillations in freely moving mice: Searching for an electrographic signature of pain

Authors: *J. ZHANG[1], S. PONSEL[1], M. PILZ[2], Y. YANOVSKY[1], J. BRANKAčK[1], A. DRAGUHN[1];
[1]Inst. of Physiol. and Pathophysiology, [2]Inst. of Med. Biometry and Informatics, Heidelberg Univ., Heidelberg, Germany

Abstract: Pain is one of the major public health concerns. However, an objective and reliable method for diagnosing pain is missing. Little is known how pain modulates brain oscillatory activities in both humans and animals. To investigate this, brain electrodes were implanted bi- and unilaterally into multiple pain-related brain regions of mice, including primary somatosensory cortex (S1), anterior cingulate cortex (ACC), insular cortex (Ins), ventroposterior lateral thalamus (VPL), parietal cortex (PAC) and olfactory bulb (OB). Electrographic oscillatory activity was recorded in freely moving animals in all brain regions, simultaneously with motor activity. Baseline recordings and controls after saline injections in one hind paw were compared to capsaicin injections which resulted in acute pain. We found increases of cross-frequency coupling (CFC) between low (1-12 Hz) and fast frequencies (80-120 Hz) in anterior cingulate cortex (ACC) and increases of coherence between specific brain regions in frequency ranges below 30 Hz. To distinguish between pain (capsaicin) and no-pain (saline) an elastic net model was applied using power, CFC and interregional coherence data separately and in combination. The elastic net permitted reliable detection of pain using three parameters, all in the frequency range below 30 Hz (which is mainly used in clinical EEG recordings in humans): Power spectrum densitiy between 6-12 Hz in ipsilateral OB, coherence at 6-12 Hz between ACC and PAC (both ipsilaterally), as well as coherence at 6-12 Hz between ipsilateral ACC and Ins. These parameters contribute to a classification with an area under the curve (AUC) of 0.74, which is significantly different from chance. These findings indicate a specific change of electrophysiological signals during acute pain in the mouse brain.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 397.04/M13

Topic: D.03. Somatosensation – Pain

Title: High frequency oscillations (HFOs) as a promising neural signature in response to noxious stimulus

Authors: *Z. WANG, Y. B. PENG;
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Abstract: High-frequency oscillations (HFOs) ranging between 80-600 Hz, is proved to be involved in normal brain function, such as, cognitive process, memory, and epileptic seizures. However, much less is known about whether HFOs are associated with pain process. In this study, we aim at exploring the HFOs features, mainly at 100-250 Hz and 250-500 Hz, in response to noxious stimulus, providing a promising neural signature of pain. We hypothesize that differential HFOs response will be detected after noxious stimulus in different brain regions, which contribute to different components of pain (e.g., anterior cingulate cortex (ACC) for emotion, and primary somatosensory cortex (S1) for sensory discrimination). Here, local field potential (LFP) was recorded from contralateral ACC, bilateral amygdala (Amg), and contralateral VTA simultaneously in male Sprague-Dawley rats (n=12). Firstly, HFOs were recorded through four electrodes localized in these four brain areas for 11 minutes as baseline. Then, rats were given 0.1mL formalin (3%) injection at the left hindpaw and continued recording for 60 minutes. Both Spike2 and MATLAB were used to analyze the generated data. A repeated measures analysis of variance (ANOVA) with LSD post-hoc test was conducted to compare the HFOs power difference: 1) between baseline and formalin periods; and 2) among these four regions. We found that the intensity of HFOs at 100-250 Hz was significantly increased after formalin injection ($p < .05$) in ACC, contralateral Amg, and VTA, which lasted up to 60 minutes, but not in ipsilateral Amg ($p > .05$). The intensity at 250-500 Hz was significantly increased ($p < .05$) in all four regions for up to 60 minutes. There was a main effect for brain regions, $F(1.35, 13.47) = 15.03$, $p = .001$. ACC responded more strongly than that of VTA, and bilateral Amg. However, for HFOs at 250-500Hz, there was no main effect for brain region, $F(1.17, 11.65) = 1.89$, $p = .197$, suggesting no significant differences responses among these four regions. In conclusion, significant intensity change of HFOs could be detected after formalin injection in various brain regions with different patterns, which demonstrated a promise that HFOs might be a new neural signature of pain.

Disclosures: Z. Wang: None. **Y.B. Peng:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 397.05/M14

Topic: D.03. Somatosensation – Pain

Support: P50 DA044121

Title: Epigenetic control of chronic pain and related brain remodeling

Authors: *M. V. CENTENO[1], M. ALAM[4,5], K. HALDAR[5,4], A. APKARIAN[1,2,3];
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Abstract: Chronic pain affects at least 100 million American adults. Treatment options remain limited and the most effective options, namely opioids, are linked to addiction and related mortality at an epidemic rate. Thus, novel, non-opioidergic treatment options are urgently needed and especially for neuropathic pain conditions. Epigenetic pathways that include modifications of histone proteins such as methylation and acetylation appear to be emerging as important determinants of chronic pain. However, despite recent progress, a comprehensive understanding of how histone modifications may impact chronic pain remains elusive. Rats with spared nerve injury (SNI) provide a validated rodent model for human chronic pain. We use this model to understand epigenetic mechanisms that control chronic pain. The SNI model induces mechanical allodynia (pain due to tactile stimuli that do not elicit a painful response under normal circumstances) as well as deficits in hippocampal function as measured by the novel object recognition assay an index of recognition memory. Inflammatory cells such as microglia, astrocytes and blood macrophages have been shown to play an important role in neuropathic pain in the SNI model. We will show how epigenetic histone modifiers decrease primary neuropathic pain-related behavior, expressed by tactile allodynia pain relief without motor or anxiety-like deficits, for an extended period of time, which also seems to be gender specific. We will further show immune cell and molecular signatures, transcriptional networks and functional pathways associated with epigenetic adaptations of the injured rat tissue. The study provides important insights into understanding the molecular mechanisms by which epigenetic factors control chronic pain and develops strategies targeting neuro-immune interactions to treat chronic pain.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 397.06/M15

Topic: D.03. Somatosensation – Pain

Support: PhRMA Foundation Starter Grant (V.D.)
Iowa Osteopathic Education and Research Funds (V.D.)

Title: Gene expression profiling of the rat hippocampus during chronic inflammatory pain

Authors: A. ASH[1], E. KOKKINOS[1], D. NERLAND[1], G. BERENBEIM[1], B. WILKE[1], L. SEMKE[1], L. POINTS[1], M. GIRGENTI[2], R. DUMAN[2], *V. DURIC[1];
[1]Des Moines Univ., Des Moines, IA; [2]Yale Univ. Sch. of Med., New Haven, CT

Abstract: Up to 50% of all clinical chronic pain patients suffer from major depressive disorder (MDD), which is significantly higher than the incidence of approximately 7% within the general population. The exact neural events that link these two neurological illnesses are still largely unknown; however, it is thought that chronic pain may produce negative effects on different limbic brain regions similar to those seen with chronic stress. In the current study, we used a genome-wide microarray analysis to examine the genetic profile of the hippocampus from male rats exposed to 21 days of peripheral inflammatory pain. Functional group analysis has identified a number of significantly dysregulated genes with known roles in either neuroinflammation or neurodegenerative processes. Bioinformatic gene network/canonical pathways analyses have identified a significant network associated with the Akt (protein kinase B) as the main hub gene. Altered activity of Akt-related signaling pathways (e.g., PI3K/Akt/mTOR) has been previously linked to both the development of depressive state and antidepressant treatment. Furthermore, expression of several dysregulated genes of interest (i.e., Gzma, Gzmk, Mis18a, S100a9, CCL5, and Lrg1) was also assessed in other limbic areas involved in mood regulation, as well as in the brains of female rats exposed to the same 21-day pain paradigm. Ongoing studies are further investigating the expression of these target genes in pain animals with or without presence of the depressive-like behavioral phenotype (i.e., high vs. low responders). The results of this study further elucidate the presence of transcriptional alterations in the hippocampus during the chronic pain state. Additionally, the dysregulation of genes involved in neuroinflammatory and neurodegenerative processes in the limbic brain areas continues to strengthen the idea that these processes may be involved in the development of mood disorders during the chronic pain state.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: This work was supported by NIH/NIDA/IRP.

Title: Lateral hypothalamic fast-spiking parvalbumin neurons modulate nociception through connections in the periaqueductal gray area

Authors: *J. N. SIEMIAN, C. B. BORJA, S. SARSFIELD, A. KISNER, Y. APONTE;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: The lateral hypothalamus (LH) contains a diverse collection of cell types crucial for orchestrating behaviors that facilitate survival. Over the past decade, tremendous progress has been made on new methods that allow systematic characterization of the function and connectivity of these heterogeneous neuronal subtypes. While studies have begun to identify LH circuits that regulate food intake and reward-related behaviors, less attention has been given to the contributions of genetically-identified LH circuits that modulate nociceptive behaviors. Here we examined how lateral hypothalamic neurons that express the calcium-binding protein parvalbumin (PVALB; LH^{PV} neurons), a small cluster of neurons within the LH glutamatergic circuitry, regulate nociception in mice. Using optogenetics to modulate neuronal activity, we found that photostimulation of LH^{PV} neurons suppressed nociception to an acute, noxious thermal stimulus, whereas photoinhibition potentiated thermal nociception. Moreover, brain slice electrophysiology recordings using channelrhodopsin (ChR2)-assisted circuit mapping (CRACM) revealed that LH^{PV} axons form functional excitatory synapses on neurons in the ventrolateral periaqueductal gray (vlPAG), a critical brain region for pain modulation. Furthermore, photostimulation of LH^{PV} axons in the vlPAG suppressed nociception to both thermal and chemical visceral stimuli. Interestingly, this antinociceptive effect appears to occur independently of opioidergic mechanisms, as antagonism of mu-opioid receptors with systemically-administered naltrexone did not abolish the antinociception evoked by activation of this LH^{PV} to vlPAG pathway. Importantly, none of the optogenetic manipulations significantly affected locomotor activity or anxiety-like behavior as measured by the open-field and elevated plus maze tests, suggesting that the role of LH^{PV} neurons is likely specific to nociception. Similar to our results for these acute pain tests, photostimulation of either LH^{PV} somata or axonal projections in the vlPAG also significantly attenuated thermal and mechanical hypersensitivity induced by a model of chronic inflammatory pain. Together, these results directly implicate LH^{PV} neurons in modulating nociception, thus expanding the repertoire of survival behaviors regulated by LH circuits.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

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Program #/Poster #: 397.08/M17

Topic: D.03. Somatosensation – Pain

Support: NIH Grant DE026749

Title: Comparison of CANE-RV and EnvA-RV when using CANE to capture nociceptive neurons in VPM

Authors: *M. UMORIN, P. R. KRAMER;
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Abstract: Genetically capturing cell populations that are active in response to a specific stimulus at a specific time would allow researchers to study functional neuronal circuits at the cellular resolution. Existing techniques, like TRAP and TRAP2, require use of a hormonal analog that has the potential to interfere with hormonal signaling. Pain signaling is different in different sexes and, thus, the use of a sex hormone analog would likely interfere with the pain mechanisms. CANE is a recently developed method that does not use sex hormones for genetic capturing. Instead, it relies on a transgenic animal that expresses the TVA receptor after a neuron is activated. We used CANE to label neurons in the ventroposterior medial nucleus (VPM) of the thalamus after injection of CFA into the TMJ or in non-injected controls. Then CANE-RV and EnvA-RV were compared with respect to the number of secondary cells labelling and retrograde tracing. Both methods resulted in low background of starter cells in non-injected controls. However, CANE-RV use resulted a significantly higher number of presynaptic labelled cells in the non-injected controls and in the CFA injected animals. Both methods had a significantly higher number of starter and presynaptic sells in pain treatment vs controls. Use of CANE-RV resulted in higher ratio of presynaptic-to-starter cells in the VPM as compared to the use of plain EnvA-RV (15.8 vs 9.0). Thus, it is possible to use diverse expression constructs available in EnvA-pseudotyped rabies but the labelling rate would be much lower.

Disclosures: M. Umorin: None. **P.R. Kramer:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: D.03. Somatosensation – Pain

Support: SRNSFG grant 217148

Title: Opioid antagonists attenuate NSAIDs-induced antinociception in anterior cingulate cortex of rats

Authors: *M. G. TSAGARELI[1], N. TSIKLAURI[1], N. TSAGARELI[1,2], I. KVACHADZE[2];
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Abstract: Anterior cingulate cortex (ACC), which is activated by noxious stimuli, is involved in pain processing, the neural mechanisms of the ACC involvement in affective pain have yet to be elaborated. To study relation antinociceptive effects induced by non-steroidal anti-inflammatory

drugs (NSAIDs) with endogenous opioid system we treated experimental rats with opioid receptor antagonists, naloxone and CTOP in the ACC pre- and post-following microinjections with NSAIDs.
We measured nociceptive thermal paw withdrawal latencies and mechanical thresholds in rats' formalin test following microinjections of NSAIDs (diclofenac, ketoprofen, ketorolac and lornoxicam), saline or opioid receptor antagonists, naloxone and CTOP in the ACC. Five minutes following intraplantar formalin injection all animals showed a significant reduction in thermal paw withdrawal latency and mechanical withdrawal threshold compared to pre-baseline values. Fifteen minutes after formalin injection, diclofenac, ketoprofen, ketorolac and lornoxicam clearly showed antinociceptive effects of NSAIDs. When pretreated with naloxone and CTOP separately, we found a significant reduction of analgesic effects of NSAIDs as well as post-treatment of these completely abolished NSAIDs-induced antinociception.In summary, we demonstrated that microinjection widely used non-opioid, NSAID analgesics, diclofenac, ketorolac anad lornoxicam injected into the rostral part of the ACC, induced antinociception in rats. The present findings support the concept that NSAIDs-induced antinociception is mediated via descending endogenous opioid system.
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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

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Program #/Poster #: 397.10/M19

Topic: D.03. Somatosensation – Pain

Support: NIH 05010
HBHL

Title: Resting-state fMRI reveals reduced brain communication in a mouse model of generalized pain

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Abstract: Fibromyalgia (FM)[1] is a disorder characterized by generalized pain. The acid saline-induced muscle (ASM)[2] model is considered an acceptable animal model of FM with widespread chronic pain. Here we use non-invasive resting-state functional magnetic resonance (Rs-fMRI) neuroimaging[3] to investigate the impact of FM-like pain in the ASM model on whole brain neuronal communication. We test whether mice receiving intramuscular pH7.2 (control) and pH4.0 (FM) treatment show distinct functional connectivity (FC) patterns and strengths. Acquisition was performed in slightly anesthetised animals under resting state using a 7 Tesla Bruker animal MRI scanner with Cryoprobe. Independent component analysis (ICA) was performed and identified 97 components, which we anatomically annotated following the Allen mouse brain atlas. Correlation analysis revealed significant reduction of FC in several brain regions, with predominant effects for retrosplenial cortex (RSP) and periaqueductal gray matter (PAG) components, known as core centers of the default mode network(DMN) and pain, respectively. Seed-to-seed analysis (hypothesis-driven) was performed using 14 pain-relevant brain regions, and connectivity analysis showed highest FC reduction for the PAG seed. Seed-voxelwise analysis (hypothesis-driven) was also performed with a focus on PAG. We found reduced PAG FC with 13 Allen Brain Atlas-based regions, and quantification of these alterations showed highest FC modifications of PAG with habenula and thalamic areas, as well as insular, anterocingular and entorhinal cortices, all involved in aversive sensory and emotional processing. Finally, von Frey experiment[4] was performed 3 weeks after fMRI scanning to score the extent of generalized pain of each subject. Pain thresholds measures by paw withdrawal were significantly lower in the pH4.0 group compared to pH7.2 group. We then correlated pain sensitivity scores with the intensity of FC reduction between PAG and RSP seeds at individual subject level, and found a trend to positive correlation, suggesting that brain connectivity across these two seed may be relevant to the behavioural pain response. In conclusion, our FC analyses concur to demonstrate that FM like-induced pain reduces brain communication with predominant effects on general connectivity (DMN) and pain centers (PAG). Consistent with human data[5-6], this study established a signature of generalized pain in the brain and shows the potential for translational MRI approach to study a chronic pain condition.
References:[1] Ueda et al, 2017; [2] Sluka et al,2001;[3] Mechling et al,2016;[4] Deuis et al,2017;[5]Coulombe et al,2017;[6] Lee et al, 2018

Disclosures: M.T. Nasseef: None. **W. Ma:** None. **J.P. Singh:** None. **N. Dozono:** None. **K. Lancon:** None. **P.A. Seguela:** None. **H. Ueda:** None. **E. Darcq:** None. **B.L. Kieffer:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 397.11/M20

Topic: D.03. Somatosensation – Pain

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Title: Imaging cortical circuitry during general anesthesia: Insights into cortical pain processing

Authors: *J. A. P. WEINRICH, M. X. BERNSTEIN, C. R. ANDOLINA, A. I. BASBAUM; Anat., Univ. of California San Francisco, San Francisco, CA

Abstract: BACKGROUND: General anesthetics work in a concentration-dependent manner on the central nervous system (CNS) to induce loss of consciousness and block the experience of pain. Interestingly, however, during nitrous oxide anesthesia and the initial stages of ether and isoflurane anesthesia, analgesia can be produced independently of loss of consciousness. In the present studies in the mouse, we monitored, over time, the *in vivo* activity of hundreds of individual cortical neurons during the induction to, and emergence from, general anesthesia. Furthermore, we compared the effects of isoflurane on bulk neural activity (pan-neuronally) versus isolated subpopulations of molecularly-defined cortical inhibitory interneurons (parvalbumin (PV+), somatostatin (SST+) or vasointestinal peptide (VIP+)). Our objective is to uncover the mechanisms through which a major volatile anesthetic, isoflurane, not only produces loss of consciousness, but also analgesia. **METHODS:** In adult mice, we monitored the activity of neurons in the anterior cingulate cortex (ACC), a region implicated in the affective component of pain. Spontaneous neural activity was continuously monitored before, during, and after isoflurane anesthesia. We used virally delivered genetically encoded fluorescent reporters of neural activity (GCaMP6f) expressed either (1) ubiquitously across neurons, or (2) specifically within molecularly-defined interneuron populations (PV+, SST+, or VIP+). Changes in GCaMP6f fluorescence captured via the Inscopix miniscopes. **RESULTS:** For induction and emergence, we find that inhibitory interneuron populations are more susceptible to the suppression of neural activity by isoflurane than the bulk population. During induction, both spontaneous neural activity and the percentage of active neurons decreases with increasing concentrations of isoflurane, however, inhibitory neurons are affected at much lower concentrations of isoflurane than the bulk population. Similarly, during emergence, neural activity resumes in at much higher concentrations of isoflurane for the bulk neural population than for inhibitory interneurons. **CONCLUSIONS:** Consistent with previous studies where the global activity of the cerebral cortex is decreased during general anesthesia, we found that isoflurane anesthesia decreases spontaneous activity within the ACC in a concentration dependent manner. However, our results clearly indicate that isoflurane-induced decreases in neural activity are not uniform across all neurons of the ACC. Rather, inhibitory interneurons exhibit increased susceptibility to isoflurane when compared to the bulk neuronal population.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Title: A partial connectome in mice of the thalamocortical input from a higher order thalamic nucleus, POm, to the secondary somatosensory cortical area, S2

Authors: *V. SAMPATHKUMAR[1], A. J. MILLER[2], S. SHERMAN[3], N. B. KASTHURI[2]; [2]Neurobio., [1]Univ. of Chicago, Chicago, IL; [3]Dept Neurobiol, Univ. Chicago, Chicago, IL

Abstract: Large volume serial EM "connectomics" has emerged as a key tool for understanding neural circuits. We applied this approach to study long distance thalamocortical connections between the higher order thalamic relay, POm, and the secondary somatosensory cortical area, S2, in the adult mouse. To trace the pathway, we injected a "cocktail" of 2 types of AAV into POm, the first to express Cre in POm neurons and a second expressing Cre-dependent APEX2 in the cytoplasm of the now Cre expressing neurons. Staining with osmium and DAB produced APEX2 electron dense precipitates, allowing us to perform multiscale EM imaging using the ATLUM approach [Kasthuri N, et al. (2015) Saturated reconstruction of a volume of neocortex. Cell 162:648-661] to reconstruct the connections of POm neurons in all layers of S2. We sampled a region of S2 that spanned the entire cortical depth of (~ 1mm x 0.5mm x 0.1mm). Cytoplasmic APEX2 expression reliably allowed for long range tracing of neuronal connectivity: APEX2 labeling was clear even in the finest axonal processes, including the upper layers of cortex, with no sign in reduction of signal from diffusion, fading, etc. 'Intersectional' viral labeling strategies in this pathway appear to not label neurons retrogradely since low resolution EM sections that span cortex show no sign of labeled cortical cell bodies. Thalamocortical synapses were found throughout all cortical layers. Furthermore, POm neurons primarily targeted spines on dendrites of the cortical neurons. Notably, each of these spines had a spine apparatus, an ultrastructural indication of strong synapses, whereas many unlabeled synapses were found on both spines without such an apparatus as well as dendritic shafts. Multiple spines on the same dendritic segments were commonly targeted by POm inputs, with up to 50% of such spines innervated by POm terminals, and individual POm axons typically provided multiple such inputs to the same dendritic segments. The question to be addressed is whether further examples

of thalamocortical inputs, including those from first order nuclei like VPm show similar or different features.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

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Topic: D.04. Somatosensation – Touch

Support: RO1NS094184
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NeuroNex
MH109100

Title: The partial connectome of the layer 5 corticothalamic projection from primary motor and sensory cortex to the higher order somatosensory thalamic nucleus, POm

Authors: *M. S. SHERMAN[1], N. KASTHURI[2], V. SAMPATHKUMAR[2], A. J. MILLER[2]; [1]Univ. Chicago, Chicago, IL; [2]Univ. of Chicago, Chicago, IL

Abstract: Electron microscopy (EM) has been used for decades to study ultrastructural details of cells, including the connections of neurons. Recent advances have combined serial EM with specific cell labeling using engineered peroxidases to distinctly label neuronal types in EM datasets with electron dense precipitates (APEX2). We applied these approaches to characterize connections from layer 5 (L5) neurons in primary S1 and M1 to the higher order somatosensory thalamic relay, POm. We use Cre dependent AAV/APEX2 viruses injected into transgenic animals expressing Cre in L5 pyramidal neurons to label L5 corticothalamic pathways. Specifically, the AAVs producing APEX2 cytoplasmic labeling were injected into M1, and APEX2 mitochondrial labeling, into S1, and appropriate staining which produced APEX2 EM precipitates. We then analyzed the connections in POm using multiscale EM imaging [Kasthuri N, et al. (2015) Saturated reconstruction of a volume of neocortex. Cell 162:648-661]. Relatively few terminals have been found so far in our material from M1, and beyond noting that these were large and did not innervate the same cells as those innervated by S1 terminals, we do not further consider these. We found that labeled terminals from S1 showed characteristic large vesicle filled boutons, as predicted from reconstructions from other species. The labeled terminals are at the large end of the size distribution of all labeled and unlabeled terminals found in POm. These labeled terminals synapsed predominantly near the first branch point of dendritic trees. We find that &gt5% of the labeled large terminals participate in triads and that neurons in POm have

different connectivity patterns, some with only labeled giant synapses throughout the dendritic arbor, some with all unlabeled giant synapses, and some cells with mixed innervation. Thus, because of their size, termination initial dendritic branch points (and thus proximally on dendritic arbors), and occasional involvement in triadic arrangements, these POm terminals have the characteristics of "driver" synapse.

Disclosures: M.S. Sherman: None. **N. Kasthuri:** None. **V. Sampathkumar:** None. **A.J. Miller:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Program #/Poster #: 397.14/M23

Topic: D.03. Somatosensation – Pain

Support: Grant number 16K10988 from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Involvement of descending pain inhibitory system mediated by orexin/orexin 1 receptor in the regulation of central post-stroke pain

Authors: *W. MATSUURA[1], S. TOKUYAMA[2];
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Abstract: [Aims]Central post-stroke pain (CPSP) is one of the secondary diseases of cerebral stroke. However, the detailed mechanism remains unclear. Recently, it is reported that orexin level of cerebrospinal fluid decreased in stroke patients and central administration of orexin-A reduces nociceptive responses in inflammatory pain model mice, suggesting that orexin play an important role in the regulation of CPSP. Furthermore, orexin 1 receptor (OX1R) is located in the rostral ventromedial medulla (RVM) and locus coeruleus (LC) which is nucleus of origin in the descending pain inhibitory system. In this study, we tested the involvement of orexin-A/OX1R in CPSP model mice.
[Methods]Bilateral carotid arteries of male ddY mice (5 weeks old) were occluded for 30 min (BCAO). Mechanical allodynia was evaluated by a von Frey filament test on day 3 after BCAO. The test was conducted at 10, 20, 30 and 60 min after the intracerebroventricular (i.c.v.) injection of orexin-A Prepro-orexin (orexin precusor) in the hypothalamus was analyzed by real-time PCR. SB334867 (OX1R antagonist) was administered i.c.v. injection 30 min before orexin-A injection. Yohimbine (α_2 receptor antagonist) and WAY100635 (5-HT_{1A} receptor antagonist) were administered intrathecal (i.t.) injection 15 min before orexin-A injection. Immunohistochemistry for OX1R and immunoreactivity for c-Fos (a neuronal activation marker) were observed in the RVM or LC on day 3 after BCAO.

[Results]The withdrawal responses to mechanical stimuli were significantly increased on day 3 after BCAO. Prepro-orexin in hypothalamus was significantly decreased as compared with sham. BCAO-induced mechanical allodynia dose-dependently suppressed by the pre-treatment of orexin-A. These effects of orexin-A were inhibited by the pre-treatment of SB334867, yohimbine and WAY100635. On day 3 after BCAO, OX1R was located and the c-Fos expression was induced in the RVM and LC after i.c.v. injection of orexin-A.
[Conclusion]These results show that BCAO induced-mechanical allodynia was controlled by descending pain inhibitory system mediated by orexin-A/orexin 1 receptor.

Disclosures: W. Matsuura: None. **S. Tokuyama:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: Grants-in-Aid for Scientific Research (C) [grant number 16K10988] from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Title: Involvement of cerebral and spinal nicotinic acetylcholine receptor mediated system in the regulation of central post-stroke pain

Authors: *S. TOKUYAMA, W. MATSUURA;
Kobe Gakuin Univ., Kobe, Japan

Abstract: [Aims]Central post-stroke pain (CPSP) is one of the complications of cerebral ischemia and of neuropathic pain syndrome. The treatment of CPSP remains incomplete due to its resistance to both pharmacological and non-pharmacological therapies in approximately half of CPSP patients. Although CPSP is a serious condition, details pertaining to underlying mechanisms are not well known, making current standard treatments only partially effective. Recently, it is reported that nicotine suppressed infact area after stroke. Nicotine suppressed sciatic nerve injury induced mechanical allodynia. Nicotinic acetylcholine α4 receptor agonist injected into locus coeruleus (LC) suppressed inflammatory pain. In this study, we tested the effect of nicotine signaling on CPSP model mouse.
[Methods]Bilateral carotid arteries of male ddY mice (5 weeks old) were occluded for 30 min (BCAO). Mechanical allodynia was evaluated by a von Frey filament test on day 3 after BCAO. On day 3 after BCAO, nicotine intracerebroventricular (i.c.v.; 10 or 20 nmol/mouse) injected. Von Frey filament test was conducted at 10, 20, 30 and 60 min after nicotine injection. Yohimbine (α_2 receptor antagonist) was administered intrathecal (i.t.; 16 μg/mouse) injection 15 min before nicotine injection. Immunoreactivity for c-Fos (a neuronal activation marker) was

observed in the LC.
[Results]The number of escape behaviors, one of mechanical allodynia, against the stimulation induced by the von Frey filament was significantly increased on day 3 after BCAO compared with that in the sham group. BCAO-induced mechanical allodynia dose-dependently suppressed by the pre-treatment of nicotine. These effect of nicotine were inhibited by the pre-treatment of yohimbine. The c-Fos expression was induced in the LC after i.c.v. injection of nicotine on day 3 after BCAO.
[Conclusion]These results suggest that nicotinic acetylcholine receptors play an important role in the regulation of BCAO induced-mechanical allodyni. Our findings are helpful for better understanding of the pathological mechanism in CPSP.

Disclosures: S. Tokuyama: None. **W. Matsuura:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Topic: D.03. Somatosensation – Pain

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Title: Role of Lipocalin-2 (Lcn2) in the limbic pain processing

Authors: E. KOKKINOS, A. ASH, D. NERLAND, B. WILKE, G. BERENBEIM, *L. SEMKE, L. POINTS, V. DURIC;
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Abstract: Clinical studies have shown a high co-morbidity between different chronic pain conditions and major depressive disorder. The exact brain mechanisms connecting these two neurological illnesses are still largely unknown. Our recent whole genome microarray high-throughput profiling has identified lipocalin-2 (Lcn2) as one of the highest upregulated genes in the hippocampus of rats exposed to 21 days of peripheral inflammatory pain (i.e., hindpaw injections of Complete Freund's Adjuvant; CFA). Lcn2 is an iron-related protein whose function was previously linked to innate immune response, as well as regulation of the cell differentiation, maturation and death. Recent studies further suggest that Lcn2 may also play an important role in emotional behaviors and cognitive function through regulation of neuronal excitability and dendritic spine formation/maturation. However, to our knowledge, Lcn2 has not been previously implicated in the limbic pain processing. Thus, in the current study, we investigated the expression of Lcn2 gene in the affective pain neurocircuitry within specific limbic brain areas. In male rats exposed to the 21-day CFA pain paradigm, robust increases (~ 2 fold) in Lcn2 mRNA

levels were observed within the contralateral hippocampus, prefrontal cortex (PFC) and anterior cingulate cortex (ACC). Similar upregulation of the hippocampal Lcn2 gene was also observed in the female animals exposed to the same pain model. Ongoing studies in our lab are further examining the activity of Lcn2 gene in pain animals with or without presence of the depressive-like behavioral phenotype (i.e., high vs. low responders). Overall, the results of this study suggest that chronic pain activates Lcn2 within several different limbic brain regions, which may contribute to the neural mechanisms underlying the development of mood disorders associated with the chronic pain state.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: NIH/NIAAA Grant F31AA027129

Title: Cell type specific midbrain and extended amygdala contributions to sex differences in pain and drug use

Authors: *W. YU, T. KASH;
UNC Chapel Hill, Chapel Hill, NC

Abstract: Chronic pain and drug abuse are comorbid disorders that manifest with differing prevalence and severity in males and females. A mechanism that explains sex-specific pain and drug interactions has yet to be identified. Recent evidence from our lab suggests that dopaminergic neurons of the ventrolateral periaqueductal grey ($vlPAG^{DA+}$) contribute to the anti-nociceptive effects of morphine and alcohol use. Following up on these studies, we found that activation of $vlPAG^{DA+}$ terminals in a primary output region, the bed nucleus of the stria terminalis (BNST), relieves thermal and mechanical nociception in males but not females. This effect persists during pathological pain, with $vlPAG^{DA+}$/BNST activation attenuating heightened pain sensitivity following treatment with the inflammatory agent Complete Freund's Adjuvant (CFA). Downstream of $vlPAG^{DA+}$ neurons, we examined a population of pain- and alcohol-sensitive corticotropin releasing factor (CRF) neurons in the BNST. Genetic deletion of CRF from the BNST reduces thermal and mechanical nociception and potentiates alcohol consumption in male and female mice. *In vivo* miniscope calcium imaging of $BNST^{CRF+}$ neurons further reveal robust and synchronized recruitment of these neurons during acute exposure to pain and alcohol in both sexes. Taken together, these findings support the notion that $vlPAG^{DA+}$

and $BNST^{CRF+}$ neurons differentially contribute to sex-specific interactions of pain and drug use. This knowledge will be informative for future approaches to treating chronic pain and drug abuse, as it identifies new morphine- and alcohol-sensitive mechanisms that are capable of attenuating pain in both a sex-dependent and -independent manner.

Disclosures: W. Yu: None. **T. Kash:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Support: NIH Grant F31DK12148401
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Title: CGRP in the left amygdala reduces bladder pain in female mice

Authors: *H. ALLEN, A. COX, B. J. KOLBER;
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Abstract: Urologic chronic pelvic pain syndrome (UCPPS) is among the most common visceral pain conditions in the United States, affecting between 5 and 10 million Americans and costing over $5 billion in healthcare annually. UCPPS patients experience debilitating symptoms that severely impact quality of life, including increased urgency and frequency of urination, intense and burning pain during bladder filling and voiding, referred pain in the back and pelvic area, and co-morbidities such as anxiety and depression. The underlying cause of UCPPS is largely unknown, and therefore treatments are poor and ineffective. Recent imaging studies have identified changes in cortico-limbic areas in UCPPS patients. The central amygdala (CeA) is a bilateral, mid-brain limbic region that processes both pain and emotion and can modulate bladder pain. Sensory neurons that relay information from the bladder to the CeA express high levels of calcitonin gene-related peptide (CGRP), a peptide with a well-established role in pain processing. Although there is ample evidence that CGRP is pro-nociceptive in the right CeA, a few studies indicate that CGRP may act anti-nociceptively in the left CeA. Here, we explored CGRP's contribution to the left CeA's anti-nociceptive role in the context of bladder pain using molecular and behavioral techniques. Immunohistochemical quantification of CGRP expression in the left and right CeA of control and bladder-sensitized mice revealed a significant decrease in CGRP expression in the left CeA of bladder sensitized animals. Additionally, infusion of CGRP into the left CeA of bladder-sensitized mice decreased abdominal mechanical sensitivity and visceromotor response to noxious urinary bladder distention for up to 60 minutes after injection.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Title: Parabrachial to central amygdala neural circuit for the modulation of pathological pain

Authors: *T. D. WILSON[1], A. ADKE[1], J. J. BECKER[3], S. MARTINEZ-GONZALES[1], Y. K. SUGIMURA[4], Y. CARRASQUILLO[2];
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Abstract: Increasing evidence over the last several years supports the central nucleus of the amygdala (CeA) as a critical center for pain processing, the modulation of pathological pain and its related affective comorbidities. We have previously shown that the CeA can bidirectionally modulate pain like behaviors in a cell-type-specific manner. Anatomical and electrophysiological experiments have further demonstrated that nociceptive inputs into the CeA come from the lateral parabrachial nucleus (lPB). The functional contribution of the PB-CeA circuit to the modulation of pain-related behaviors, however, has not been established. To answer this question, we used intersectional approaches in combination with chemogenetics, optogenetic-assisted circuit mapping and behavioral assays. The sciatic nerve cuff model of neuropathic pain and the formalin model of inflammatory pain were used in mice to measure spontaneous pain-related responses to inflammation as well as hypersensitivity to cold, heat, pinch and tactile stimulation of the paw after nerve injury or paw inflammation. The results from our experiments specifically demonstrate that CeA cells expressing somatostatin and CeA cells expressing PKCδ receive monosynaptic inputs from the lPB. We also show that inhibition of CeA-projecting PB neurons decreases spontaneous responses to formalin as well as nerve injury-induced hypersensitivity to cold, heat and pinch stimuli. Interestingly, while hypersensitivity to tactile stimuli one day after paw inflammation was reversed by inhibition of the CeA-projecting PB cells, hypersensitivity two weeks after nerve injury was unaffected. Ongoing experiments aim at determining whether pain plasticity in the PB-CeA pathway is cell-type-specific. We also want to determine if the modulation of tactile hypersensitivity by this pathway is time-dependent or pain-type-specific (neuropathic vs inflammatory).

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

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Topic: D.03. Somatosensation – Pain

Support: NRF-2015R1C1A1A01053484
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Title: Inhibition of neuropathic pain by glial regulation in the insular cortex

Authors: *S. CHOI[1,2], K. KIM[1,2], M. CHA[1], S.-K. HONG[3], B. LEE[1,2];
[1]Dept. of Physiol., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Brain Korea 21 PLUS Project for Med. Science, Yonsei Univ., Seoul, Korea, Republic of; [3]Div. of Bio and Hlth. Sci., Mokwon Univ., Daejeon, Korea, Republic of

Abstract: The insular cortex (IC), one of the brain areas that process motivational and emotional aspect of pain information. Studies on the role of neuroglia have been carried out in various pain researches, but the majority of researches have been done at the spinal cord. We hypothesize that glial cells may mediate the neuronal alteration through regulating synaptic physiology of cortical neurons in the IC. The aim of this study was to reveal the associations between neuroglia and neurons in the IC on neuropathic pain (NP) condition. Fluorocitrate (FC) and minocycline (MC) were bilaterally administered into the IC at different injection time points. We conducted two experiments, one is injection of FC (1 nM) and MC (20 uM) into the IC for 7 days (NP 0-7 days) and the other is application of drugs in the chronic pain stage (NP 8-14 days) for 7 days. The behavioral tests were performed before and after drug application. Western blot analysis was performed to evaluate expression changes of glial cells in the IC. As a result, the early inhibition model of glial cells showed analgesic effect as increasing mechanical thresholds. Especially, MC-treated group which belongs to early inhibition model showed a significant pain alleviation effect despite the drug withdrawal. The chronic pain stage inhibition model showed significant analgesic effect which was confirmed by behavioral tests during the period that FC and MC were applied. Interestingly, GFAP, increased as much as vehicle in the MC-treated group of the early inhibition model. These data suggest that glial cell regulation in the IC has a pain relieving effects during the development of chronic pain. Suppression of glial cells just after the nerve injury could delay the neuronal changes associated with chronic pain development. This study was supported by the Basic Research Program through the National Research Foundation (NRF) funded by the Ministry of Science and ICT (NRF-2015R1C1A1A01053484 and 2017R1A2B3005753)

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

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Title: Chronic pain promotes integration of sensory and affective cortical circuits

Authors: *A. K. SINGH[1], Q. ZHANG[2], X. GUO[4], D. PATEL[4], R. TALAY[4], L. HU[4], E. ROBINSON[4], H. KEMPRECOS[4], E. MARTINEZ[4], Z. CHEN[1], J. WANG[3];
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Abstract: Pain is an integrated sensory and affective experience. Cortical mechanisms for this sensory and affective integration, however, are not known. Here we combine optogenetics with *in vivo* electrophysiology in free-moving rats to investigate a direct projection from the primary somatosensory cortex (S1), which receives and encodes the sensory information of pain, to the anterior cingulate cortex (ACC), an area crucial for processing the affective, or aversive, component of pain. We identify nociceptive information flow between these two regions and show that whereas only a small fraction (8%) of ACC neurons receives direct S1 inputs, these neurons display exceptional responsiveness to noxious stimuli. Furthermore, activation of S1 axon terminals in the ACC can recruit new ACC neurons to respond to noxious stimuli, as well as to increase the firing rates of the individual pain-responsive neurons. At the behavioral level, this direct S1 to ACC projection contributes to the aversive response to pain. Importantly, in the chronic pain state, there is an increase in the connectivity between these two cortical areas, as manifested by a higher number of ACC neurons that respond to S1 inputs and the magnified contribution of these neurons to the nociceptive response in the ACC. This increased cortical connectivity in turn contributes to enhanced aversive behavior associated with chronic pain. These results provide direct evidence for cortical integration of sensory and affective pain pathways; they also show that elevated cortico-cortical nociceptive information flow contributes to chronic pain and may thus be a target for neuromodulatory therapies.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 397.22/M31

Topic: D.04. Somatosensation – Touch

Support: European Research Council (ERC-2015-CoG-682422)
Centre national de la recherche scientifique (CNRS)
Independent Research Fund Denmark

Title: Posterior insular cortex encodes non-painful thermal perception

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Abstract: Fast and accurate perception of surface temperature is important for forming a somatosensory percept during object touch. Anatomical, functional imaging and lesion studies in humans and rodents have suggested different cortical areas are involved in non-painful thermal perception (Craig et al. 2000, Milenkovic et al. 2014, Bokiniec et al. 2018, Beukema et al. 2018, Greenspan et al. 2008), however, a consensus has not yet been reached. Here we used the mouse forepaw system to investigate the cortical processing of non-painful temperature stimuli. Widefield calcium imaging data indicate that a region of posterior insular cortex (pIC) is activated by brief (2s) warming and cooling of the forepaw. While responses to warming and cooling of the forepaw spatially overlap, responses to stimulation of different body parts are somatotopically organized. The widefield thermal response amplitude correlates with stimulus size but shows distinct temporal dynamics, with warming responses having a longer latency than cooling. Two-photon single cell imaging reveals that a partially overlapping population of neurons respond to cooling and warming with similar temporal dynamics to that observed at the population level. Finally, optogenetic manipulations in trained mice show that pIC is required for non-painful warm and cool perception. Taken together, our data suggest that pIC is the primary cortical region for non-painful thermal sensation.

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Disclosures: M. Vestergaard: None. **M. Carta:** None. **J.F.A. Poulet:** None.

Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 397.23/M32

Topic: D.03. Somatosensation – Pain

Support: MOST106-2629-B-010-001-MY3

Title: Modulating pain by auditory neural entrainment

Authors: *R.-J. HUNG[1], I. LOW[1], T.-F. LIN[1], H.-K. FU[3], H.-Y. LI[1], L.-F. CHEN[1,4,2], J.-C. HSIEH[1,4,2];
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Abstract: Perception of pain is protective. However, when pain persists, it can become detrimental to one's emotion, cognition, and quality of life. Pain can be associated with a decrease of alpha power. People who are sensitive to pain are more likely to have lower peak alpha frequency. It has been shown that antecedent alpha-wave entrainment may decrease the subsequent experimentally induced pain intensity and pain-evoked potentials. This study aimed to investigate the efficacy and the central mechanisms of auditory neural entrainment (ANE) for pain modulation. We hypothesized that ANE can induce alpha oscillations in the neural substrates for pain processing and thereby reduce pain intensity. Twenty-nine healthy volunteers (mean age 25.24 ± 3.82 years; 14 males) were recruited in this study. The study was a within-subject design. All participants received 15-min auditory stimuli as ANE protocol and underwent pressure pain tests (Medoc, Israel) as a model of chronic pain. In experimental condition, subjects listened to the binaural beats during ANE phase; in control condition, subjects listened to the pure tone during ANE phase. Electroencephalographic signals were recorded during the experiment using a 64-channel actiCAP system (Brain Products, Munich, Germany). ANE-induced analgesia was statistically examined using Wilcoxon's signed-rank tests. Almost all participants developed more intensified pain during the second pain test as compared to the first pain test (possibly due sensitization). However, listening to the binaural beats lessened the degree of sensitization in 15 participants (responders). In addition, the responders showed significantly higher peak alpha frequency after ANE in parietal ($Z = 2.31$, $p = .02$) and occipital ($Z = 2.07$, $p = .04$) regions. Our study unveiled different phenotypes and EEG endophenotypes for ANE-analgesic effect.

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Poster

397. Pain: Thalamus, Cortex, and Amygdala Processing

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: NIH Medical Scientist Training Program
A. Alfred Taubman Medical Research Institute

Title: Zona incerta stimulation in humans differentially modulates perception of heat pain and thermal grill sensation

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Abstract: Deep brain stimulation (DBS) for the treatment of pain classically targets sensory thalamus, periaqueductal gray, and periventricular gray matter. Long term success rates of DBS treatment of neuropathic pain due to stroke, peripheral neuropathy, and brachial plexus injury, are unsatisfactory. Strong cytoarchitectural, animal, and clinical evidence point to zona incerta as a promising new target for analgesic neuromodulation. This study directly examines the effect of zona incerta stimulation on perception of heat pain and thermal grill sensation in human volunteers. Parkinsonian patients with subthalamic DBS implants traversing zona incerta were asked to rate perceived pain evoked by heat or thermal grill stimuli applied to the volar forearm. Pain ratings with and without concurrent DBS were compared to determine the analgesic effects of zona incerta neuromodulation. Preliminary results show decreases in evoked heat pain and increases in thermal grill pain during DBS. These results suggest that zona incerta DBS may differentially modulate perception of heat pain and thermal grill sensation, encouraging further investigation of zona incerta as a DBS target for treatment of pain.

Disclosures: C.W. Lu: None. **D.E. Harper:** None. **S.E. Harte:** None. **P.G. Patil:** None.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

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Program #/Poster #: 398.01/M34

Topic: D.03. Somatosensation – Pain

Support: NIH Grant 5R01GM120519 to C.D.M

Title: Early postnatal exposure to general anesthesia agent causes chronic neuropathic pain via activation of mTOR signaling pathway in pain circuits

Authors: *Q. LI, R. P. MATHENA, N. EREGHA, C. D. MINTZ;
ACCM, Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Previous studies raise a concern that childhood exposure to general anesthetics (GA) may produce cognitive deficits. Our animal studies indicated that early exposure to isoflurane (Iso) causes lasting impairments in learning and memory functions and impedes neuronal development and myelin formation in the mouse hippocampus. Iso aberrantly increases the activity of mTOR and treatment with rapamycin (Rapa), an inhibitor of the mTOR, restores the developmental abnormalities resulting from this exposure. Here we ask whether Iso might also alter the development of the sensory pathways mediating pain perception, which are regulated by mTOR activity. At P7, 14 mice were exposed to 1.5% Iso and 7 were separated from the dams to serve as controls. From P21-P35, Rapa or vehicle was injected (i.p.) to Iso exposed mice. During P56-P63, pain behavior tests (von Frey, tail flick, and formalin injection) were performed and mice were sacrificed at P63. Sections of brain and L4-6 spinal cord (SC) were immunostained (IHC; n=4) and fresh tissue of insular cortex (IC) and dorsal SC were conducted for Western blotting (WB; n=3) with following antibodies: c-fos, p-S6, NeuN, p-mTOR, t-mTOR. In pain behavior tests, Iso significantly decreased hind-paw withdrawal threshold (1.9 g vs. 1.6 g, $p<0.05$) and time to tail-flick (10.2 sec vs. 7.6 sec, $p<0.05$). Rapa treatment reversed these effects (von Frey: 1.8 g; tail flick: 9.9 sec, $p<0.05$). Following formalin injection in hind paw, Iso exposed mice spent increased time licking the injection site (314.3 sec) compared to controls (233.4 sec, $p<0.05$); and Rapa decreased this time (265.1 sec) during phase II (15-30 min). IHC revealed 12.1/mm^2 c-fos+ nuclei in control IC and 79/mm^2 in Iso group ($p<0.001$); and Rapa decreased this number to 31.6/mm^2 ($p<0.01$). 75.4/mm^2 p-S6+ neurons were seen in control and 161.3/mm^2 in Iso group ($p<0.05$). This number was decreased in Rapa treated mice (92/mm^2, $p<0.05$). In dorsal SC (lamina I+II), the number of p-S6+ neurons was increased by Iso (2/mm^2 vs. 4.77/mm^2, $p<0.01$) and it was restored with Rapa (2.5/mm^2, $p<0.05$). WB results indicated that ratio of band intensity of phospho-mTOR over total-mTOR increased with Iso compared to control in IC (29.4% vs. 103.6%, $p<0.001$), and decreased with Rapa (58.7%, $p<0.05$). Iso exposure to early developmental mice causes aberrant increase in neuronal activity, mTOR signaling, and expression of key excitatory synaptic receptors in the IC and superficial dorsal SC in adult. Rapa treatment attenuates these effects. Chronic hyperalgesia is observed in Iso exposed mice and Rapa rescues this function. Together these data suggest early GA exposure may alter the development of pain pathways.

Disclosures: Q. Li: None. **R.P. Mathena:** None. **N. Eregha:** None. **C.D. Mintz:** None.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

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Program #/Poster #: 398.02/M35

Topic: D.03. Somatosensation – Pain

Support: National Health and Medical Research Council of Australia Project Grant APP1144429

Title: The effect of GlyT2 inhibitors on glycinergic neurotransmission in the spinal cord dorsal horn

Authors: *C. A. NATALE[1], T. RAWLING[3], S. N. MOSTYN[1], K. AUBREY[2], R. J. VANDENBERG[1], M. J. CHRISTIE[1];
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Abstract: Evidence suggests that nociceptive and innocuous somatosensory pathways are kept separate within the spinal cord dorsal horn due to the presence of a feed-forward glycinergic gate. In animal models of neuropathic pain this glycinergic function is decreased resulting in allodynia, a condition in which a usually innocuous stimulus is perceived as painful. A novel way of treating this condition is to enhance glycinergic neurotransmission within the dorsal horn, thereby reinstating the glycinergic gate. Here we investigate the ability of a novel class of GlyT2 inhibitors, derived from the endogenous lipid, N-Arachidonyl glycine, to inhibit the GlyT2 subtype of glycine transporters (GlyT) and enhance glycine signalling. This work aims to identify how these novel inhibitors effect glycinergic neurotransmission in the spinal cord dorsal horn in a normal physiological state as well as in animal pain models. The lumbar enlargement section of the spinal cord was removed from anaesthetised rats and sliced using a vibratome. Cell responses to exogenous glycine application were recorded from interneurons in lamina II of the spinal cord dorsal horn via whole-cell patch-clamp electrophysiology. A novel GlyT2 inhibitor known as Oleoyl-D-Lysine was compared to the previously characterised GlyT2 inhibitors, ORG25543 and ALX1393. Both the exogenous and tonic glycine responses are increased in the presence of ORG25543 and ALX1393. In contrast, the novel inhibitor, Oleoyl-D-Lysine had no effect on the current induced by exogenous glycine, but did stimulate a significant increase in tonic current. For both ORG25543 and Oleoyl-D-Lysine the increase in tonic current was positively correlated with the size of the exogenous glycine response, with statistically significant increases in tonic current being present in cells with glycine responses of 200pA or greater. ALX1393 did not follow this trend. Overall these results show that all 3 GlyT2 inhibitors tested reach their desired target in spinal cord slice recordings, as they are able to enhance glycinergic neurotransmission. The difference in effect of ALX1393, ORG25543 and Oleoyl-D-

Lysine is likely linked to different mechanisms employed by each drug to inhibit GlyT2. We will further investigate the effects of Oleoyl-D-Lysine and its mechanism of action to understand its potential as an alternative treatment for allodynia and neuropathic pain.

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Poster

398. Central Nervous System Mechanisms in Pain

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Program #/Poster #: 398.03/M36

Topic: D.03. Somatosensation – Pain

Support: KAKENHI 19K07856
KAKENHI 16K09001
KAKENHI 25460729

Title: Expression analysis of BEGAIN mRNA and protein in the nervous systems

Authors: *T. KATANO[1], K. KONNO[2], K. NISHIDA[1], M. WATANABE[2], K. SAKIMURA[3], S. ITO[4], T. KOBAYASHI[1];
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Abstract: Expression level of synaptic proteins are regulated in the physiological and/or pathological state. In our previous study, we identified brain-enriched guanylate kinase associated protein (BEGAIN) as an increased protein in the PSD fraction of the spinal dorsal horn after spared nerve injury. In the spinal cord, BEGAIN protein was highly localized at the synapse of dorsal laminae IIi-IIIo and it was not detected in the ventral horn. However, BEGAIN-positive neurons were detected in both ventral and dorsal horn of the spinal cord by *in situ* hybridization. In order to clarify this discrepancy between protein and mRNA, we carried out expression analysis of BEGAIN. In mice, two splice variants of BEGAIN (Began-203 and 204), which may encode proteins as 619 and 824 amino acids, respectively, have been demonstrated (Ensemble database; http://asia.ensembl.org/index.html). To confirm the expression of these splice variants, we performed quantitative RT-PCR for each splice variant and western blotting in the hippocampus, spinal ventral horn, dorsal horn and dorsal root ganglia, and detected both variants in all the tissues by quantitative RT-PCR. Western blotting using anti-BEGAIN antibody demonstrated that Begain-203 of approximately 68 kDa was expressed in the hippocampus and spinal dorsal horn, but not ventral horn and dorsal root ganglia. On the other hand, Begain-204 of approximately 90 kDa was not detected in any tissue by western blotting. These results demonstrate that the expression pattern of BEGAIN protein differs from that of

RNA (coding and non-coding) in mouse. Further analyses of these splice variants may clarify the translational control of BEGAIN in the nervous systems.

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Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

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Topic: D.03. Somatosensation – Pain

Support: National Natural Sciences Foundation of China (81701112, 31871065, 81870886)
China Postdoctoral Science Foundation Grant (2018M633527)

Title: Sex specific effects of paclitaxel on genes expression in prefrontal cortex

Authors: ***L. LIANG**, J.-X. WEI, F. GAO, B. NAGENDRA, L. TIAN, H.-R. WANG, L. XU, F.-Q. HUO, S. LU;
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Abstract: Background: Neuropathic pain and cognitive impairment are most popular adverse effects of chemotherapy drugs in nervous system. More recently, paclitaxel has been found to be related to peripheral neuropathy and cognitive impairment in various domains including working memory, information processing speed, and visual attention. Prefrontal cortex (PFC) is one of the area which is fragile to chemotherapy drugs and contributes to pain and cognitive function. However, the role of PFC in paclitaxel induced-cognitive impairment and peripheral neuropathy are not clear, and there is currently no treatment for this condition. The purpose of this study was to examine the gene expression changes of whole transcriptomes in PFC in the development of chemotherapy-induced cognitive impairments and neuropathic pain. Methods: Chemotherapy-induce neuropathic pain and cognitive impairment were established by intraperitoneal injections of paclitaxel (4 mg/kg) as 4 consecutive injections every other day. Mechanical pain behavior was examined by recording paw withdrawal frequencies (PWFs) in response to 0.16g calibrated von Frey filaments. Cognitive function was examined by new object recognition test and Morris water maze test. RNA-Seq assay and an in-depth gene expression analysis was performed to determine the effect of paclitaxel treatment on genes expression in the PFC in both male and female mice. RT-qPCR was used to verify genes expression in subareas of PFC including medial PFC and ventrolateral orbital cortex in both sexes. Results: Mice with paclitaxel injection showed significant mechanical pain hypersensitivities, impaired cognitive function in new object recognition test and Morris water maze test. Analysis from paired control and paclitaxel-treated PFC showed that total 1798 DEGs were differentially expressed by paclitaxel treatment in male

and female mice. There is an increased tendency for genes related to inhibitory synaptic transmission while a decrease in genes related to excitatory synaptic transmission. Conclusions: This study suggests that paclitaxel-induced genes expression changes may be related to neuropathic pain and cognitive impairments. Precise mechanisms in sex dimorphisms may help to improve the translational relevance to clinical populations.

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Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 398.05/M38

Topic: D.03. Somatosensation – Pain

Support: Asahi Kasei Pharma
Boston Scientific

Title: An EEG-AI classifier of chronic pain

Authors: *M. M. EDHI[1,2], J. LEVITT[1], K. S. SCARFO[1], A. G. CARAYANNOPOULOS[1], J. W. GU[3], K. H. SRIVASTAVA[3], B. A. CLARK[3], R. ESTELLER[3], C. Y. SAAB[1,2]; [1]Neurosurg., Rhode Island Hosp., Providence, RI; [2]Carney Inst. for Brain Science/Brown Univ., Providence, RI; [3]Boston Scientific, Valencia, CA

Abstract: Due to the pain epidemic and related opioid crisis, the development of pain biomarkers is urgently needed. We hypothesized that electroencephalography (EEG) contains physiological information relevant to pain, which could be leveraged to build a diagnostic algorithm. We obtained resting state EEG from 3 groups of human subjects: G1) healthy (n=20), G2) chronic lower radiculopathy pain (n=20, visual analogue scale or VAS >5, aged and gender matched to healthy), and G3) chronic lower back pain (n=17, VAS >5, treatment-resistant pain and candidates for spinal cord stimulation). First, we investigated differences between G1 and G2 in the following categories: power spectra in single channels, coherence between channel pairs, and phase amplitude coupling between frequency band pairs, however, results showed no statistical difference. We then trained a support vector machine (SVM) algorithm which resulted in a cross-validated binary classification accuracy of 80% and greater than 100% of randomly shuffled training and testing iterations. Second, we investigated the performance of a SVM trained on data from G1 and G2 using 'test' data from G3. The SVM predicted G3 to fall within the G2 class with 100% accuracy, suggesting that the EEG features in these patients are distinct from those in healthy subjects. Third, we trained a SVM using data from G1, G2 and G3, resulting is 3-way SVM classification with 70% accuracy and leave-one-out cross validation. We

concluded that, while conventional statistics show no difference in standard wavelet analysis of EEG, some of these features nonetheless contain pain-related information that enables a computer algorithm to predict clinically distinct pain states.

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Poster

398. Central Nervous System Mechanisms in Pain

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Distinguished Professor Program of Jiangsu, and Postgraduate Research & Practice Innovation Program of Jiangsu Province Grant KYCX18_2196

Title: Dorsal raphe nucleus glutamatergic neurons innervate mesolimbic system and regulate chronic pain

Authors: *S. HU, D. LIU, Q. ZHANG, J. YANG, M. ABDUL, S. XIA, J. CAO;
Jiangsu Province Key Lab. of Anesthesiol., Xuzhou Med. Univ., Xuzhou, China

Abstract: Numerous researches have indicated that the mesolimbic system, consisting of ventral tegmental area (VTA) and nucleus accumbens (NAc), plays a vital role in the modulation of chronic pain. Our previous study has suggested that the maladaptations in VTA-NAc dopaminergic (DA) neurons can mediate the pathophysiological processes of nociception. However, the mechanisms by which the VTA DA neurons are over-activated under nociceptive condition are still unclear. Here, we demonstrated the dorsal raphe nucleus (DRN) neurons innervated VTA-NAc DA neurons with the tans-synaptic tracing strategy mediated by modified rabies virus. The vitro electrophysiological recordings confirmed that the spontaneous firing rates of DRN neurons innervating VTA-NAc DA neurons (DVN) were increased in a mouse model of chronic pain (sciatic nerve chronic constriction injury, CCI). In naïve mice, photoactivation of the DVN was sufficient to induce a nociceptive phenotype which could last

for 2 days when repeated photoactivation was given. We also validated that the DRN were mostly glutamatergic (GLU) rather than serotonergic (SER). In parallel, photoinhibition of the DRN-VTA GLU pathway relieved pain in CCI mice. Meanwhile, photoactivation of this pathway mimicked pain-like behavior in naïve mice. However, DRN-VTA SER pathway was incapable of eliciting the same effects which were driven by the GLU pathway. Besides, the EPSCs in VTA-NAc DA neurons were increased by stimulation of VTA-projecting DRN neuronal terminals with optogenetics, which could be blocked by bath perfusion of artificial cerebrospinal fluid containing NBQX. In addition, increased amplitude and decreased paired-pulse ratio (PPR) of light-evoked EPSCs were observed in the VTA-NAc DA neurons in CCI mice. The AMPA/NMDA ratio of VTA-NAc DA neurons innervated by DRN neurons was increased. Furthermore, the intra-VTA injection of NBQX reversed the nociceptive phenotype induced by photoactivation of DVN but AP-5 could not express the same effect. Hence, we conclude that the GLU innervations from the DRN to mesolimbic DA neurons are involved in nociception modulation. Both glutamate released from presynaptic DRN neurons and glutamate receptor on postsynaptic VTA-NAc DA neurons can mediate the modulatory roles of the DRN-VTA-NAc pathway in chronic pain. Our results provide a potential target for therapeutic intervention for chronic pain.

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Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

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Program #/Poster #: 398.07/M40

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS099245

Title: Suppression of the inhibitory amygdala-parabrachial pathway contributes to chronic pain

Authors: C. RAVER, O. UDDIN, Y. LI, Y. JI, N. CRAMER, R. MASRI, *A. KELLER; Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The parabrachial nucleus (PB) is a midbrain area involved in acute pain transmission. PB communicates with diverse brain regions controlling sensation, homeostatic functions, and emotional processing, including the central nucleus of the amygdala (CeA). We have recently shown, in rats, that PB neural activity is amplified after chronic constriction injury of the infraorbital nerve (CCI-ION), a model of neuropathic pain. Here, we test the hypothesis that decreased inhibitory input from CeA to PB contributes to PB neuronal amplification, and to chronic pain. Adult male and female animals were randomly assigned to CCI-ION and control

groups; experimenters were blind to their condition. Anterograde and retrograde tracers in mice and rats revealed a dense inhibitory projection from CeA to PB. We used RNA-scope to neurochemically identify PB-projecting CeA neurons, finding that they include somatostatin, dynorphin, and corticotropin releasing hormone positive neurons. Whole-cell patch clamp experiments confirmed that inputs from CeA to PB are inhibitory, and, critically, that this inhibition is suppressed in CCI-ION conditions. Corroborating our previous findings in rats, PB neural activity is increased *in vivo* in CCI-ION mice. Finally, we show the functional relevance of this pathway: pain behaviors increase in rats after suppressing the inhibitory CeA to PB pathway, and pain behaviors are relieved by activating the projection in CCI-ION animals. These findings suggest that changes in the CeA to PB pathway could be a major driver in developing and maintaining chronic pain.

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Poster

398. Central Nervous System Mechanisms in Pain

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Topic: D.03. Somatosensation – Pain

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Title: Modulation of mechanosensory vibrissal responses in the trigeminocervical complex by stimulation of the greater occipital nerve

Authors: *N. GARCÍA MAGRO[1], Y. MARTIN[2], P. NEGREDO[1], A. NUNEZ[1], C. AVENDANO[1];
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Abstract: Referred and refractory cervico-cephalic pain is a subject of growing interest because of its high prevalence and severity. The discovery of an area of convergence of afferents from occipital and trigeminal nerves in the upper cervical and medullary dorsal horn provided an appealing substrate to study the neural mechanisms of these painful conditions. Anatomically, this area, known as 'trigeminocervical complex' (TCC), extends from the spinal caudal trigeminal nucleus (Sp5C) to segments C_2-C_3 in the spinal cord[1]. Neuromodulation of occipital or trigeminal nerves has become widely used as a treatment for chronic refractory neurovascular headaches as migraine or cluster headache. The stimulated nerves project to the upper cervical spinal cord or brainstem, where they coincide with other afferents coming from vessels or other

tissues of the regions affected. In this study we aimed at investigating the acute effects that the electrical stimulation of the greater occipital nerve (GON) elicited on the responses of neurons in the TCC to the mechanical stimulation of the ipsilateral vibrissal pad. Experiments were performed on urethane-anesthetized adult male Wistar rats. Following a laminectomy, Sp5C and the upper two segments of the spinal cord were exposed. Unit recordings were obtained from neurons in laminae I-III of the TCC. The GON was isolated and stimulated unilaterally by a monopolar electrode. The responses to the stimulation of vibrissae by 20 ms air pulses were analyzed before and after GON stimulation. Recordings were acquired and analyzed with Spike 2 software. Stimulation of the GON generated a short-lasting response in Sp5C. The stimulation of GON 100 ms before the vibrissal stimulation caused a 33% facilitatory effect on the response to vibrissae in controls. This facilitation was dependent on NMDA receptors, being blocked by local injection of 1µl of AP5 (50µM). Under these conditions, an inhibitory effect was unmasked, which was dependent on the activation of $GABA_A$ receptors and was blocked by the local application of bicuculline (1µl , 20 mM). Currently in progress, a similar recording-stimulation study is carried out on rats showing trigeminal neuralgia-like condition induced by chronic constriction of the infraorbitary nerve. In these animals, the GON-induced facilitation of vibrissal responses disappears, being replaced by inhibitory effects. These results show that, at least in acute settings, the stimulation of GON is able to modulate the responses in TCC to sensory input from a distant location, and that this modulation is changed in rats that present a chronic facial pain.

[1] García-Magro et al. (2018), *J Comp Neurol*, 526.

Disclosures: **N. García Magro:** None. **Y. Martin:** None. **P. Negredo:** None. **A. Nunez:** None. **C. Avendano:** None.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 398.09/M42

Topic: D.03. Somatosensation – Pain

Support: Conacyt, PN-5098

Title: Role of α_5GABA_A receptors in diabetic neuropathy and modulation of the Hoffmann reflex and primary afferent excitability

Authors: J. HERNÁNDEZ-REYES[1], A. SALINAS-ABARCA[2], G. VIDAL-CANTÚ[2], G. RAYA-TAFOLLA[1], D. ELÍAS-VIÑAS[3], V. GRANADOS-SOTO[4], *R. DELGADO-LEZAMA[1]; [1]Physiology, Biophysics and Neurosci., [2]Dept. de Farmacobiología, [3]Ingeniería Eléctrica, Cinvestav, IPN, CDMX, Mexico; [4]Dept. De Farmacobiologia, Cinvestav, Coapa, Ciudad de Mexico, Mexico

Abstract: There is evidence that the loss of spinal GABAergic inhibition participates in painful diabetic neuropathy. However, the role of extrasynaptic α_5 subunit-containing $GABA_A$ (α_5GABA_A) receptors in this process is unknown. The purpose of this study was to investigate the role of α_5GABA_A receptors in diabetes-induced tactile allodynia, loss of rate dependent depression (RDD) of the Hoffmann reflex (HR) and modulation of primary afferent excitability. A combination of behavioral (threshold withdrawal), electrophysiological (HR, RDD and excitability test) and immunohistochemical techniques was used in this study. Experiments were carried out in female Wistar rats. Intraperitoneal administration of streptozotocin (STZ) induced tactile allodynia. Intrathecal injection of α_5GABA_A receptor inverse agonist, L-655,708, produced tactile allodynia in naïve rats whereas it reduced allodynia in STZ-treated rats. In healthy rats, electrical stimulation of the tibial nerve at 5 Hz induced RDD of the HR, although intrathecal treatment with L-655,708 (15 nmol) abolished RDD of the HR. STZ induced the loss of RDD of the HR, while intrathecal L-655,708 (15 nmol) restored RDD of the HR. For the excitability test the antidromic compound action potential (cAP) was evoked in the tibial nerve by the intraspinal electrical stimulation at the level of the lumbar enlargement. The sural and the peroneal nerves were used as a conditioned stimulation. The interstimulus interval of 30 ms between the conditioned and the test stimulation was selected to assess the action of L-655708 on the phasic and tonic excitability of primary afferent fibers. L-655,708 (15 nmol) increased tonic excitability of the primary afferents without affecting the phasic excitability produced by the primary afferent depolarization. α_5GABA_A receptors were immunolocalized in superficial laminae of the dorsal horn and L4-L6 DRG. STZ increased mean fluorescence intensity and percentage of neurons expressing α_5GABA_A receptors in dorsal horn and L4-L6 DRGs in ten-week diabetic rats. Our results suggest that spinal α_5GABA_A receptors modulate the HR, play an antinociceptive and pronociceptive role in healthy and diabetic rats, respectively, and are tonically active in primary afferents.

Disclosures: J. Hernández-Reyes: None. **A. Salinas-Abarca:** None. **G. Vidal-Cantú:** None. **G. Raya-Tafolla:** None. **D. Elías-Viñas:** None. **V. Granados-Soto:** None. **R. Delgado-Lezama:** None.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 398.10/M43

Topic: D.03. Somatosensation – Pain

Support: NIH R01 DE021996
NIH F32 NS100438

Title: TLQP-62, a neuropeptide upregulated by nerve injury, facilitates spinal BDNF release

Authors: *A. G. J. SKORPUT[1], J. L. COOK[1], R. GORE[1], M. S. RIEDL[1], L. VULCHANOVA[2];
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Abstract: BDNF-dependent central sensitization contributes to the development and maintenance of mechanical allodynia in rodent models of neuropathic pain. The signaling mediators leading to increased BDNF bioavailability and its cellular source remain controversial. The neuropeptide TLQP-62 is derived from the precursor protein VGF (non-acronymic), which in the superficial dorsal horn of the spinal cord is localized predominantly in primary afferent central processes. Peripheral nerve injury induces rapid and robust upregulation of VGF in primary afferent neurons, and an increase in the level of TLQP-62 in the spinal cord. Exogenous TLQP-62 application induces potentiation of glutamatergic responses in the spinal cord and calcium transients in primary afferent neurons. Several lines of evidence suggest a functional relationship of TLQP-62 and BDNF in hippocampus. For example, TLQP-62 induces BDNF-dependent plasticity that functions in learning and memory. In addition, VGF signaling increases the expression of BDNF, and vice versa in an autoregulatory feedback loop. Based on these observations, here we test the hypothesis that TLQP-62 facilitates the release of spinal BDNF. Using an *in-situ* ELISA approach, we are examining the effect of TLQP-62 on BDNF release from spinal cord slices, cultures of dorsal root ganglion (DRG) neurons, and cultures of adult spinal microglia. Our results are consistent with TLQP-62-induced facilitation of activity-dependent release of BDNF from spinal cord slices and DRG cultures. This suggests that BDNF bioavailability in the spinal cord following peripheral nerve damage may be the result of increased TLQP-62 levels. In combination with our previous work, these results suggest that TLQP-62 and BDNF may participate in an autoregulatory feedback loop that maintains a state of heighted synaptic plasticity in the spinal cord dorsal horn to facilitate central sensitization and the manifestation of tactile allodynia.

Disclosures: A.G.J. Skorput: None. **J.L. Cook:** None. **R. Gore:** None. **M.S. Riedl:** None. **L. Vulchanova:** None.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

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Program #/Poster #: 398.11/M44

Topic: D.03. Somatosensation – Pain

Support: MRC Grant MR/P00668X/1

Title: Alterations in pre limbic - periaqueductal grey functional connectivity regulates the temporal development of neuropathic pain in rats

Authors: *R. DRAKE, R. APPS, A. PICKERING, B. LUMB;
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Abstract: The descending pain modulatory system (DPMS) regulates acute pain and its maladaptation is thought to be important in chronic pain. However, the cause of this maladaptation is not well understood. The medial prefrontal cortex (mPFC) and midbrain periaqueductal grey (PAG) are key components of the DPMS with the PAG orchestrating modulation of nociceptive processing within the spinal dorsal horn via brain stem nuclei. Brain imaging studies in humans show functional connectivity changes between the mPFC and PAG during endogenous pain modulation and alterations in this functional connectivity that is related to chronic pain development. However, it is not known whether these alterations are causally related to the development of the chronic pain. To address this question of causality, we selectively transduced pre-limbic cortex-PAG projection neurones with both channelrhodopsin2 and the inhibitory DREADD, hM4Di using a retrograde CAV-Cre + Cre-dependent AAV strategy. This allows selective activation and inhibition of neurones in rats undergoing sensory testing. In uninjured animals, optoactivation (20Hz, 10ms) of PL-PAG neurones produced a bi-lateral increased in thermal withdrawal latency (Stim vs no-stim, 10.9±0.8 vs 8.6±0.7 seconds, p=0.0006, paired t-test, n=7) but had no effect in control rats (no CAV-Cre, n=4). Systemic clozapine-N-oxide (2.5mg.kg^{-1} i.p), DREADD agonist, had no effect on withdrawal latencies to thermal stimulation in either group. The same animals had tibial nerve ligation (under recovery anaesthesia) to produce a neuropathic pain model (chosen because of its gradual (~1 week) progression which is dependent on descending inhibitory noradrenergic control (Hughes et al 2013)). DEADD inhibition of PL-PAG projections revealed the contribution of PL-PAG communication to the pain phenotype. At early time points (day 3-7), inhibition unmasked a latent mechanical hypersensitivity (day 3 von Frey withdrawal threshold vehicle vs CNO, 10.3±2.5 vs 2.9±1.6g, p=0.02, Two-Way RM ANOVA with Sidak's post-test, n=4) but not in control animals. Conversely, systemic CNO delivery at late time (21 days post-TNT) points had little effect on mechanical withdrawal thresholds in experimental or control animals which is consistent with the concept that the DPMS fails over time in this model and implicating the PL-PAG causally in the development of the neuropathic pain phenotype.

Disclosures: R. Drake: None. **R. Apps:** None. **A. Pickering:** None. **B. Lumb:** None.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

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Topic: D.03. Somatosensation – Pain

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Title: A cortico-accumbens CRF-ergic circuit for pain sensation

Authors: *W. ZHAO[1,2], X. WANG[1,2], Y. MA[1,2], G. ZHANG[1,2], Y. YU[1,2], B. FAN[1,2], J. YANG[1,2], A. MANNAN[1,2,3], H. ZHANG[1,2], J.-L. CAO[1,2,3];
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Abstract: Corticotropin-Releasing Factor (CRF) is a neuropeptide which is closely related to pain perception. However, the mechanism through which it regulates pain behaviors, especially at the microcircuit level remains to be unknown. The nucleus accumbens (NAc), an important target brain region of CRF heavily influence the pain modulation in animal and human being studies. The present study was designed to screen the sources of CRF-ergic projections in the NAc, and to explore the potential functional role of interested CRF-ergic pathways in modulating pain sensation. To determine the upstream sources of CRF-ergic projections in the NAc, a retrograde adeno-associated virus (AAV) encoding CRF promoter with an enhanced green fluorescent protein tag was injected into the NAc of naïve mice. Our immunofluorescent staining in consecutive whole brain slices demonstrated that the medial prefrontal cortex (mPFC) was the predominant source of NAc CRF-ergic projections. To investigate whether artificially activating the cortico-accumbens CRF-ergic projection in the NAc was sufficient enough to induce pain-like behaviors, AAV-encoding Cre-recombinase with CRF promoter and Cre-inducible Channelrhodopsin (AAV-DIO-ChR2) were injected into the NAc and mPFC respectively, to target the projection-specific cortico-accumbens CRF-ergic neurons. Behavioral tests that were repeated at the interval of 5-days (10Hz 15mW 10ms) demonstrated that the optical activation of the cortico-accumbens CRF-ergic projections in the NAc decreased the thermal paw withdrawal latency (PWL) and 50% mechanical paw withdrawal threshold (50%PWTs) in naïve mice. In neuropathic pain mice in which chronic constrictive injury (CCI) of the sciatic nerve was performed shows a significant increase in c-fos protein within cotico-accumbens CRF neurons, indicating hyperactivity of this circuit in pathological pain. In our proceeding inhibitory optogenetic experiment, replacing AAV-DIO-ChR2 with Cre-inducible Halorhodopsin (AAV-DIO-NpHR), increased PWL and 50% PWT were observed when the yellow laser was turned on. Interestingly, the analgesic effect observed above could also be achieved by pharmacologically antagonizing CRF receptors in the NAc. Ongoing experiments are more exploring the role of NAc CRF receptor in mediating the pain behaviors induced by activating the cortico-accumbens CRF circuit. In summary, these studies identified the previously unknown circuit for regulating pain sensation of the CRF-ergic neurons that project from mPFC to NAc, and these studies also

help in providing the novel molecular targets for translational drug development for chronic pain treatment.

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Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 398.13/N2

Topic: D.03. Somatosensation – Pain

Support: Canadian Institutes of Health Research - Master Award
University of Toronto Centre for Study of Pain
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Canadian Institutes of Health Research operating grant MOP130555

Title: Tn pain relief leads to a bilateral volumetric increase in hippocampal subfields

Authors: *A. NOORANI[1], S.-P. HUNG[1], J. ZHANG[1], M. HODAIE[2];
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Abstract: Hypothesis
Trigeminal neuralgia (TN) is the most prevalent type of chronic neuropathic facial pain. Recent findings suggest that the limbic system, specifically the hippocampus (HC), is involved in processing of chronic pain, and associated with decreased HC volume. However, there are few models that can adequately study the structural changes in the HC in chronic pain. TN serves as a unique model to assess these alterations after successful pain relief as it is highly amenable to surgical treatment. We hypothesize that relief of pain status results in a volumetric HC increase, in particular subfields important in connecting the pain circuits to HC such as Cornu Amonis 2 (CA2) and CA4.
Methods
Patients with T1 anatomical imaging before and 6 months after surgical treatment were retrospectively studied. FreeSurfer 6.0 was used to segment the HC subfields and a residual approach was employed to account for individual variability. HC subfield volumes were contrasted between responders (75% pain reduction) and non-responders at pre and post-surgery using standard T-test.
Results
71 TN (45F, age = mean ± SD, 66.1 ± 12.5) patients were grouped as 46 responders (29F, age = 65.0 ± 12.3), and 25 non-responders (16F, age = 68.3 ± 12.7). Unique subfield HC changes

related to pain relief consisted in a contralateral volumetric increase in GC (P=0.048) and CA4 (P=0.047) in responders. Ipsilaterally, molecular Layer (P=0.030), GC (P=0.007), CA2/3 (P=0.018), and CA4 (P=0.006) showed a volumetric increase in responders.

Conclusion

Our findings suggest that there are HC subfields volume increases associated with TN pain relief, characterized by a bilateral increase in CA4 and GC. Considering the known association between chronic pain, stress and associated HC structural abnormalities, our data points to a dynamic nature of HC subfields. Furthermore, we suggest that these structural alterations may relate to increased neuronal density/neurogenesis in HC.

Disclosures: A. Noorani: None. **S. Hung:** None. **J. Zhang:** None. **M. Hodaie:** None.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 398.14/N3

Topic: D.03. Somatosensation – Pain

Title: Alleviating neuropathic pain by overexpression of a non-functional colony stimulating factor-1 receptor

Authors: S. V. GUSHCHINA[1,2], P. K. YIP[1], G. A. PARRY[1], H. SIVAKUMAR[1], J. LI[1,3], M. LIU[1], *X. BO[1];
[1]Ctr. for Neuroscience, Surgery and Trauma, Queen Mary Univ. of London, London, United Kingdom; [2]Dept. of Cytology, Histology and Embryology, Ogarev Mordovia State Univ., Saransk, Russian Federation; [3]Dept. of Lab. Medicine, Taihe Hosp., Hubei Univ. of Med., Shiyan, China

Abstract: Proliferation and activation of microglia have been implicated to play an important role in the development of neuropathic pain following peripheral nerve injury. We have taken a molecular therapy approach to attenuate microglial proliferation to alleviate neuropathic pain. Colony stimulating factor-1 receptor (CSF1R) is crucial for microglial proliferation. We generated an adeno-associated viral vector (serotype 9) expressing a non-functional soluble CSF1R (AAV9/sCSF1R) as a decoy to block the binding of CSF1 to CSF1R. AAV9/sCSF1R and the control vector AAV9/GFP were intrathecally injected into the lumbar spine of adult C57BL/6 mice. Two weeks later, these mice were subjected to partial sciatic nerve ligation or sham operation. Normal mice and mice with sham operation or sciatic nerve ligation without viral injection were also used as controls. Before and after the surgery on sciatic nerves, mice were tested for mechanical pain sensation. Animals were sacrificed 3 weeks post operation. GFP and sCSF1R were found to be highly expressed in lumbar DRG and spinal cords in AAV9-injected mice. Significant increase in the number of macrophages in lumbar DRG and microglia

in the dorsal and ventral horns of lumbar spinal cords were observed in the mice subjected to sciatic nerve ligation or AAV9/GFP injection plus nerve ligation. In the AAV9/sCSF1R injected mice with sciatic nerve ligation, the microglial densities in the dorsal and ventral horns and macrophage density in DRG were significantly lower compared with the mice subjected to sciatic nerve ligation or AAV9/GFP plus nerve ligation, indicating that sCSF1R expression is able to limit the microglial and macrophage proliferation induced by nerve ligation. Behavioural test showed that sCSF1R expression significantly reduced the nerve ligation-induced mechanical allodynia. The results implicate that over-expression of sCSF1R may represent a novel approach in long-term alleviation of neuropathic pain.

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Poster

398. Central Nervous System Mechanisms in Pain

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Topic: D.03. Somatosensation – Pain

Support: Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (NRF-2017R1D1A1B04035645)
Korea University Fund (K1220201)

Title: Role of the interaction between GluN2B subunit and PSD-95 in neuropathic pain after peripheral nerve injury

Authors: *Y. KIM[1,2], Y. YOON[1,2];
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Abstract: N-Methyl-D-aspartate receptor 2 (NMDAR GluN2) has glutamate binding sites, especially GluN2B subunit located at sensory regions in central nervous system such as the forebrain and the spinal dorsal horn. Therefore, blocking of GluN2B is gaining attention to reduce neuropathic pain with minimal side effects. However, the signal transduction associated with GluN2B in neuropathic pain is not well known. L5 spinal nerve was ligated using 6/0 silk (L5 SNL) under the isoflurane inhalation in male Sprague-Dawley rats (220-250 gram). We analyzed the temporal changes in GluN2B, its phosphorylation residue at Ser1303 and Tyr1472 in the ipsilateral side of the dorsal spinal cord after SNL. Protein expression of GluN2B increased from 6 hours to 4 days and p-Ser1303 expression increased up to 2 weeks after injury. Co-immunoprecipitation was used to examine the interaction between p-Ser1303 and CaMKII, PKC or PSD-95, respectively. The interaction between p-Ser1303 and CaMKII was robustly enhanced from 6 hours to 4 days, and that between p-Ser1303 and PSD-95 was increased from 4

days to 2 weeks after injury. Mechanical paw withdrawal threshold was measured before and after intrathecal administration of relevant drugs. Inhibitors of GluN2B or CaMKII more effectively increased mechanical paw withdrawal threshold in the early phase than the later phase, and reduced the interaction between p-Ser1303 and PSD-95 at that time. These results demonstrate that GluN2B translocation onto the postsynaptic density may play a role in the long term maintenance of neuropathic pain.This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (NRF-2017R1D1A1B04035645) and the Korea University Fund (K1220201).

Disclosures: **Y. Kim:** None. **Y. Yoon:** None.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

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Topic: D.03. Somatosensation – Pain

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FCT project PTDC/NEU-NMC/1259/2014 (POCI-01-0145-FEDER-016588)

Title: Low threshold-afferent-driven inhibition of spinal lamina I neurons

Authors: Y. ANDRIANOV[1,3], D. SHEVCHUK[1,3], N. TRETIAK[1,3], B. V. SAFRONOV[4], N. V. VOITENKO[1,3], ***P. V. BELAN**[2,3];
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Abstract: Spinal lamina I neurons receive inputs from nociceptive Aδ- and C-fiber afferents and relay these inputs to the supraspinal pain processing centers. Pre- and postsynaptic inhibition of these neurons play an important role in processing of painful stimuli. Although the low-threshold fibers are believed to mediate both types of inhibition, their roles in shaping the input-output characteristics of lamina I neurons is poorly understood. Here, we did the whole-cell patch-clamp recording from lamina I neurons in an isolated spinal cord preparation with an attached dorsal root. We have developed a novel method to evoke the Aδ/C-fiber-mediated EPSCs and IPSCs with or without simultaneous activation of the low-threshold fibers. Our results show that the low-threshold afferents can mediate a complete or partial pre- and postsynaptic inhibition of the mono- and polysynaptic Aδ- and C-fiber inputs to lamina I neurons. Different combinations of the low-threshold-afferent-driven presynaptic inhibition were present in the majority of lamina

I neurons, whereas the postsynaptic inhibition was rare. Importantly, the low-threshold-afferent-driven inhibition controls the number of action potentials generated by the lamina I neurons. Thus, we demonstrate that the pre- rather than postsynaptic low-threshold-afferent-driven inhibition shapes the output characteristics of lamina I nociceptive neurons.

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Poster

398. Central Nervous System Mechanisms in Pain

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Program #/Poster #: 398.17/N6

Topic: D.03. Somatosensation – Pain

Support: NRF-2015R1C1A1A01053484
NRF-2017R1A2B3005753

Title: Alleviation of neuropathic pain by the inhibition of mTOR complex in the insular cortex

Authors: *K. KIM[1,2], S. CHOI[1,2], M. CHA[1], S.-K. HONG[3], J. LEEM[1], B. LEE[1,2];
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Abstract: In the pain matrix, the insular cortex (IC) is mainly involved in discriminative sensory and motivative emotion. Recent studies have shown that the mTOR kinase is major regulator of protein synthesis and it could be involved in the regulation of synaptic plasticity and memory formation in the central nervous system. This study was conducted to determine the changes in pain behavior and downstream effectors by mTOR inhibition. In addition, the dynamic changes in the spatiotemporal patterns of the IC activities were analyzed and compared before and after inhibition of mTOR complex in the IC after nerve injury. Under isoflurane anesthesia, the neuropathic surgery was conducted to Sprague-Dawley rats and the rats were anesthetized with isoflurane. The behavioral tests were performed before and after microinjection of mTOR inhibitors (Torin1 and XL388) and vehicle. To assess the spatiotemporal patterns of the IC activities, the optical imaging was conducted. The response of neuronal activities was recorded and these signals were analyzed. Western blot was carried out in order to ascertain the expression changes in mTOR and its downstream effectors. As a result, mTOR inhibitors showed the pain-relieving effect four hours after microinjection of mTOR inhibitors in behavioral test. In optical imaging, the Torin1- and XL388-injected groups showed decreased signals and reduced activation area contrary to the vehicle group. The phosphorylation of downstream effectors of mTOR complex, such as P70S6K and 4EBP, were significantly increased in the vehicle-treated

group. However, they were decreased in the Torin1- and XL388-treated groups. The other phosphorylated downstream effectors of mTOR complex, Akt and PKCα, also increased in the vehicle-treated group while significantly reduced in the mTOR inhibitor-treated groups. In addition, the expression level of phosphorylated mTOR significantly increased in the vehicle-treated group but, it was decreased in the Torin1- and XL388-treated groups. These findings suggested that the pain-relieving effect of Torin1 and XL388 was manifested by modulation of mTOR complex. This study was supported by the Basic Research Program through the National Research Foundation (NRF) funded by the Ministry of Science and ICT. (NRF-2015R1C1A1A01053484 and 2017R1A2B3005753)

Disclosures: K. Kim: None. **S. Choi:** None. **M. Cha:** None. **S. Hong:** None. **J. Leem:** None. **B. Lee:** None.

Poster

398. Central Nervous System Mechanisms in Pain

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Program #/Poster #: 398.18/N7

Topic: D.03. Somatosensation – Pain

Support: MOE Tier 1 - NUHS Joint Grant Call FY17 – 1st call-04

Title: Putative roles of secretogranin V and its metabolites in human osteoarthritic chronic pain

Authors: *F. C. K. TAN[1], I. K. M. CHEAH[2], C. LI[3], W. LIU[1], H. J. NEO[1], C. M. LOW[4,3], S. KHANNA[4], T. L. LEE[3], L. K. TI[3];
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Abstract: Chronic pain can result in social and psychological problems such as inability to work, disrupted social relationship, depression and suicidal thoughts. Chronic pain is difficult to manage, due to incomplete understanding of the complex modulating pathways in nociception and the side effects that limit the use of conventional analgesics such as NSAIDs and opioids. A major cause of chronic pain is osteoarthritis (OA), affecting 10% to 15% of people aged above 60 worldwide (WHO; 2013), is characterized by the loss of joint cartilage that leads to chronic pain and loss of function in the knees and hips. Through HPLC analysis of small peptides in pooled cerebrospinal fluid (CSF) from 20 OA patients, we identified a peptide metabolite of secretogranin V (7B2) protein, designated 7B2CT. 7B2 is involved in the maturation and activation of prohormone convertase 2 (PC2). 7B2CT, however, inhibits the activity of PC2. To activate PC2, 7B2CT peptide which is 31 a.a. (amino acids) in length must be cleaved by PC2 in the secretory granules into two fragments, 7B2CT-N (N-terminal, 18 a.a.) and 7B2CT-C (C-terminal, 13 a.a.). To quantify these peptides in individual patient's CSF, we employed liquid

chromatography coupled with mass spectrometry (LC-MS) and optimized the methodology to detect 7B2CT, 7B2CT-N and 7B2CT-C using 10ul of individual patient's CSF per run. Comparison between the CSFs of OA patients with chronic pain and control subjects without pain indicated that 7B2CT levels were significantly elevated in Asian OA pain patients in a small sample size trial (n=4; T-Test; $p<0.01$). Next, we investigated the binding site(s) of 7B2CT-N and 7B2CT-C by using biotinylated-7B2CT-N and biotinylated 7B2CT-C peptide to bind to mouse brain section in a binding assay. We found that 7B2CT-C, but not 7B2CT-N, binds strongly to the thalamus region of the mouse brain suggesting that 7B2CT may also bind in this brain region. Our findings suggest that 7B2CT may be a novel biomarker for OA chronic pain and 7B2CT (and its metabolites) may be involved in induction and/or maintenance of OA pain by acting as a neuromodulator released from the CNS during chronic pain in OA. These data may help in the isolation of an interacting partner for 7B2CT and in the development of a new class of anti-nociceptive drugs.

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Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 398.19/N8

Topic: D.03. Somatosensation – Pain

Title: Comparing the firing patterns of superficial dorsal horn neurons evoked by robotically automated and human manual brushing and Von Frey hair stimulation

Authors: *D. C. LEE[1], J. E. LEE[2], K. LEE[1], Z. KAGAN[1], K. BRADLEY[1];
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Abstract: Investigations into the response of spinal dorsal horn (SDH) neurons to afferent inputs typically employ various types of mechanical stimulation to peripheral structures (e.g., a rodent paw). Most often, these stimuli are applied manually to a receptive field; manual application may contribute to inconsistent variation in the parameters of the stimulus: temporal timing, duration, location, etc. We compared the spinal dorsal horn neural response between manual and custom robot-based brushing/Von Frey stimulation system.A robot with four degrees of freedom was programmed to brush the hind paw of an anesthetized rat. Multichannel electrodes were placed in the ipsilateral SDH (lamina II-III, the spinal segment L4-6) to monitor single unit firings evoked by a similar brushing motion and Von Frey stimulation performed by the robot and from a trained examiner. The robot brushing was programed to brush in an arc motion at different depths (0.5, 1, 2 and 3mm), speeds (50, 100 and 200mm/s) and directions (proximal to distal vs. distal to proximal). Von Frey hair stimulation was applied with 2, 6, 10 and 15g hair for 1.5s.

Qualitatively, automated brushing and Von Frey generated consistent and repeatable patterns of multiunit SDH activity. Manual forward brushing was most similar to the automated brushing with depth=2mm, speed=100mm/s in terms of firing rate and duration. Manual Von Frey duration had during of 0.76±0.12s with intension of 1 s stimulation duration, while automated Von Frey had duration of 1.4±0.02s. Mean firing rate from manual Von Frey was ~10% higher than automated stimulation. Interestingly, it appeared that most recorded neurons, activated by manual brushing or Von Frey hair were also fired by automated brushing, but certain neurons were activated more by manual mode or the other. Standardization of the sensory stimulus using this novel robot tool may allow for fewer trials of applied afferent input and greater sensitivity to detect subtle changes in response in sensory experiments or intervention such as spinal cord stimulation.

Disclosures: **D.C. Lee:** A. Employment/Salary (full or part-time):; Nevro. **J.E. Lee:** None. **K. Lee:** A. Employment/Salary (full or part-time):; Nevro. **Z. Kagan:** A. Employment/Salary (full or part-time):; Nevro. **K. Bradley:** A. Employment/Salary (full or part-time):; Nevro.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 398.20/N9

Topic: D.03. Somatosensation – Pain

Support: Saint Louis University Start Up Fund (DS)
Training Grant T32GM008306-28 (CMH)

Title: S1PR1 induced mechano-hypersensitivities are mediated by alterations in adenosine signaling

Authors: *F. LAURO, L. A. GIANCOTTI, Z. CHEN, T. M. DOYLE, C. M. HARADA, D. SALVEMINI;
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Abstract: Chronic pain is a global burden and unfortunately its treatments are frequently insufficient or non-existent. Underlying mechanisms that drive molecular pain pathways remain to be defined and new therapeutic targets are needed. We have recently reported that intrathecal (i.th) injection of sphingosine-1-phosphate (S1P) in rodents causes mechanical and thermal hypersensitivities by activating the S1P1 receptor subtype (S1PR1). Conversely, blocking S1PR1 through genetic and pharmacological approaches, inhibit the development of neuropathic pain states. We have also demonstrated that S1PR1 activation engages molecular signaling pathways downstream of the nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome (NLRP3) leading to the production of the potent inflammatory and

neuroexcitatory cytokine, interleukin-1β (IL-1β). Recent studies suggest that IL-1β can increase the expression of adenosine kinase (ADK). ADK is the key intracellular enzyme regulating intra- and extracellular adenosine concentrations and is regarded as the essential "upstream" regulator of adenosine neurotransmission; increased ADK activity reduces adenosine signaling. We found that mechano-allodynia caused by i.th injection of IL-1β was blocked by an ADK inhibitor (ABT702). Reduced adenosine signaling at the A3 adenosine receptor subtype (A3AR) in the spinal cord contributes to the development of neuropathic pain states; selective A3AR agonists are potent analgesics. The interactions between sphingolipids and adenosine signaling are not known. Here, we show that the ability of S1PR1 antagonists to reverse neuropathic pain (caused by constriction of the sciatic nerve), is prevented by an A3AR antagonist suggesting the existence of potential cross talks. In support, we found that the development of mechano-allodynia caused by i.th. injection of a selective S1PR1 agonist was blocked by an ADK inhibitor; this led to reduced activation of NLRP3 activation. Collectively our data provides a first evidence of a functional connection between S1P and adenosine signaling and suggest that S1PR1 antagonists may exert their beneficial effects by restoring adenosine signaling at the A3AR warranting further investigations.

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Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 398.21/N10

Topic: D.03. Somatosensation – Pain

Support: NS101880
GM120844

Title: Calcineurininhibition increases glutamatergic input to spinal dorsal horn neuronsand pain hypersensitivity through α2δ-1-bound NMDA receptors

Authors: *Y. HUANG, S.-R. CHEN, H. CHEN, Y. LUO, H.-L. PAN;
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Abstract: Calcineurin inhibitors such as tacrolimus (FK506) is clinically used as immunosuppressants in organ transplant patients. However, these drugs can cause severe pain in some patients, commonly referred to as calcineurin inhibitor-induced pain syndrome (CIPS).

Although calcineurin inhibition increases NMDA receptor (NMDAR) activity in the spinal cord, the underlying mechanism remains unclear. In this study, we showed that systemic administration of FK506 in mice significantly increased the frequency of miniature excitatory postsynaptic currents (EPSCs) in spinal dorsal horn neurons and potentiated the amplitude of NMDAR-mediated EPSCs monosynaptically evoked by dorsal root stimulation and puff NMDA elicited currents in dorsal horn neurons. These effects were blocked by inhibiting α2δ-1 with gabapentin or α2δ-1Tat peptide, which interrupts the interaction between α2δ-1 and NMDA receptors. In α2δ-1 KO mice, treatment with FK506 failed to increase NMDA receptor-mediated glutamatergic input to spinal dorsal horn neurons. FK506 treatment significantly increased the amount of α2δ-1-NMDAR complexes at synaptic sites in the spinal cord. Furthermore, FK506 treatment-induced tactile allodynia and mechanical hyperalgesia were largely reduced in α2δ-1 KO mice and in mice with GluN1 conditional KO in primary sensory neurons. In addition, systematic administration of gabapentin or intrathecal injection of α2δ-1Tat peptide reversed pain hypersensitivity in FK506-treated mice. Together, our findings indicate that α2δ-1-bound NMDA receptors mediate calcineurin inhibition-induced synaptic NMDA receptor hyperactivity in the spinal cord and pain hypersensitivity.

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Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

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Program #/Poster #: 398.22/N11

Topic: D.03. Somatosensation – Pain

Title: Identification of the function dorsal horn calretinin-expressing interneurons to the processing of pain inputs

Authors: *H. PETITJEAN[1], F. B. BOUROJENI[2], D. TSAO[1], A. DAVIDOVA[1], S. G. SOTOCINAL[1], J. S. MOGIL[1], A. KANIA[3], R. SHARIF NAEINI[1];
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Abstract: The dorsal horn of the spinal cord (DH) is the first relay center of somatosensory information originating from the periphery. In the superficial DH laminae I-II, nociceptive information is processed by a complex network of excitatory and inhibitory interneurons whose function and connectivity remain poorly understood. In this study, we examine the role of calretinin-expressing interneurons (CR neurons) in the processing of innocuous and noxious sensory inputs. These neurons are mainly located in lamina II, where they receive direct inputs from the central endings of nociceptive fibers and polysynaptic inputs from touch-sensitive Aβ fibers. Their activation by either chemogenetic or optogenetic stimulation produces mechanical

allodynia and nocifensive behaviors. Furthermore, we examined the position of CR neurons in the circuitry of the DH in which there are ideally positioned to modulate pain projection neurons in lamina I. In conclusion, we propose a new neuronal pathway in which CR neurons are positioned at the junction between nociceptive and innocuous circuits and directly ascending pain pathways of the dorsal horn.

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Poster

398. Central Nervous System Mechanisms in Pain

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Topic: D.03. Somatosensation – Pain

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Title: Defining pre- and post-synaptic Ca^{2+} signaling in the spinal cord in pain sensitization

Authors: *A. KEYES[1], C. WARWICK[1], L. SHUTOV[1], V. KROTOV[3], P. BELAN[3], Y. USACHEV[1,2];
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Abstract: Chronic pain affects approximately 100 million Americans and only a minority of patients experience satisfactory relief of their pain with currently available pharmaceutics. One type of chronic pain caused by direct injury to the nerve is called neuropathic pain, and it affects ~10% of the overall population. Despite the prevalence, the underlying mechanisms of neuropathic pain are not well-defined, and better understanding of the mechanisms that promote central sensitization after injury could lead to better treatment of this condition. Among various mechanisms, central sensitization, or the enhancement of synaptic transmission in the spinal cord, is thought to be especially important in the pathogenesis of neuropathic pain. Primary afferents, responsible for transducing painful stimuli, terminate in the spinal cord at what is called the first sensory synapse. This synapse is a key regulator of pain signaling, and aberrant processes at this synapse can lead to an amplification of pain. As most studies have only examined the post-synaptic signaling of this synapse via patch-clamp recordings, the critical questions regarding the role of presynaptic changes in Ca^{2+} signaling in central sensitization have not been addressed. Furthermore, there are many questions remaining regarding the relationship between pre- and post-synaptic changes in Ca^{2+} signaling and Ca^{2+} signaling in microglia in central sensitization. We have developed a method to examine Ca^{2+} signaling in the spinal cord

with high spatial (~0.5 μm) and temporal (~100 ms) resolution using an *ex vivo* intact spinal cord preparation and multiphoton microscopy. Using this method, we are able to simultaneously monitor pre- and post-synaptic Ca^{2+} signaling in intact spinal cord from the mice that express Pirt-GCaMP3 in central terminals of primary afferents and Thy1-RGECO in dorsal horn neurons, and Ca^{2+} signaling in mice that express GCaMP5G-TdTomato in microglia. This approach will facilitate our ability to define pre- and post-synaptic mechanisms of central sensitization in the spinal cord.

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Poster

398. Central Nervous System Mechanisms in Pain

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Program #/Poster #: 398.24/N13

Topic: D.03. Somatosensation – Pain

Support: NIH R01 AT009401

Title: Neuromodulation of pain processing in the dorsal horn circuit

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Abstract: Almost one of five adults suffer from chronic pain. Today, chronic pain is primarily treated with neuropharmacology, which may have negative side effects (e.g., addiction to narcotics), and lose efficacy after long-term use. Alternatively, chronic pain is also treated with neurostimulation—such as spinal cord stimulation or deep brain stimulation—which has great potential but has been associated with suboptimal efficacy and limited long-term success as its mechanisms of action are unclear. Understanding the mechanisms of pain processing to predict its modulation by therapies is therefore essential toward pain management, yet current computational models suffer from several limitations. They are either too simple (such as the static gate control theory) and lack fundamental dynamical functions of the dorsal horn circuit, or they are too complex (such as high dimensional conductance-based models) and lack the functional intuitions that are necessary for the design of new treatments as their analysis becomes untractable. Using mathematical models that combine computational economy and physiological interpretability, we investigated how the cellular and synaptic mechanisms in the dorsal horn circuit—the first central relay station for pain processing—contribute to its function.
At the cellular level, deep dorsal horn neurons can switch their intrinsic firing properties—tonic discharges, plateau potentials, or rhythmic bursting. This change in neural activity is captured in

our model by a modulation of the balance between negative and positive conductances in distinct timescales. A first ultraslow variable acts as a positive feedback (ultraslow regenerative ionic channels) and is responsible for the acceleration in the depolarized state and the deceleration in the hyperpolarized state. A second ultraslow variable acts as a negative feedback (ultraslow restorative ionic channels) and is responsible for the interburst period. In addition, at the circuit level, these firing modes correspond to specific functional states of information transfer at the circuit level in which dorsal horn neurons can faithfully transmit, greatly enhance, or block the transfer of nociceptive information. Our tractable model is a new step forward in understanding the early stages of pain processing in normal and pathological conditions and creating a testbed for finding potential targets of novel drugs or neurostimulation.

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Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: D.03. Somatosensation – Pain

Support: PAPIIT-RA205018

Title: Multidimensional approach of experimental chronic neuropathic orofacial pain: From behavioral to neuronal correlates

Authors: *C. D. MONTES-ANGELES, E. PERRUSQUIA-HERNÁNDEZ, R. D. ANDRADE-GONZÁLEZ, I. O. PÉREZ-MARTÍNEZ;
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Abstract: Chronic orofacial neuropathic pain (CNOP) is defined as an alteration of the trigeminal somatosensory system. Injuries from dental treatments, cancer and accidental injuries are the main causes of the development of this disease, which can involve modifications in both peripheral and central nervous system. The CNOP causes a decrease in the quality of life of who suffers in different dimensions, such as sensory, emotional and cognitive. From this multidimensional approach, it has been found that CNOP produces sensory impairment, depression and inability to adapt to the requirements and challenges of the environment. The aim of this project is to set the foundations for multidimensional assessment in a mouse CNOP model. All of the procedures presented were performed in accordance with the ethical standards of the National Autonomous University of Mexico committee on animal experimentation. Following a constriction injury of mental nerve in C57BL/6 mice, spontaneous and evoked nocifensive responses were assessed, in order to characterize the development of allodynia and hyperalgesia, sensory signs of CNOP. Responses during the escape/avoidance paradigm reflect

the emotional component of pain as a sign of emotional adaptation to environment. Behavioral paradigms assessed the adaptation to unexpected stimuli (switch task) and to changes in the environmental requirements (set-shifting and reversal-set-shifting tasks). These evaluations show that there are cognitive alterations after the CNOP. The present work provides the behavioral basis for the cortical and subcortical study of neuronal activity associated with cognitive adaptation processes when there is CNOP. The authors declare that they do not have conflict of interest.

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Poster

398. Central Nervous System Mechanisms in Pain

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: D.03. Somatosensation – Pain

Support: Krembil Foundation

Title: Sex specific DNA methylation pattern in spinal cord and periaqueductal gray (PAG) before and after peripheral nerve injury

Authors: *S. GHAZISAEIDI[1,3], P. SHOOSHTARI[4], E. CHATER-DIEHL[5], Y. TU[3], S. ASSI[1,3], A. RAMANI[4], A. S. SENGAR[6], R. WEKSBERG[5], M. BRUDNO[2,4], M. W. SALTER[7,1]; [1]Physiol., [2]Computer Sci., Univ. of Toronto, Toronto, ON, Canada; [3]Neurosciences and Mental Hlth., [4]Ctr. for Computat. Med., [5]Genet. & Genome Biol., [6]Neurosciences & Mental Hlth., The Hosp. for Sick Children, Toronto, ON, Canada; [7]Neurosciences & Mental Hlth. Program, Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Chronic pain has been labelled the silent health epidemic afflicting hundreds of millions of people worldwide. The most debilitating and poorly treated form of chronic pain is neuropathic pain. Existing treatments for chronic neuropathic pain are minimally effective and may be contributing to the current opioid crisis. The lack of novel therapies is directly related to the complex nature of pain signaling. Our previous work has revealed that there are multiple different cellular and molecular mechanisms that form the basis of pain processing in rodents. These critical differences are also susceptible to genetic variability and are sexually dimorphic. DNA methylation is one of the main mechanisms for sexual differentiation in the CNS. We evaluated the DNA methylome in spinal cord and periaqueductal gray (PAG) of male and female Sprague Dawley rats after spared nerve injury (SNI) by reduced representation bisulfite sequencing (RRBS). We selected methylated regions using Methyl Kit that were considered to be differentially methylated regions (DMRs) by the following criteria: adj. p.val <0.05,

differential methylation > |10%|, and width >3 CpG. Enrichment analysis was then performed on DMRs using Genomic Regions Enrichment of Annotations Tool (GREAT). Here we report that in spinal cord, 2,811 and 6,144 genomic regions are differentially methylated between males and females for naïve and SNI conditions, respectively. GREAT linked these regions to 3,486 and 6,005 genes, 2,371 of which are shared by the two condition. In the PAG, we found 5,448 and 3,631 genomic regions are differentially methylated between males and females DMRs for naïve and SNI conditions, respectively. These DMRs are linked to 5,627 and 4,265 genes, respectively, 2,684 of which are shared. We observed robust sex specific DNA methylation in both spinal cord and PAG in the naïve state and, after SNI, both tissue methylomes were altered in sex specific manners. Together, our findings support a hypothesis that DNA methylation is important for the sexual differentiation of the CNS and may be the foundation for sex differences in neuropathic pain mechanisms.

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Poster

398. Central Nervous System Mechanisms in Pain

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Title: Susceptibility and resistance to chronic pain: Sex is important

Authors: *M. R. GUIMARÃES, C. MIRANDA, A. M. CUNHA, S. ROQUE, M. ESTEVES, N. D. ALVES, N. SOUSA, M. CORREIA-NEVES, A. ALMEIDA, H. LEITE-ALMEIDA; Neurosci. Res. Domain, Life and Hlth. Sci. Res. Inst. (ICVS), Braga, Portugal

Abstract: Chronic Pain (CP) is an integrative phenomenon that results from dynamic interactions between sensory and emotional, motivational and cognitive domains. In fact, several studies using rodent models of CP revealed adaptations in brain regions related with anxiety, depression, cognitive dysfunction and nociception. However, little is known about their cross-relations prior to injury. In this work we aim to evaluate behavioral and immunological parameters associated with CP resistance/susceptibility in male and female rats.Male and female Sprague dawley (SD) rats were used in all experiments since 15% of these animals do not

manifest pain-related behaviors. Prior to installation of a modified spared nerve injury (mSNI), sociability (SB - neutral arena), emotional (EPM, elevated-plus maze; FST, forced swimming test) and impulsivity decision making (VDS, variable delay-to-signal) were assessed. Then, mechanical allodynia (proxy of neuropathic pain) was measured weekly to evaluate the progression of hypersensibility. After 4 weeks blood was collected for fluorescence-activated cell sorting (FACS) and the sucrose preference (anhedonia) test was performed.We observed that: 1) mSNI promotes a wide range of mechanical allodynia thresholds particularly in males; 2) High threshold (HT) animals are at baseline less depressed and more social; 3) HT animals had higher hedonic behavior than LT. We also show that females are more prone to develop hypersensitivity than males although no sex differences were observed between male and females in GFAP staining in spinal cord. Finally, resistance to pain manifestation was associated with increased sociability and decreased depressive-like behavior prior to the pain-triggering injury and with lower levels of T lymphocytes. Overall, our experiments show that in pain protection after a neuropathic injury is associated with better emotional state and lower levels of T lymphocytes, indicating that immune system and emotional state are crucial for pain manifestation.

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Poster

398. Central Nervous System Mechanisms in Pain

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MR130053

Title: Resolving the molecular identity and connectivity of amygdalar neural ensembles active during pain

Authors: *D. J. BERG, C. CHEN, B. AHANONU, M. CHEN, S. QUAKE, M. SCHNITZER, G. SCHERRER;
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Abstract: The amygdala critically contributes to emotional valence assignment during pain experience. By means of time-lapse *in vivo* calcium imaging and neural activity manipulation in freely behaving mice encountering noxious stimuli, we recently identified a neural ensemble that encodes pain unpleasantness in the basolateral amygdala (BLA) (Corder, Ahanonu, et al., 2019). Here we examined the connectivity of this BLA neural ensemble using viral tracing methods and the transcriptome of individual BLA neurons constituting the ensemble with single cell RNA-sequencing.
We first induced Cre activity in BLA nociceptive neurons, using TRAP2 mice injected with intraplantar formalin or stimulated with pinprick. To trace inputs to and from BLA nociceptive neurons, we injected the BLA with GFP-expressing mutant rabies viruses with retrograde transsynaptic spread properties combined with helper adeno-associated viruses. Additionally, we used adeno-associated viruses to express GFP and synaptophysin-mRuby to visualize BLA nociceptive neuron cell bodies and their axon terminals. We found that the BLA nociceptive ensemble receives convergent synaptic input from several brain regions, including the anterior cingulate cortex (ACC) and insular cortex, which are thought to play an important role in pain affect. We determined that outputs of the BLA nociceptive ensemble includes the ACC, nucleus accumbens, claustrum and striatum.
To identify novel therapeutic targets, we sequenced the transcriptomes of individual nociceptive BLA neurons. TRAP2 mice were crossed with Ai14 mice (*Rosa26*$^{LSL\text{-}tdTomato}$), to fluorescently label neurons active during pain. To distinguish and positively select tdTomato+ BLA nociceptive neurons, we microdissected the BLA, dissociated neurons, and acquired labeled neurons using fluorescence-activated cell sorting (FACS). We generated high-quality full-length gene expression profiles of individual neurons using Smart-seq2 and Illumina sequencing. Initial scRNA-seq experiments and unsupervised clustering analysis shows amygdalar subpopulations expressing known markers, including *CamKIIa* and the negative-valence marker gene *Rspo2*, and reveals the molecular architecture of amygdala neurons that encode pain emotional component. This transcriptomic analysis will provide a comprehensive catalog of surface proteins specifically expressed by BLA neurons encoding pain unpleasantness for the development of therapeutics to treat pain affect.

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Poster

398. Central Nervous System Mechanisms in Pain

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Topic: D.03. Somatosensation – Pain

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Title: Maladaptive cortical plasticity associated with neuropathic pain after SCI

Authors: *G. H. BLUMENTHAL[1,2], V. BRACCHI-RICARD[3], B. NANDAKUMAR[1], J. R. BETHEA[3], K. A. MOXON[1];
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Abstract: The role of the primary somatosensory cortex (S1) in the development of chronic neuropathic pain (CNP) remains unclear. Some studies have shown that increased cortical reorganization is associated with increased intensity of CNP, and that alleviation of some CNP symptoms can be achieved through reduction or reversal of this maladaptive plasticity. Conversely, others have shown that CNP is associated with a preserved representation of the painful region of the body. Much of this research was conducted in human subjects with variable injuries using neuroimaging methods, and was limited in that the painful region of the body was not directly stimulated. A well-controlled animal study would help shed light on the role of cortical plasticity in the development of CNP. To address this, we used a rat model of mid-thoracic (T10) spinal cord contusion injury in which a proportion of animals developed at-level CNP, allowing for a comparison of cortical reorganization in the S1 of injured animals that developed CNP to injured animals that did not. This is important because it allows us to control for the confounding factor of injury. Animals were tested for at-level CNP once a week for 5 weeks post-injury. At-level CNP was assessed by probing the dorsal trunk with a 26g von Frey filament within a body grid system, allowing us to precisely and consistently stimulate the trunk across all animals and localize the painful region to thoracic dermatomes. Aversive supra-spinal responses were measured. Animals were then separated into experimental groups based on the presence or absence of at-level CNP. At week 6, animals in both groups underwent sensory mapping in which receptive fields were measured from cortical cells both within and surrounding the putative trunk S1. In a separate cohort of animals, fresh tissue from S1 was analyzed to assess differential regulation of genes related to plasticity using RNAseq followed by qPCR. In our model, at-level CNP alone developed in dermatomes T4-T10 in 19% of animals, while 66% of animals developed no CNP anywhere. The remaining animals developed pain in the forepaws and/or hindpaws. In assessing S1 reorganization, we found that in animals that developed at-level CNP, the representation of the painful region of the trunk expanded into the putative representation of the forelimb when compared to both injured animals without CNP as well as naïve controls. Additionally, in animals with at-level CNP, mRNA from genes associated with both CNP and plasticity such as P2X7R were upregulated in S1 compared to injured animals without CNP and naïve controls, suggesting that this reorganization may be in part due to transcriptional changes at the cortical level.

Disclosures: G.H. Blumenthal: None. **J.R. Bethea:** None. **V. Bracchi-Ricard:** None. **K.A. Moxon:** None. **B. Nandakumar:** None.

Poster

398. Central Nervous System Mechanisms in Pain

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 398.30/N19

Topic: D.03. Somatosensation – Pain

Support: AIHS Graduate Studentship
Eyes High Doctoral Recruitment Scholarship
CIHR Foundation Grant to Gerald W. Zamponi

Title: The role of dopamine in the processing of chronic pain signals in the mouse prefrontal cortex

Authors: *S. HUANG, E. GAMBETA, C. THOMAS, N. GODFREY, Z. ZHANG, S. BORGLAND, G. ZAMPONI;
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Abstract: The medial prefrontal cortex (mPFC) is a brain region involved in coordinating consciousness and is important in mediating pain arousal. Previous studies have revealed that the mPFC undergoes plasticity during development of chronic pain. Pain and reward systems encode opposing motivational salience value, however, through a shared neuronal circuit. Dopamine is a key neuromodulator in the reward system and has been implicated in the modulation of the pain axis. The mPFC receives dopaminergic inputs, and stimulation of these inputs has been shown to modulate the plasticity of the mPFC and aversive behavior. In this study, we use optogenetic approaches in conjunction with a transgenic mouse line to selectively activate dopaminergic inputs in the mPFC during neuropathic pain states. We found that phasic but not tonic optogenetic activation of these dopaminergic inputs significantly reduced mechanical hyperalgesia. Conditional place preference tests of affective pain consistently showed that mice with neuropathic pain exhibit a preference to chambers in which they were conditioned with optogentically released dopamine. Furthermore, c-fos expression suggested that this modulation is mediated by activation of mPFC principal neurons. This is in accordance with previous findings showing that decreased activity of principal neurons has a causal relation to chronic pain. Together, our findings indicate a novel role of mPFC dopamine signaling in pain modulation.

Disclosures: S. Huang: None. **E. Gambeta:** None. **C. Thomas:** None. **N. Godfrey:** None. **Z. Zhang:** None. **S. Borgland:** None. **G. Zamponi:** None.

Poster

399. Chemosensory Processing II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 399.01/N20

Topic: D.05. Olfaction and Taste

Title: Dual GABA/acetylcholine basal forebrain input on a specific periglomerular cell subtype in the olfactory bulb

Authors: *D. DESAINTJAN;
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Abstract: GABAergic and cholinergic long-range projections from the basal forebrain (BF) potentially orchestrate inhibition in the olfactory bulb with synaptic connections on diverse interneuron subtypes in multiple layers. In the glomerular layer, BF afferents provide a prominent GABAergic synaptic input onto three subclasses of type 2 periglomerular (PG) cells (Sanz Diez et al., JPhysiol 2019). The impact of these inputs is unclear as previous studies concluded that GABA is depolarizing in PG cells but could nonetheless be inhibitory by shunting excitatory signals. To further investigate this question, BF modulation of PG cells spiking activity was examined using loose cell-attached recordings. BF axons were stimulated using optogenetics in olfactory bulb slices from adult mice. ChR2-EYFP was selectively expressed in BF neurons using stereotaxic virus injection in dlx5/6-cre mice. This led to the expression of ChR2-EYFP in multiple populations of BF GABAergic neurons as well as in cholinergic neurons that are known to be also GABAergic. Despite the high connection rate found in dlx5/6;ChR2-EYFP mice, light stimulation (1 ms) had no noticeable effect on a majority (>50%) of the recorded PG cells which were totally silent or fired only occasionally. PG cells that were modulated by the light-evoked BF input had diverse responses. In a minority of cells, BF inputs were excitatory and induced a single time-locked action potential (n=8) or a single delayed action potential (n=11). However, in the majority of cells (n=70), the firing was transiently inhibited by the BF input. Firing inhibition persisted in the presence of antagonists of the glutamate, nicotinic or muscarinic receptors but was abolished by gabazine, an antagonist of the $GABA_A$ receptors (n=12). The duration of this inhibition was highly variable across PG cells (range 100-900 ms) and in a subset of neurons (n=32), spiking inhibition was followed by a robust increase of firing. Increase in spike frequency peaked ~1 s after the light stimulus and lasted several seconds. This excitation persisted in the presence of gabazine or of NBQX/D-AP5 but was blocked by atropine or scopolamine, two antagonists of mACh receptors (n=9). This dual GABA/ACh modulation of firing was recorded in 7 cells that were then characterized in the whole-cell configuration. All were regularly firing type 2 PG cells, responding to the stimulation of the olfactory nerve with a long-lasting barrage of EPSCs (duration >1s) and to the stimulation

of the BF axons with a slow IPSC (decay 195±50 ms). Thus, the results identify a specific subtype of type 2 PG cells as the selective target of GABA and ACh inputs from the BF.

Disclosures: D. Desaintjan: None.

Poster

399. Chemosensory Processing II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 399.02/N21

Topic: D.05. Olfaction and Taste

Support: OIST Graduate School

Title: Protein kinase inhibitor beta may be a specific marker for mitral cells in the olfactory bulb

Authors: A. KOLDAEVA[1], C. ZHANG[1], S. TAKAHASHI[2], H. MATSUNAMI[3], *I. FUKUNAGA[1];
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Abstract: In many sensory systems, streams of information processing become segregated even at early stages. In investigating what information is conveyed and how distinct streams are used to guide behavior, molecular markers for distinct cell types are often indispensable. In the olfactory system, the information stream is thought to first segregate in the olfactory bulb (OB) into at least two streams, conveyed by mitral and tufted cells (MCs and TCs). These two neuron classes differ in anatomical and physiological properties, thus suggestive of unique gene expression patterns. MCs and TCs are likely to play distinct roles, but no specific driver mouse line for MCs exists yet. Here we took advantage of recent advances in single cell expression profiling to search for a marker that distinguishes MCs from TCs. We obtained the expression patterns of mouse OB neurons from a publicly available single-cell RNAseq data (Zeisel et al., 2018). To identify a potential mitral cell marker, we searched for a gene that is expressed reliably by MCs, but not TCs. We defined a putative MC cluster to be one that expresses Tbx21, but has the lowest CCK expression levels. Of the 10,745 OB cells analyzed, 1,682 cells expressed CCK and 47 cells expressed Tbx21, with some overlap between two populations. The CCK expressing cells constitute a large cluster among the OB cell population with sub-clusters, some of which may correspond to distinct types of TCs. We found no clear candidate marker that distinguishes these sub-clusters. On the other hand, we found 66 genes that are more abundant in the putative MC cluster. Of the candidate genes, we found that protein kinase inhibitor beta (Pkib) may be a specific marker for MCs. Using a threshold (7 counts), we found that Pkib is expressed in 72% of the putative MCs, and in 0% of the putative TCs. Immunohistochemistry and in-situ hybridization for Pkib confirm that, indeed, Pkib is present densely in the majority of cells in the

MC layer, but only at low levels or not at all among cells in other layers. We have generated a knock-in mouse where IRES-Cre is inserted immediately after Pkib open reading frame, and are currently testing its suitability as a mouse line for investigating unique contributions that MCs make in olfactory processing.

Disclosures: A. Koldaeva: None. **C. Zhang:** None. **S. Takahashi:** None. **H. Matsunami:** None. **I. Fukunaga:** None.

Poster

399. Chemosensory Processing II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 399.03/N22

Topic: D.05. Olfaction and Taste

Support: ERC

Title: Flexible categorization in the mouse olfactory bulb

Authors: *E. KUDRYAVITSKAYA, E. MAROM, D. PASH, A. MIZRAHI;
Dept. of Neurobio., The Edmond and Lily Safra Ctr. For Brain Sci. (ELSC), The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Categorical perception is a canonical feature of sensory systems. In earlier studies of category perception learning, distinguishable objects on each side of the category boundary are often treated as the same. In real life, however, even when we spontaneously group two items into the same category, we do not necessarily treat them as identical for all purposes. In our study, we tested the impact of learning on category formation in the mouse OB. We first examined how odour category is represented in the mouse OB using two-photon calcium imaging of Mitral Cells (MCs) in head-fixed awake mice. We imaged MCs responses to morphed monomolecular odours Ethyl Butyrate (EB) into Ethyl Tailgate (ET), through a series of intermediate mixtures (100/0, 90/10, 80/20, 20/80, 10/90, 0/100). We show that in naïve mice OB, MCs respond naturally in a categorical manner by grouping morphed odour stimuli based on the relative stimulus strength. MCs activity pattern changed abruptly with the morphing from one odour to another, consistent with known literature.
We next investigated wherever neurons in the OB could encode category information in a flexible way, could previously grouped stimuli be learned apart, and can primary category responses be changed with task demands. To answer these questions, we challenged mice with two learning tasks that use the same odours in different categorical logic. 1. An easy, 1-descision boundary task where animals learned to classify binary odour mixtures in two groups according to the dominant component in the mixture. 2. A hard task with multiple decision boundaries intermingled along the space of binary odour mixtures. We show that mice were able to learn

both tasks with the high accuracy and also switch between the tasks. Moreover, our data shows that odor representations by MCs are flexible, switching between the two different categorical schemes. Specifically, when mice were engaged in the 1-descision boundary task, MC odour responses reflected the perceptual boundary. This sharp 2-category transition was completely abolished in the multi boundary task and new task specific boundaries were formed instead. Our data thus underscores that categorical representation of odours in the mouse OB is dynamic and can be reorganized following learning.

Disclosures: **E. Kudryavitskaya:** None. **E. Marom:** None. **D. Pash:** None. **A. Mizrahi:** None.

Poster

399. Chemosensory Processing II

Location: Hall A

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Program #/Poster #: 399.04/N23

Topic: D.05. Olfaction and Taste

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Wellcome Trust (110174/Z/15/Z)

Title: Large-scale functional mapping of population dynamics in the main olfactory bulb by CHIME recordings

Authors: *M. KOLLO[1], R. R. RACZ[1], T. WARNER[1], A. M. OBAID[2], M.-E. HANNA[3], J. J. HARRIS[1], M. R. ANGLE[3], J. MUELLER[4], A. HIERLEMANN[5], N. MELOSH[2], A. T. SCHAEFER[1];
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Abstract: The characterization of population dynamics in mammalian sensory and motor circuits at single spike resolution has been challenging due to the discrepancy between the laminar, stratified architecture of neuronal networks and the axial arrangement in electrophysiological probes providing sufficiently large numbers of recording sites. To address this problem, here we used CHIME (CMOS hosted in-vivo microelectrodes), a technique allowing high signal-to-noise, parallel recordings from 100s-1000s of electrical recording sites over large areas at sub-millisecond time resolution to record population activity from distributed

populations of principal cells in the mouse main olfactory bulb. High signal-to-noise ratio (<10μV noise floor) was achieved due to the high conductivity of core metals in glass-ensheathed microwires allowing for the realization of ultrathin metal cores (down to <1 um) and negligible stray capacitance. The metal recording sites were electrodeposited with gold and IrOx to maximize the specific recording area. This enabled ultra-low access impedance with a geometric recording area of 1-2 μm. Damage to the blood-brain-barrier upon insertion was found to be minimal by post-hoc histology. Our results show that combining microwire bundles and CMOS arrays allows for a highly scalable neuronal recording approach, which addresses the need for application-specific electrode site arrangement as well as the mismatch between the three-dimensional architecture of the brain and largely two-dimensional microfabrication techniques.

Disclosures: **M. Kollo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent: Electrochemical probe (US20180067075A1). **R.R. Racz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent: Electrochemical probe (US20180067075A1). **T. Warner:** None. **A.M. Obaid:** None. **M. Hanna:** A. Employment/Salary (full or part-time):; Paradromics Inc.. **J.J. Harris:** None. **M.R. Angle:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Paradromics Inc. **J. Mueller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MaxWell Biosystems. **A. Hierlemann:** None. **N. Melosh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Paradromics Inc. **A.T. Schaefer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Paradromics Inc..

Poster

399. Chemosensory Processing II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 399.05/N24

Topic: D.05. Olfaction and Taste

Support: NIH DC015137

Title: Hebbian model of the structural plasticity in the olfactory bulb

Authors: J. H. MENG[1], S. SAHA[2], K. SAILOR[2], P.-M. LLEDO[2], *H. RIECKE[1];
[1]Applied Mathematics, Northwestern Univ., Evanston, IL; [2]Lab. for Perception and Memory, Pasteur Inst., Paris, France

Abstract: How animals can learn to discriminate between different sensory stimuli, e.g. similar odors, is an intriguing question. In rodents, the olfactory bulb, which is the first brain area to receive sensory input from the nose, exhibits structural synaptic plasticity even in adult animals: reciprocal connections between excitatory mitral cells and inhibitory granule cells are persistently formed and eliminated. Here we present a Hebbian-type model to understand how this synaptic structural plasticity can contribute to the learning of discrimination tasks by enhancing differences between representations of similar odors.Recent work on spine fluctuations has shown that the experimentally observed age-dependence of spine stability can arise from a Hebbian mechanism that stabilizes synapses connecting strongly active cells [1]. Further, spine dynamics in the olfactory bulb is correlated with the activity of pre-synaptic mitral cells and post-synaptic granule cells [2, 3]. Based on these experiments, we assume that synaptic structural plasticity follows Hebbian-type rules: co-activation of a mitral and granule cell pair leads to the formation of a synapse connecting them; if the mitral cell is highly active, but not the granule cell, the connecting synapse is removed; if the mitral cell activity is low, the synapse is unchanged. In addition, the total number of synapses of each granule cell is limited.Here we first show that our model can explain the striking experimental observation [4], that upon familiarizing the animal with a pair of very similar odors the difference between the bulbar representations of these odors is enhanced, while learning a pair of dissimilar odors reduces their differences. Correspondingly, the number of mitral cells that respond differently to the dissimilar odors decreases, but it stays the same for the similar odors. Second, the model quite generally leads to a bimodal distribution of granule cell activity, which is also seen in ongoing experiments. Third, our model predicts that the learned bulbar network structure will be remembered unless additional, interfering discrimination tasks are presented.
[1] K. Sailor et al., Neuron 91 (2016) 384.
[2] V. Breton-Provencher et al., Nat Commun 7 (2016) 12659
[3] V. Breton-Provencher et al., J Neurosci 34(5) (2014) 1748-59
[4] M.W. Chu, W.L. Li, T. Komiyama, Neuron 92 (2016) 174.

Disclosures: **J.H. Meng:** None. **S. Saha:** None. **K. Sailor:** None. **P. Lledo:** None. **H. Riecke:** None.

Poster

399. Chemosensory Processing II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 399.06/N25

Topic: D.05. Olfaction and Taste

Support: NIH-NIDCD grant DCR01- DC-009817 (RCA)

Title: Cholinergic modulation of local and top-down GABAergic inhibition in the olfactory bulb

Authors: P. S. VILLAR, *R. HU, R. C. ARANEDA;
Biol., Univ. of Maryland, College Park, MD

Abstract: Precise regulation of an extensive network of interneurons in the olfactory bulb is crucial to odor processing, with the largest population corresponding to granule cells (GCs). Regulation of GCs occurs by the cooperative action of local and top-down inputs to GCs that fine-tune the encoding of odor signals. Within the GC layer, local GABAergic neurons, the deep short-axon cells (dSACs), are thought to provide inhibition to distal processes of GCs suggesting they control dendritic excitability in CGs. We have previously shown that long-range GABAergic projections originating in the basal forebrain (BF) are a source of important inhibition to GCs, with an innervation pattern that suggests perisomatic inhibition of GCs. Despite the importance of these inhibitory mechanisms in GCs, little is known of their regulation. Interestingly, the distribution pattern of BF cholinergic and GABAergic fibers shows extensive overlap in the GC layer, suggesting that acetylcholine can regulate inhibition onto GCs. To explore this possibility, we examined the effect of muscarinic acetylcholine receptor (mAChRs) activation on spontaneous inhibitory currents in GCs (sIPSCs). Under resting conditions, GCs exhibit a low frequency of events (0.23 ± 0.05 Hz, n=29), with an average amplitude of 17.5 ± 1.8 pA. Bath application of the non-selective mAChRs agonist muscarine (Mus; 10 µM), produced a significant increase in sIPSC frequency (control, 0.25 ± 0.05 Hz; Mus, 0.39 ± 0.5 Hz; n=11, p=0.02), which we hypothesized occur by an increase in inhibitory inputs from dSACs. In agreement with this possibility, application of Mus depolarized dSACs, an effect that is abolished in the presence of a selective M3-mAChR antagonist (4-DAMP 100 nM) (Mus, $\Delta V = 5.7 \pm 1.1$ mV; Mus + 4-DAMP, $\Delta V = 0.3 \pm 0.5$ mV; n=4, p=0.03). Importantly, 4-DAMP blocked the increase in sIPSCs frequency in GCs elicited by Mus (Mus, 0.28 ± 0.07 Hz; Mus + 4-DAMP, 0.31 ± 0.08 Hz; n=7, p=0.2). To examine the cholinergic modulation of BF inhibition to GCs, we expressed channelrhodopsin in BF GABAergic neurons. In contrast to the muscarinic effect on local inhibition in GCs, activation of mAChRs reduced the amplitude of the optogenetically evoked IPSCs from GABAergic axons (control, 116 ± 7 pA; Mus, 25 ± 7 pA; n=4, p=0.0009). This reduction in amplitude was blocked by AFDX-385 (300 nM), an antagonist of M2/M4 mAChRs. These results indicate that the activation of the BF cholinergic neurons can locally regulate the inhibition of GCs. We propose that the selective activation of M2/M4 and M3 mAChRs can produce a shift in the weight of top-down perisomatic vs. local distal GABAergic inhibition in GCs.

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Poster

399. Chemosensory Processing II

Location: Hall A

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Program #/Poster #: 399.07/N26

Topic: D.05. Olfaction and Taste

Support: NIH R01 DC014367
NIH R01 DC014701

Title: Dynamics of spike time encoding in the olfactory bulb

Authors: *J. C. WERTH, T. A. CLELAND;
Psychology, Cornell Univ., Ithaca, NY

Abstract: Gamma oscillations are generated within the earliest central circuits of the olfactory system, and reflect synchronized activity across ensembles of principal neurons, such as the mitral and tufted cells (collectively, MTCs) of the olfactory bulb. The regulation of spike timing in these neurons - particularly including the synchronization of spikes in activated ensembles - and the phases of these spikes with respect to the underlying gamma oscillations are potentially critical bases for neural information coding. Understanding the fundamental mechanisms underlying these phenomena is therefore essential for understanding the construction of olfactory representations. However, it remains unclear which aspects of this dynamical regulation of spike timing are important for odor encoding and olfactory bulb function, in part due to the limited capacity to control the intensity and timecourse of odor delivery on fine spatial and temporal scales.
We investigated the spike-timing properties of MTCs using an *in vitro* bulbar slice preparation mounted on a 120-channel planar multielectrode array (MEA), while delivering “artificial odor” stimuli using spatiotemporally patterned optogenetic stimulation. These “artificial odor” stimuli evoke persistent gamma oscillations in the local field potential and also narrow the phase constraint of MTC action potentials with respect to gamma. Individual MTCs varied considerably in their responses to stimulation; while the mean phase constraint across all recorded MTCs increased significantly, clearly some MTCs became strongly phase-locked while others were relatively unaffected. Importantly, the MTCs that became phase-locked to the gamma oscillation depended on which “artificial odor” was presented. We present and discuss several alternative metrics for the spike timing-dependent analysis of MTC activity.

Disclosures: J.C. Werth: None. **T.A. Cleland:** None.

Poster

399. Chemosensory Processing II

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: D.05. Olfaction and Taste

Support: NIH Grant U19MH114831

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CZI Collaborative Computational Tools for the Human Cell Atlas

Title: Epigenomic identity of cell types in the olfactory bulb and peduncle with single cell resolution

Authors: *W. I. DOYLE[1], E. J. ARMAND[1], F. XIE[2], R. FANG[3], A. C. RIVKIN[4], R. CASTANON[4], J. R. NERY[4], X. HOU[5], K. SMITH[6], S. PREISSL[5], C. LUO[4,9], B. TASIC[7], M. BEHRENS[10], B. REN[11], H. ZENG[8], J. R. ECKER[4,9], E. A. MUKAMEL[1];
[1]Cognitive Sci., [2]Physics, [3]Bioinformatics and Systems Biol., UCSD, La Jolla, CA; [4]Genomic Analysis Lab., Salk Inst. for Biol. Studies, La Jolla, CA; [5]Ctr. for Epigenomics, Univ. of California San Diego, La Jolla, CA; [6]Mol. Biol., [7]Mol. Genet., [8]Structured Sci., Allen Inst. for Brain Sci., Seattle, WA; [9]Howard Hughes Med. Inst., La Jolla, CA; [10]CNL-B, The Salk Inst. for Biol. Studies, La Jolla, CA; [11]Cell. and Mol. Med., Univ. of California San Diego, Ludwig Inst. for Cancer Res., La Jolla, CA

Abstract: Olfactory information processing requires the coordinated activity of neuronal circuits in multiple brain regions, including the olfactory bulb and peduncle which represent the first stages of processing of olfactory sensory inputs. These circuits contain multiple cell types with functional roles defined by their morphology, connectivity, and gene expression. Although recent single cell transcriptome (scRNA-seq) analyses identified cell types in the olfactory bulb by their patterns of gene expression, it remains unclear how these patterns are established and regulated through development. Here, we integrated large-scale single-cell transcriptomic with epigenomic datasets to investigate the molecular signatures of neurons in the olfactory bulb and peduncle. The accessibility of chromatin and the methylation status of genomic cytosines (DNA methylation) can influence transcription factor binding, leading to functional changes in gene expression. The Center for Epigenomics of the Mouse Brain Atlas (CEMBA), a BRAIN-Initiative funded consortium of laboratories, has produced a dataset that includes single cell resolution profiles of DNA methylation (snmC-seq, >5,000 cells) and chromatin accessibility (snATAC-seq, >40,000 cells) in the olfactory bulb and peduncle. We used these data to identify cell types by their epigenomic landscape, including multiple distinct types of granule cells and periglomerular cells in the olfactory bulb, several types of peduncle-specific inhibitory neurons, and a broad variety of excitatory neuron types. By comparing gene body DNA methylation with gene expression, we verified that these cell types are consistent with those determined by recent single-cell transcriptomic atlases as reported in Tepe et al. (2018) Cell Reports and Zeisel et al. (2018) Cell. For each cell type, we identified putative gene regulatory elements (enhancers) by overlapping sites of low DNA methylation with regions of open chromatin. These regions contain DNA sequences recognized by transcription factors that regulate cell type identity in the olfactory bulb and peduncle. Using scRNA-seq datasets from the olfactory bulb and peduncle generated by the Comprehensive Center on Mouse Brain Cell Atlas at the Allen Institute for Brain Science, we found cell type specific expression of genes regulated by the predicted cell type specific enhancers. In this study we have produced the first epigenomic-based cell atlas of

neurons and identified key sites of regulation for cell type identity in two key sensory processing areas, the olfactory bulb and peduncle.

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Poster

399. Chemosensory Processing II

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 399.09/N28

Topic: D.05. Olfaction and Taste

Support: NIH Grant R01DC013797

Title: A search for neural metrics of odor identity perception

Authors: *H. NAKAYAMA[1], D. RINBERG[2];
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Abstract: In the mammalian olfactory bulb, stimulus information from individual olfactory sensory neurons is transmitted into anatomically localized structures called glomeruli. Odorants evoke stimulus-specific spatiotemporal patterns of glomerular activation. One of the central questions in sensory neuroscience is how similarities between patterns of neural activity is related to perceptual differences between sensory stimuli. Here, we propose potential metrics of the spatiotemporal patterns of glomerular activation and develop a behavioral paradigm to test perceptual odor similarities and their relationship to the neural metrics.
We performed wide-field calcium imaging of odor responses from a larger number of glomeruli at high temporal resolution (~100 glomeruli at 100 frames/sec). Since potential neuronal metrics for odor identity perception are supposed to be concentration-invariant, we compare different metrics based on the stability across odor concentration changes. We found that the rank order of response onset latencies of glomeruli is more stable than other neuronal metrics including mean or peak level of activations.
To assess the relevance of different metrics of glomerular activation odor percepts, we designed a novel odor generalization task for mice that allows us to measure perceptual similarities between odors. Mice were trained to discriminate a target odor from many non-target odors at variable concentrations. Subsequently, novel odors are presented, and their perceptual similarities to the target odor are evaluated based on the similarities in behavioral readouts. We find consistent changes between behaviors and some of neural metrics. Our approach for the

combination of neuronal and behavioral measurement of similarity enables systematic investigation and dissociation of potential neural codes for odor perception.

Disclosures: H. Nakayama: None. **D. Rinberg:** None.

Poster

399. Chemosensory Processing II

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Program #/Poster #: 399.10/N29

Topic: D.05. Olfaction and Taste

Support: RO 4046/2-1 and /2-2
GRK2416

Title: Dynamic inhibition of mice olfactory bulb output by the anterior olfactory nucleus

Authors: R. MEDINACELI QUINTELA, L. WALLHORN, J. BAUER, ***M. ROTHERMEL**; RWTH Aachen Univ., Aachen, Germany

Abstract: The anterior olfactory nucleus (AON) is the most anterior part of the olfactory cortex. Its extensive connections to lower and higher brain centers render the AON an interesting model system. Here, we examined the effect of AON modulation on olfactory processing both on a cellular and a behavioral level in anesthetized and awake mice.
First, we tested for behavioral consequences of extrinsically modulating AON activity. Mice implanted with an optical fiber targeting ChR2-expressing AON neurons were trained to report the presence of odorants. Stimulated optically, without odorant presentation, these mice failed to lick showing that activation of AON neurons in awake animals was probably not perceived as an odorant equivalent cue. AON activation during odorant presentation however, reliably suppressed odor detection. This AON mediated effect was constant across odors and concentrations. Testing different optical stimulation durations revealed that the AON modulates odor detection on a fast timescale.
In order to shed light on potential mechanisms, we next investigated if top-down projections from the AON modulate olfactory bulb (OB) output neuron activity using electrophysiological recordings in anesthetized mice. Optogenetic activation of AON axon terminals in the OB led to a significant decrease in odor evoked mitral/tufted (MT) cell spiking. The population time course showed a fast reduction of MT cell activity during light stimulation that was followed by a long lasting increase, reminiscent of OB offset responses. AON stimulation effects were independent of the strength and polarity of the odorant response and were observed across a variety of odors. Averaged normalized sniff-triggered spike histograms showed a decrease in both baseline and peak spike rate, consistent with an AON mediated effect on odor sensitivity rather than an influence on signal-to-noise ratio.

Taken together, the strong inhibition of MT cell activity observed during electrophysiological recordings might explain the suppression of odor detection observed in the behavioral experiments. These results support the hypothesis that the AON acts as a strong source of top-down input to the OB. Future experiments will focus on studying the effects of light-driven AON inhibition on OB output.

Disclosures: M. Rothermel: None. **R. Medinaceli Quintela:** None. **L. Wallhorn:** None. **J. Bauer:** None.

Poster

399. Chemosensory Processing II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 399.11/N30

Topic: D.05. Olfaction and Taste

Title: Utilizing 3D spatial transcriptomics to generate a comprehensive map of glomerular identity in the mouse olfactory bulb

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Abstract: Determining the spatial organization of odorant receptor (OR) gene expression in the mouse olfactory bulb (OB) can reveal important insights into OB subdomain structure, define neighbor relationships among glomeruli, and characterize odorant-OR interactions *in vivo*. However, only a few glomeruli have been genetically identified thus far. Here, we use Spatial Transcriptomics (ST) to develop a comprehensive map of glomerular identity in the OB. Spatial Transcriptomics provides visualization and quantification of gene expression in individual histological sections of tissue. mRNA capture probes with spatial barcodes are printed in spots on a glass slide and sections of OB tissue are placed on the printed microarrays. mRNA from the tissue is captured by the barcoded probes and sequenced. This sequence information is then aligned with the printed spots, resulting in a spatial map of gene expression in the tissue. Here, we will present preliminary data that validate ST as capable of accurately and precisely determining the identity of GFP-tagged glomeruli in OB sections of transgenic mice. We will also present ST arrays with improved spatial resolution and show that improved specificity of OR detection can be obtained by using degenerate OR capture probes. Finally, our preliminary

results show that, by performing ST on all sections of the OB, we are able to construct a three-dimensional map of gene expression and glomerular identity.

Disclosures: **N.L. Klimpert:** None. **A. Mollbrink:** None. **K. Thrane:** None. **C. Bosch:** None. **A.T. Schaefer:** None. **J. Lundeberg:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author on patents owned by Spatial Transcriptomics covering the technology. **A. Fleischmann:** None.

Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

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Title: Odor-dependent spatial patterns of odor identity coding in the olfactory bulb

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Abstract: Most sensory systems are topographically organized, where sensory neurons and projections are spatially arranged, and their functional properties systemically vary with their spatial position. By contrast, in the olfactory system, neurons and connections exhibit a spatially scattered and disordered pattern, which supports the generalization that functional similarities and positions in the olfactory system are not associated with each other. However, in the olfactory bulb (OB), mitral and tufted cells receive input from sensory neurons through glomeruli, which converge on axons of olfactory receptor neurons expressing the same receptors, to form a spatially arranged glomerular map on the surface of the OB. Whether the position of neurons in the OB conveys functional information and/or contributes to the odor coding remains unknown. In order to gain insights into OB spatial properties, we implanted 16-channel multi-electrode arrays to the ventral mitral cell layer along the anterior-posterior axis of the mouse OB.

A wide-area of neural population activity was recorded in head-fixed awake mice while eight monomolecular odorants were presented. Decoding analyses revealed that odors were represented with diverse odor-dependent spatial patterns across the OB. Some odors were accurately decoded within a specific local region while others required the involvement of broad regions. The data and the associated analysis support the idea that spatial positions of neurons in the OB may be associated with their function in encoding odor identity.

Disclosures: **B. Li:** None. **P. Liu:** None. **S. Pun:** None. **F. Wan:** None. **M. Vai:** None. **A. Klug:** None. **A. Li:** None. **T.C. Lei:** None.

Poster

399. Chemosensory Processing II

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NIH Grant F31 DC016485 (JMR)
NIH Grant R01DC004553 (RRR)

Title: MCHR1 localizes to primary cilia of olfactory bulb granule cells and modulates glomerular responses to odors

Authors: *K. R. JASSO[1], J. M. ROSS[2], H. KULAGA[4], M. L. FLETCHER[3], R. R. REED[5], J. C. MCINTYRE[1];
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Abstract: Sensory perception relies on the detection of external stimuli, but is dependent on modulation of the involved cells from periphery to brain. This process allows stimulus detection to match the demands of an organisms metabolic and hormonal states. Olfactory sensory neurons send axons that innervate the olfactory bulb (OB) where they transmit information about environment odors. Centrifugal innervation of the OB from several brain regions modulates this process, altering responses to odors. An area that has received limited research is hypothalamic modulation of signaling in the OB although hypothalamic neuronal populations, including melanin-concentrating hormone receptor (MCH) neurons, innervate the OB. MCH is known to regulate several olfactory-related behaviors, such as food finding and mating. In rodents there is a single MCH receptor, MCHR1, which is enriched in neuronal primary cilia. Cilia are

evolutionarily conserved organelle that functions as a signaling center projecting from nearly every cell type. While ciliary signaling is critical for neuronal development, the role of primary cilia in regulation of neuronal function, or neuromodulation, is less clear. A detailed anatomical understanding of MCHR1 expression in the OB is required to elucidate its potential modulatory effects on olfactory signaling. Using antibody labeling, we find that MCHR1 localizes to cilia of interneurons in the granule cell and glomerular layers. To further confirm this, and generate a potential tool for future studies, we used CRISPR/CAS9 to fuse mCherry to the n-terminus of MCHR1 (mCherry:MCHR1). Expression of mCherry:MCHR1 matches that of endogenous MCHR1, localizing to the primary cilium of granule cells and a subset of periglomerular cells. MCHR1, and mCherry:MCHR1, is not detected in the primary cilia of mitral cells, the primary output neurons of the olfactory bulb. To test the neuromodulatory role of MCHR1 in olfactory signaling, we have performed functional imaging experiments of the OB in response to odorant stimulation. Using epifluorescence imaging, we find that glomerular responses are enhanced following administration of an MCHR1 antagonist, SNAP-94847, ($t(157) = 5.425$, $p < 0.0001$) while application of MCH decreases glomerular responses ($t(99) = 10.98$, $p < 0.0001$). Together these data suggest that MCH/MCHR1 signaling contributes to modulation of olfactory function. Furthermore, our newly generated mouse line will aid in studying the role of MCHR1 in other brain regions and will aid in determining the importance of ciliary localization in modulating neuronal function.

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Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

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Diamond Light Source beamtime proposal MT20274

Title: Ultrastructure and function of a genetically-identified mouse glomerular column studied by correlative *in vivo* physiology, synchrotron X-ray tomography and volume electron microscopy

Authors: *C. BOSCH PIÑOL[1], T. ACKELS[1,3], A. PACUREANU[5], C. PEDDIE[2], M. BERNING[6,7], N. RZEPKA[7], M.-C. ZDORA[8,4], M. STORM[8], I. WHITELEY[1,3], L. COLLINSON[2], T. W. MARGRIE[9,3], A. T. SCHAEFER[1,3];

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Abstract: The logic of neural processes is encoded by physiological events mediated by nm-sized synapses that are distributed across mm-scale volumes of brain tissue. Therefore, a holistic approach bridging those temporal and spatial gaps is required in order to obtain a mechanistic understanding of brain function.
Here we describe a correlative experiment that involves *in vivo* physiology, rapid, high-resolution synchrotron X-ray tomography (SR-CT) and serial block-face volume electron microscopy (EM) to study neural computation in the first processing stage of the mammalian olfactory sensory system: the glomerular columns in the olfactory bulb (OB).
The projection neurons of the OB have a several µm-thick apical dendrite reaching into the glomerulus that defines the neuron's input signal. Their cytoplasm incorporates heavy metals poorly compared to the immediate neighbouring tissue, allowing to trace and resolve dendrites in near-micron-resolution histological datasets. This structural signature could be retrieved from neurons with known physiology by using a correlative *in vivo* - to - SR-CT - to - EM approach: We presented an array of 48 odours *in vivo* while recording the activity of a neuronal population of interest, surrounding a genetically-identified glomerulus, using volume 2-photon microscopy. Then, that same circuit was dissected, landmarks recorded and the tissue was stained with heavy metals. At this point, SR-CT could deliver datasets in which projection neuron apical dendrites could already be traced across 3*3*0.6 mm-sized samples, providing key contextual information of the underlying neural circuit. Finally, these samples were trimmed down to ~1mm^3 cubes so targeted regions could be imaged at nm resolution with EM. We show that consecutive imaging with these techniques is possible and can be conducted efficiently.
Combining SR-CT with volume EM holds significant promise for the wider systems neuroscience research. Here, we report biologically-relevant features in other brain areas that can be resolved by this technique: axon bundles in the striatum and CA1 pyramidal neuron apical dendrites in the hippocampus. With several synchrotron sources across the world being upgraded to provide higher flux and increased number of beamlines for biological imaging, SR-CT can become a tool to bridge scales for neuroscience research in a routine manner.
Altogether, we present an efficient correlative workflow that allows interrogating a neural circuit of interest from both the functional and ultrastructural level, bridging volume *in vivo* physiology of hundreds of neurons, sub-µm histology across several mm^3 and volume ultrastructure at the nm scale.

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Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

Support: MH109280
NARSAD

Title: Functional role of NRG1/ErbB4 signaling in odor discrimination

Authors: *Z. TAN[1], Y. LIU[1], F. LIU[3], W. XIONG[1], L. MEI[2];
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Abstract: ErbB4 and its receptor neuregulin-1 (NRG1) are both risk genes of schizophrenia and major depression disorder. The NRG1/ErbB4 signaling has been implicated in neural development, GABA release regulation, and synaptic plasticity. Mutation of either NRG1 or ErbB4 leads to behavioral deficits that are associated with schizophrenia. Schizophrenia patients also show olfactory system syndromes. Here we show that ErbB4 is highly expressed in olfactory bulb (OB), in particular in GABAergic neurons that are not positive for parvalbumin, calretinin, calbindin, and tyrosine hydroxylase. The ErbB4+ neurons in the OB can be classified into three distinct groups, each with unique electrophysiological properties, integrated into different local circuits and potentially have distinct functions. Group1 neurons receive direct input from olfactory sensory neuron (OSN); Group2 neurons receive input from external tufted cell; and Group3 neurons do not receive direct input from OSN. Projection neurons (mitral and tuft cell) in the OB receive direct input from ErbB4+ cells. Importantly, NRG1 increases GABA release onto mitral cells, which might be a mechanism of impaired odor discrimination of Erbb4-/- mice. Thus, NRG1/ErbB4 signaling modulates GABA release in the OB, which is critical for odor discrimination.

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Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

Support: DARPA grant HR00111990034 to MB
an intramural grant from NIH-NICHD to MS

Title: Robustness and complexity of olfactory coding are endowed by distinct mechanisms of neural plasticity

Authors: *M. A. STOPFER[1], S. HANEY[2], B. KIM[1], Z. ALDWORTH[1], N. RULKOV[3], M. BAZHENOV[2];
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Abstract: Oscillatory synchrony and temporal coding are prominent features of olfactory processing: information about olfactory stimuli is encoded by populations of transiently synchronized neurons whose identities progressively change across oscillation cycles. We found that responses of olfactory receptor neurons (ORNs) in the locust are more complex than previously documented. ORNs respond to odor stimuli with multiple distinct response types (i.e., onset responding, offset responding, and inhibitory). Further, each response type undergoes a different form of sensory adaptation, with onset responses decreasing, and offset responses increasing, during high frequency or lengthy odor pulses. During natural plume-like stimuli, odor filaments arrive at the animal with varying frequencies and durations. Using computational models of an ORN array implementing different receptor types with properties tuned to match those recorded *in vivo*, we found that the variety of the ORN response types and adaptation properties substantially improve classification performance for plume-like stimuli. We further examined how information from the ORN array interacts with a well-characterized mechanism of plasticity in the antennal lobe (AL): fast odor learning mediated by progressive buildup of stimulus specific synaptic inhibition. We found that distinct ORN response types are critical for orchestrating complex temporal patterning across the population of AL neurons, and that fast odor learning in the AL is required for the maintenance of robust temporal coding during plume-like stimuli. Our study predicts that a combination of receptor adaptation at the periphery and inhibitory facilitation in the AL formats olfactory information into an efficient — complex and robust — spatiotemporal code.
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Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

Support: NIH U19 NS107464
NIH T90DA043219

Title: Precise optical probing of perceptual detection in olfactory circuits

Authors: *J. V. GILL[1,2], G. M. LERMAN[2], S. SHOHAM[2,3], D. RINBERG[2,1];
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Abstract: Establishing causal links between patterns of neuronal activity and perception is crucial for understanding brain function. The mouse olfactory system is emerging as an ideal model for investigating spatiotemporal neuronal coding, given its ease of access for recording and manipulation, as well as its behavioral relevance for the animal. Recent studies have revealed that fine temporal scales are essential to olfactory information processing, but it is unknown which precise features of this code are behaviorally accessible. Recently, we developed a system for simultaneous large-scale 2-photon calcium imaging and holographic optogenetic stimulation in the olfactory bulb of awake, behaving mice, permitting recording and manipulation of groups of neurons at cellular and single action potential resolution, with millisecond precision. With this system we have measured odor-evoked activity in broad populations of mitral and tufted cells (MTCs), the projection neurons of the olfactory bulb, as well as interneurons and demonstrated the use of this system for closed-loop optogenetic feedback, mimicking natural, stimulus-evoked activity temporally patterned across an intrinsic biological rhythm (respiration). We performed a series of theory-driven experiments to establish the basic rules of MTC code readability by higher brain areas. Specifically, we ask what features of spatiotemporal neuronal activity in the olfactory bulb are *detectable* and *discriminable* by downstream circuits to guide behavior. We found that mice can detect single action potentials evoked synchronously across <20 olfactory bulb neurons, while ruling out detection of indirect effects using a novel optical sham-photostimulation technique. Additional experiments explored how this sensitivity changes as a function of synchrony, respiration phase, cell type and the odor tuning of targeted neurons. Finally, we characterized the effects of rapid and longer-term plasticity induced by cellular stimulation and behavioral training on network activity.

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Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

Support: NIH R01 DC013329
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Title: Optophysiological mapping of synaptic inputs to adult-born granule cells in the olfactory bulb

Authors: *J. L. WALLACE[1], J. D. ZAK[2], V. N. MURTHY[3];
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Abstract: New neurons appear only in a few regions of the adult mammalian brain and form synapses with preexisting cells to become integrated into functioning circuits. Adult neurogenesis is abundant in the rodent olfactory bulb (OB), where new neurons assume the identity of inhibitory interneurons, including granule cells. Adult-born granule cells (abGCs) receive feedforward inputs from both the principal cells of the OB (mitral and tufted cells) as well as feedback synapses from olfactory cortex. However, it is still unclear how these synapses are formed and refined in an input-specific manner to contribute to the functional activity of these cells *in vivo*, which we recently characterized (Neuron 96:883). By expressing channelrhodopsin in either feedforward or feedback inputs to abGCs in OB slices, we now describe the pattern of synaptic development and refinement of these inputs and relate this to action potential output in abGCs. We find that feedback inputs develop early, and there is no significant change in the amplitude of optogenetically-evoked minimal excitatory postsynaptic potentials or the estimated number of fibers converging onto single abGCs over development. Furthermore, activation of these inputs can already evoke action potentials in young abGCs (7-10 days after viral labeling of migrating neuroblasts). Functional feedforward inputs can also be detected in some cells at this stage and we are currently characterizing their spatial extent using patterned illumination with single-glomerulus resolution. These experiments will uncover the time course of development of different synaptic inputs to abGCs under normal conditions and under controlled perturbations.

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Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

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Title: Active sampling optimizes processing of fluctuations in odor concentration

Authors: A. PARABUCKI[1], A. BIZER[2], ***R. SHUSTERMAN**[3];
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Abstract: We actively shape our sensory input through our sampling behavior. Further, we adjust our sampling behavior to *optimally* sample an area or object of interest. However, the benefit of this flexibility in sampling behavior is unknown - does it facilitate sensory processing? In olfaction animals control sniff rate: in rodents, sniffing varies between 2 to 10 Hz. However, how modulating sniff rate benefits neural processing is not known.
Increases in sniff rate are especially prominent during odor tracking tasks. Odor tracking entails sampling and comparing odor concentrations across various points in space and time. Consequently, to track odors animals should have an ability to compare odor concentrations across sequential sniffs (hereafter referred to as ΔC_t). Our recent work has revealed that ΔC_t representation begins in the OB. A subset of mitral/tufted cells (MTCs), the projection neurons of the OB, respond to odor concentrations in a history-dependent manner (Parabucki et al., 2019).
Here we show that fast sniffing improves ΔC_t processing in the olfactory system. Sniff rate strongly affects the processing of time-varying input, enhancing the contrast between concentrations. When we present identical concentration on two consecutive cycles, the odor information acquired during the second sniff cycle is redundant and therefore it is filtered out during fast sampling. Further, we predict that faster sniffing will improve the behavioral perception of ΔC_t.

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Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

Support: 1F31DC016483-01A1
2R01DC000566-29A1

Title: Phase referenced LFP power carries information on odor identity in proficient animals in the olfactory bulb

Authors: *J. LOSACCO, D. RESTREPO;
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Abstract: The accurate perception of odor identity can influence everything from quality of life to survival itself. The local field potential (LFP) in olfactory bulb (OB) represents a loose gestalt of the odor in terms of incoming sensory signal as well as top-down modulation of the odor's representation.
We studied the LFP in awake, behaving mice performing the go no-go odor discrimination task where the thirsty mouse learns to respond to the rewarded stimulus to obtain a water reward. In the past it was shown that single unit neural activity and LFPs measured in the OB carry information necessary to differentiate odorants. Here we studied whether aligning the LFP power to the phase of the theta oscillations makes a difference in the ability to predict odor identity. We used a Hilbert transform to obtain the phase of theta oscillations and wavelet analysis to quantify the power of high frequency oscillations. In addition, we used phase amplitude coupling (PAC) analysis to assay changes in the strength of coupling of high frequency bursts with theta oscillations. High frequency/theta PAC has been shown to occur for oscillations in the OB (Rojas-Libano et al., 2014).
We found that as the animal learns to differentiate the odorants the variance of the peak theta angle for bursts increases for the unrewarded odorant. We then determined the proficiency of prediction of the odorant using linear discriminant analysis (LDA) using the LFP power referenced to either the peak or the trough of the mean amplitude distribution over theta phase. We find that LDA for peak-referenced LFP power predicts the odorant when the animal is proficient for odorant discrimination. Prediction is closer to shuffled control when the animal is naïve, or when the LDA is performed with the trough-referenced LFP power.

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Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

Support: NIH Grant R00DC013305
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Title: Processing of intermittent odor plumes through population activity between glomerular networks in the mouse olfactory bulb

Authors: *S. M. LEWIS[1], L. XU[4], M. F. TARIQ[2], V. GOPAL[5], A. SEMINARA[6], M. STERN[3], D. H. GIRE[1];
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Abstract: Although mice can navigate and locate resources using turbulent airborne odor plumes how they achieve this is poorly understood. One of the main challenges for animals navigating in these regimes is the intermittent contact with odor stimuli in a fluctuating plume. Population activity at the glomerular level is thought to help process this complex spatial and temporal information and create representations that can be used for effective navigation and source localization. However, how varying levels of intermittency impact odor representation in the first stages of the mouse olfactory system is not known. Using a miniaturized odor sensor combined with wide field calcium imaging techniques in head-fixed mice we precisely tracked plume dynamics and investigated glomerular response profiles in response to this fluctuating input. We found multiple distinct representations of intermittent plumes in the olfactory bulb, with some glomeruli reliably signaling intermittent plume contact at short timescales (seconds) and others signaling over longer time courses (tens of seconds). We will discuss how these results apply to navigation across a variety of odor landscapes and present data connecting intermittency-based processing to the physics of odor transport within a variety of environments that mice encounter during natural foraging behaviors.

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Poster

399. Chemosensory Processing II

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Support: NIH Grant DC005259
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KIST 2E267190

Title: The olfactory bulb contributes to the accommodation of odor responses: The input-output transformation

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Abstract: Humans and other animals exhibit adaptation to odorants. It remains unclear whether the olfactory bulb, the brain structure that mediates the first stage of olfactory information processing, participates in generating this perceptual adaptation. Olfactory bulb glomeruli are regions of neuropil that contain input and output processes; olfactory receptor neuron nerve terminals (input) and mitral/tufted cell apical dendrites (output). Differences between the input and output of a brain region define the function(s) carried out by that region. We compared the activity signals from the input and output to repeated odor stimulation across a range of odorant concentrations. Repeated odor stimulation of the same concentration resulted in a decline in the output maps, while the input remained relatively stable. These results suggest that the mammalian olfactory bulb may also participate in the perception of adaptation. This approach was previously used to show that the bulb contributed to the perception of concentration invariance of odor recognition and should be useful for understanding the role of the olfactory bulb in additional olfactory perceptions.

Disclosures: D.A. Storace: None. **L.B. Cohen:** None.

Poster

399. Chemosensory Processing II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 399.23/N42

Topic: D.05. Olfaction and Taste

Support: BBSRC, BB/L020637/1
NERC, NE/L007371/1
BBSRC, BB/P025528/1

Title: Estrogen regulates olfactory glomerular development and neuronal activity of the olfactory bulb via specific glial progenitor cells

Authors: *A. TAKESONO, P. SCHIRRMACHER, M. J. WINTER, A. SCOTT, J. M. GREEN, O. LEE, H. WAI, T. KUDOH, C. R. TYLER;
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Abstract: In vertebrates, estrogen plays critical roles in the development and function of neurons in the developing brain, however, the precise cellular mechanisms promoting these functions are unclear. Employing the use of the Ca^{++} sensor transgenic zebrafish line, elavl3:GCaMP6s, we found that estrogen signalling has a crucial role in regulating neuronal activity in the olfactory

bulb (OBs). We show that estrogen suppresses intrinsic neuronal activity in regions of the anterior forebrain, notably in the olfactory bulb (OB), and conversely an estrogen receptor antagonist hyper-activates this neuronal activity during embryo development. Using an ERE:GFP transgenic zebrafish line we show that estrogen responsive cells in the brain of embryo-larvae predominantly occur in the OBs suggesting these cells (we have named estrogen-responding olfactory bulb/EROB cells) may regulate OB neuronal activity. Interestingly, EROB cells are not neurons but a type of glial progenitor cell. Ablation of the EROB cells supresses the estrogen-mediated neuronal inhibition in the OB and also impedes OB glomerular development. This occurs through a failure to establish terminal connections between olfactory sensory neurons and the OB glomeruli. We further show that altering estrogen activity during embryogenesis disrupts the olfaction-mediated avoidance response in later larval stages. Our findings thus reveal a novel mechanism in which estrogen regulates neuronal development and activity through specific glial progenitor EROB cells in the OB.

Disclosures: **A. Takesono:** None. **P. Schirrmacher:** None. **M.J. Winter:** None. **A. Scott:** None. **J.M. Green:** None. **O. Lee:** None. **H. Wai:** None. **T. Kudoh:** None. **C.R. Tyler:** None.

Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

Support: DARPA HR0011-18-2-0024
CRCNS: NSF 1724221

Title: Contextual changes of olfactory bulb dynamics by cortical inputs

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Abstract: Olfactory bulb networks integrate sensory information conveyed by sensory neurons with “internal” information conveyed by cortical and neuromodulatory areas. The role of neuromodulatory inputs to the OB has been widely studied experimentally and computationally and coherent ideas about their function exist. In contrast, information conveyed by cortical areas to the OB is sparse but increasing due to new experimental techniques. We here use our fully developed OB-cortex model to systematically investigate the computational impact of cortical to bulbar projections. We include known details of this projection such as target neurons, transmitter released and receptors and investigate unknown parameters such as the distribution of these projections, ratios of inputs to different cell types, timing of inputs with respect to bulbar

dynamics. Our model shows that cortical inputs can have strong effects on bulbar signal to noise ratio, bulbar dynamics and bulbar odor representations. These effects depend strongly on cortical dynamics and activation, with cortex receiving olfactory as well as contextual inputs. We specifically show that beta-range dynamics can be generated in the PC due to intrinsic pyramidal cell properties: cortical feedback to OB interneurons can then entrain bulbar dynamics in the beta range. Interestingly beta range dynamics in the PC depend on feedback interactions between pyramidal cells, assumed to be strengthened during olfactory learning. As a consequence, beta oscillations in the PC and OB emerge during learning and are dependent on cortical feedback projections to the OB, as shown experimentally by Martin and Ravel (2004). Our modeling suggests a locus for beta range dynamics in the PC, imposed on OB by feedback projections, whereas gamma range dynamics would be based in OB and imposed onto PC.

Disclosures: C.L. Linster: None. **L.M. Kay:** None.

Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

Support: NIDCD R01-DC-014367
DARPA HR0011-18-2-0024

Title: Elaborating the role of olfactory bulb granule cell excitation in gating beta oscillations

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Abstract: The local field potential (LFP) of the rat olfactory bulb (OB) exhibits two distinct regimes of oscillations associated with different physiological and behavioral contexts. Gamma oscillations (40-100Hz) are associated with either odor sniffing (65-100Hz) or alert immobility (40-65Hz), and beta oscillations (15-30Hz) occur after learning and odor sensitization. Previous modeling and experimental work suggests that gamma oscillations arise from the local dendrodendritic synaptic interaction between the excitatory mitral cells (MCs) and inhibitory granule cells (GCs). However, beta oscillations require intact top down inputs to enhance GC excitability but are also generated at the dendrodendritic synapse. In particular, increasing GC excitability prolongs the activation of the NMDA receptors and voltage dependent calcium channels (VDCCs), which can mediate graded inhibition at the beta frequency because of their slow decay time constants. Our current, more detailed, model investigates specific processes (at different levels of abstraction) that regulate GC excitability to show their differential impacts on

the network oscillation. The model contains single compartment MCs receiving external input, and two-compartment GCs, which can receive cholinergic modulation or input from the piriform cortex (PC) as the top down input. The PC current is modeled either as constant, sinusoidal, or Poisson. The model suggests two distinct regimes within the beta band, high (25-30Hz) and low (20-25Hz), attained by different processes and/or modifications to the parameters. Not all means of increasing excitation lead to the most observed 20 Hz beta oscillation. Increasing excitation on GCs initially produces high power in low gamma frequency but eventually drives the network to oscillate in the high beta frequency range, unable to produce the low beta. Sinusoidal PC currents or a Poisson PC synaptic current with a sinusoidal firing rate, by contrast, can entrain the LFP, provided the driving frequency is in the beta-low gamma range, which is consistent with the experimental result that the LFPs of PC and OB have high coherence in the beta-low gamma but not in the high gamma range. Finally, ACh modulation can also slow down the network and produce 20Hz oscillation.

Disclosures: Z. Zheng: None. **L.M. Kay:** None.

Poster

399. Chemosensory Processing II

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Program #/Poster #: 399.26/N45

Topic: D.05. Olfaction and Taste

Support: DARPA HR0011-18-2-0024
CRCNS: NSF 1724221
NIH R01 DC014701

Title: Effects of olfactory bulb dynamics on cortical odor responses

Authors: *O. D. ESCANILLA[1], K. MAMA[1], M. EINHORN[2], T. A. CLELAND[2], C. LINSTER[3];
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Abstract: Olfactory bulb (OB) networks integrate afferent information conveyed by sensory neurons with “internal” information conveyed by cortical and neuromodulatory areas. The roles of neuromodulatory inputs to the OB have been widely studied, both experimentally and computationally, yielding clear proposals about their respective functions. Our computational modeling studies suggest a strong influence of bulbar dynamics and spike synchronization on cortical odor responses and dynamics. Behavioral data have shown that changes in bulbar processing evoked by manipulating bulbar neuromodulatory activity affect olfactory memory acquisition, specificity and duration. Modeling work has suggested that the loci of some of these memories are in brain areas receiving direct input from the OB. We here test these hypotheses by

recording electrical field potentials in the OB as well as unit activity in the piriform cortex (PC) while locally manipulating olfactory bulb neuromodulatory activity in awake, behaving rats. Rats were tested in a custom-designed cage equipped with pressure sensors and olfactometer inputs enabling the recording of respiratory activity and precise odor delivery without the need for prior behavioral conditioning. We recorded OB and PC activity in response to odor presentations while locally infusing cholinergic, noradrenergic and serotonergic agonists and antagonists. We observed changes in OB field potentials reflecting changes in spike synchrony and oscillatory dynamics, and correlate these with changes in PC spike responses. For example, when cholinergic tone in the OB is increased by infusing neostigmine, cortical odor responses increase in frequency. We then incorporate this information into the computational model to investigate how changes in OB dynamics and cortical responses modulate odor learning and to make detailed behavioral predictions.

Disclosures: O.D. Escanilla: None. **C. Linster:** None. **M. Einhorn:** None. **K. Mama:** None. **T.A. Cleland:** None.

Poster

399. Chemosensory Processing II

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Topic: D.05. Olfaction and Taste

Support: R01-DC017876
BRAIN 1R01NS111673-01

Title: The logic of olfactory bulb outputs revealed by high-throughput single-neuron projection mapping using sequencing

Authors: *Y. CHEN[1], X. CHEN[1], J. M. KEBSCHULL[1], A. NARASIMHAN[1], A. KOULAKOV[2], A. M. ZADOR[3], D. F. ALBEANU[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]Zador Lab., [2]Cold Spring Harbor Lab., Cold Spg Hbr, NY

Abstract: The olfactory bulb relays information about odor objects represented by olfactory sensory neurons through its output neurons, the mitral and tufted (M/T) cells, to higher brain areas. These areas have been proposed to perform distinct functions ranging from odor detection and localization, guiding spatial navigation to odor identification and innate, or learned, stimulus value assignment. Understanding the logic of information flow from the bulb to the rest of the brain is crucial for unraveling the computations performed by olfactory circuits.
Current state-of-the-art microscopy-based neuroanatomy is limited in throughput, and therefore difficult to scale up for robust statistical analysis of neuronal projections at cellular resolution.

Using MAPseq, a novel high-throughput method for mapping single-neuron projections via barcode sequencing, we investigated the single-cell projection patterns of 2,250 mitral and tufted cells (5 mice) on both the dorsal and ventral surface of the olfactory bulb. Our data recapitulate the distributed, but distinct projection patterns of mitral and respectively tufted cells cells previously observed using single-neuron tracing. Furthermore, we identified structured modules of projections from mitral cells to distinct domains along the anterior-posterior axis of the piriform cortex. These modular structures contained well-defined collaterals to non-piriform areas, and were reproducible across animals. Currently, we are further validating the projection patterns observed by single-neuron tracing using oblique light sheet microscopy. Our results indicate that information flows from the bulb to higher brain areas in a structured, non-random fashion.

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Poster

400. Peripheral Vestibular System

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 400.01/O1

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R21-DC013181

Title: MTK analysis of interactions between crista junctions in "tethered" and "untethered" regions of mitochondria in the subcuticular plate region of type I vestibular hair cells

Authors: N. LABAN[1], A. KAMBALYAL[2], R. BAHARI[1], G. PERKINS[4], ***A. LYSAKOWSKI**[3];
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Abstract: Cytoskeletal tethers connecting mitochondria to other organelles such as the endoplasmic reticulum have been well studied and are known to have an important role in molecule and ion transport (Pernice et al., 2018). However, the existence and function of tethers connecting mitochondria to other mitochondria are not as widely confirmed. We have observed such tethers in mitochondria bordering the stereociliar rootlets of a type 1 vestibular hair cell. These mitochondria were three-dimensionally reconstructed using electron microscope tomographic images and the 3D modelling program, IMOD (Kremer et al., 1996). The tomograms and models show a collection of mitochondria whose lamellar cristae are aligned forming what seems to be a mega-mitochondrial structure. Tethers were observed in some neighboring regions between these mitochondria and absent in others. Using the MTK function

of IMOD, a proximity nearest neighbor analysis was conducted to examine the relationship of neighboring crista junction pairs that were separated by two outer mitochondrial membranes and the intramembranous space. To confirm the presence of these novel connecting structures, the distances between crista junctions of neighboring mitochondria in a tethered zone were compared to distances found in an untethered zone by analyzing the histogram and data produced by the MTK function. It was observed that crista junctions in the tethered regions were in closer proximity compared to the crista junctions in the untethered regions. Thus, tethers between mitochondria allow for the flow of ATP and Ca^{2+} ions through an alignment of cristae from adjacent mitochondria, along with an alignment of their crista junctions. Both observations were made in mitochondria below the cuticular plate in type I vestibular hair cells. Increased production and concentration of ATP in this region could feed ATP into the cuticular plate for the regeneration of stereociliar rootlets or provide energy for the mechanotransduction process.

Disclosures: **N. Laban:** None. **A. Kambalyal:** None. **R. Bahari:** None. **G. Perkins:** None. **A. Lysakowski:** None.

Poster

400. Peripheral Vestibular System

Location: Hall A

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Program #/Poster #: 400.02/O2

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R21-DC013181

Title: Structural and functional characteristics of mitochondria in efferent boutons

Authors: *V. BABU[1], N. LABAN[2], J. KULAGA[2], J. LESUS[2], G. PERKINS[4], A. LYSAKOWSKI[3];
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Abstract: The synthesis of ATP, the primary energy source in the body, is an important role played by mitochondria in every cell of the body. Inner ear vestibular hair cells have especially high energy demands because they have a high resting discharge. ATP is produced by ATP synthase found in mitochondrial cristae. Because little research has focused specifically on inner ear mitochondria, this study investigates the structural and functional differences among mitochondrial subpopulations in inner ear hair cells. Three different populations of vestibular mitochondria are found: large ones under the cuticular plate in central type I hair cells, medium-sized ones in hair cells, afferent boutons and calyces, and small mitochondria in efferent boutons. In this study we focused on the third population, those in efferent boutons. We used IMOD software (Univ. Colorado) to create 3D reconstructions of electron microscopic (EM)

tomograms. Mitochondria in efferent boutons have been shown (Lysakowski et al., SFN Abst, 2017) to have the smallest surface areas and volumes of the mitochondrial subpopulations. We find that the small, densely packed mitochondria in efferent boutons possess only tubular cristae that are polarized towards dense core vesicles (DCVs). Cristae have been shown to interact with the inner mitochondrial membrane at points termed crista junctions (CJs) (Rabl et al., JCB 185:1047, 2009), and polarization occurs when more CJs are on the side of the mitochondrion facing a specific structure than on the side facing away. Thus, CJs likely enable transport of ATP and Ca^{2+} to regions of interest; for example, mitochondria in efferent boutons are polarized towards DCVs likely because they serve as a calcium stores and energy sources for vesicle release. Furthermore, we also found cytoskeletal tethers connecting DCVs and mitochondria in efferent boutons. To further investigate this association between DCVs and mitochondria, we used MTK (a proximity density analysis function in IMOD) to measure the level of interaction between CJs, DCVs, and tethers. Surprisingly, there was no polarization of CJs toward the active zone. In conclusion, efferent boutons are densely packed with mitochondria that appear to provide energy for DCV release, if not synaptic transmission.

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Poster

400. Peripheral Vestibular System

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Program #/Poster #: 400.03/O3

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R21-DC013181

Title: Structural differences in mitochondria adjacent to the cuticular plate in vestibular hair cells

Authors: *H. SATTAR[1], V. BABU[2], K. ARIAS[2], M. PATEL[2], J. KULAGA[3], J. LESUS[3], G. PERKINS[4], A. LYSAKOWSKI[3];
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Abstract: In mitochondria, cristae have been shown to interact with the inner mitochondrial membrane at points termed crista junctions (CJs) (Rabl et al., J Cell Biol 185:1047-63, 2009). These CJs likely enable transport of ATP and Ca^{2+} to regions of interest. A previous study observed CJ polarization in the auditory brainstem towards synapses that require large amounts of energy (Perkins et al., J Neurosci 30:1015-26, 2010). This study aims to investigate the structural and functional similarities among mitochondrial populations in inner ear hair cells, specifically with regard to CJs. IMOD software (Kremer et al., J Struct Biol 116:71-6, 1996) was

used to create 3D-reconstructions of mitochondria in order to analyze crista junction density with respect to structures with higher energy demands. Two models of type I hair cell mitochondria from a previous study (Lysakowski et al., SFN Abstracts, 2017) were used in this study to compare structural qualities in mitochondria in the hair cell subcuticular region. Two of the type II hair cell mitochondria in the present study that face the cuticular plate have already been reconstructed and a few more are in the process. To determine if the type II hair cell mitochondria were polarized towards the cuticular plate, we counted the number of crista junctions on the sides of the mitochondria facing towards and away from the CP using our 3D model. The CJ density on the side of the mitochondria associated with the CP was found to be significantly larger than that of the side facing away. This is similar to what was observed in type I hair cells and shows that this subpopulations of mitochondria with different sizes in different cell types possess a similarity in the presence of CJs, which may promote a similar function of delivering ATP. This occurrence in type I and type II hair cells differs to what has been observed in ribbon-associated mitochondria CJs. The polarization of the CJs suggest that they are a structural entity representing energy molecule output and delivery systems.

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Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R01-DC006685
NIDCD R21-DC016443
NSF GRFP 1747505

Title: Phase-locking of calyx-bearing vestibular afferent neurons and the role of quantal and non-quantal transmission

Authors: *M. M. IVERSEN[1], B. R. POPE[1], R. D. RABBITT[2];
[1]Univ. of Utah, Salt Lake City, UT; [2]Bioengineering, Univ. of Utah, Salt Lake Cty, UT

Abstract: Introduction
Dimorphic and calyx-only vestibular afferent neurons respond to auditory frequency (AF) sound and vibration by firing action potentials at precise times relative to the stimulus waveform (phase-locking). Calyx-bearing afferents synapsing on type I hair cells respond to AF stimuli up to several kilohertz, and are much more sensitive than bouton-only afferents synapsing on type II hair cells[1]. A unique feature of these AF sensitive afferents is that they receive both quantal (Q)

and non-quantal (NQ) transmission from hair cells[2]. It is unknown how, or if, these two forms of synaptic input combine to excite AF phase-locked action potentials in vestibular afferent neurons. In this report, we examine *in silico* the potential contributions of Q and NQ transmission to AF phase-locking in vestibular afferent neurons.

Methods

An integrate-and-fire model of a space-clamped vestibular afferent neuron was constructed with appropriate thresholds and after-hyperpolarization characteristics[3]. Q and NQ synaptic inputs from vestibular hair cells modulated at auditory frequencies were used to drive action potential generation. The NQ input was simulated using a compartmental model of ionic buildup in the synaptic cleft and modulation of a passive NQ current[4,5]. The quantal input was simulated with quantized, log-normally distributed, excitatory postsynaptic currents (EPSCs) with modulated multiquantal release (MEPSCs)[6,7]. The potential contributions of non-quantal and quantal inputs were examined numerically.

Results

Simulations indicate that Q transmission is principally responsible for phase-locking in the auditory frequency range and NQ transmission improves response fidelity by moving the voltage toward action potential threshold. Q synaptic inputs phase-lock with high vector strength at auditory frequencies and increase in strength when stimulus level dependent multi-quantal release is included. The NQ current has a cycle-by-cycle component that is present at low frequencies, but drops off as frequency increases, possibly indicating NQ's importance in low-frequency signal transmission.

Conclusion

Results offer an explanation why calyx-bearing vestibular afferents are the most sensitive to AF sound and vibration, and why these afferent neurons are preferentially excited in clinical tests that use AF stimuli.

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[1] Curthoys, *Front Neurol* (2017).
[2] Holt, *J Neurophysiol* (2007).
[3] Kalluri, *J Neurophysiol* (2010).[4] Contini, *J Physiol* (2017).[5] Highstein, *Proc Natl Acad Sci USA* (2014).[6] Highstein, *J Neurophysiol* (2015).[7] Li, *Neuron* (2014).

Disclosures: M.M. Iversen: None. **R.D. Rabbitt:** None. **B.R. Pope:** None.

Poster

400. Peripheral Vestibular System

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Topic: D.06. Auditory & Vestibular Systems

Support: National Council of Science and Technology (CONACyT) grant Fronters de la Ciencia 1544.

Title: Histamine potentiates ASIC currents in isolated neurons of the vestibular ganglia of the rat

Authors: *R. VEGA[1], S. SÁNCHEZ[3], E. SOTO[2];
[1]Inst. of Physiol., [2]Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [3]Inst. of Physiol., Benemerita Univ. Autonoma de Puebla, Puebla, Mexico

Abstract: The Acid Sensing Ion Channels (ASIC) are ligand-dependent ion channels activated by pH changes that belong to the family of ENac/DEG (Epithelial Sodium Channel/Degenerin). ASICs are involved in a large number of processes such as pain, auditory and visual processing, memory and learning, and also in pathological processes such as epilepsy and cerebral ischemia among others, this makes them targets of interest to be studied. It has been shown that ASICs are expressed in vestibular afferent neurons so they could be participating in the modulation of the excitability of these neurons. A significant number of peripheral vestibular disorders still lack a complete physio-pathogenic explanation and adequate pharmacological treatment. Histamine is a neurotransmitter whose effect is mediated by at least four receptors (H1-H4). Histamine antagonists have been used as a treatment for vestibular disorders although its peripheral mechanism of action is unclear. It has recently been shown that histamine and related amines modulate the ASIC current in heterologous systems. Therefore, we proposed to study the effects of histamine on the ASIC current of vestibular afferent neurons of the rat. The experiments were performed using the patch clamp technique in the whole cell configuration to record native ASICs present on vestibular ganglia neurons. To activate the ASIC currents neurons were perfused by a puff of acid solution (5s, pH 6.1). To determine the effect of histamine ASIC currents were recorded in control and then perfused during 55 s with histamine (1nM and 1 M) and co-perfused during 5s with the acid pH. We found that the co-application of histamine 1 nM and 1 μM with the acid pH increased the ASIC current 10.1 ± 2.3% (n = 6) and 31.3 ± 7.0% (n = 8). To determine whether or not histamine receptor activation mediates potentiation of the ASIC currents, we used GDP-β-S blocker (500 μM) we found that the potentiating effect of histamine does not change (49.0 ± 6.8%, n = 7) with the G protein inhibitor, which suggests that the effect of histamine on the ASIC current is not mediated by activation of second messengers, as it would happen if it were mediated by the activation of histamine receptors. Based on these results, we conclude that histamine enhancement of the ASIC current seems to be due to a direct interaction between the ASIC and histamine. Who significant is this action is in the overall effect of histamine is still not defined but surely should be taken in consideration while studying amine related drugs.

Disclosures: **R. Vega:** None. **S. Sánchez:** None. **E. Soto:** None.

Poster

400. Peripheral Vestibular System

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Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD Grant R01DC012347)
NIGMS IMSD grant

Title: Impact of diverse voltage-gated sodium currents on firing patterns of vestibular afferent neurons

Authors: *S. BAEZA LOYA, R. EATOCK;
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Abstract: The peripheral vestibular system transmits sensory information with two populations of primary vestibular afferent neurons (VAN), which differ in the regularity of action potential (AP) timing. The two kinds of AP timing (regular and irregular) represent different encoding strategies (rate and temporal encoding, respectively) that are optimized for different kinds of sensory information. *In vivo*, the differences between regular and irregular VANs may reflect synaptic inputs as well as diverse intrinsic ion channel expression. In isolated VANs, the difference in regularity correlates with differences in firing patterns evoked by current steps: regular neurons respond with sustained firing and irregular neurons with transient firing. We use isolated VANs to examine whether differences in voltage-dependent sodium (Na_V) currents contribute to differences in step-evoked firing patterns. Previous work indicates expression of all Na_V channel α and β subunits and at least 4 transient (inactivating) Na_V currents with different voltage dependence and time course. We are testing whether VAN Na_V currents also occur in persistent and resurgent forms, which are smaller, non-inactivating Na_V currents. In whole-cell recordings, some VAN expressed persistent Na_V current in response to slow voltage ramps, and other VAN expressed resurgent Na_V current in response to repolarizing voltage steps after a depolarization. These non-inactivated modes of Na_V current may help to depolarize VAN after each spike, promoting the sustained firing that distinguishes regular VAN, and ultimately shape sensory encoding strategies in the vestibular inner ear.

Disclosures: S. Baeza Loya: None. **R. Eatock:** None.

Poster

400. Peripheral Vestibular System

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ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD 1R01DC008846
NIH NIDCD 1R01DC013798

Title: Infrared photo-sensitivity is modulated by TRPV4 in type I vestibular hair cells in *in vivo* rat model

Authors: *F. M. RACITI[1], W. JIANG[2], S. RAJGURU[3];
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Abstract: Pulsed infrared radiation (IR) is being investigated as a non-invasive technique for altering the activity of excitable cells such as nerves and muscles. However, the mechanism of action of IR and its target have not yet been fully explained. Previous studies suggest that IR induces intracellular [Ca2+] changes as a result of activation of temperature-dependent Transient Receptor Potential (TRP) channels. We hypothesized that IR-activation of TRPV4 channels modulates Ca2+ release from intracellular compartments leading to glutamate release from vestibular hair cells and the observed excitatory and inhibitory post-synaptic responses.To define the contributions of different cell types and the mechanism of light stimulation in the vestibular neuroepithelium, we combined measurements of the vestibulo-ocular reflex during pulsed IR stimulation with local pharmacological treatments in adult female rats. Bilateral eye movements were recorded and characterized during pulsed IR stimulation (λ=1860nm, 200μs, 200Hz, various radiant exposures) of vertical semicircular canals in vivo in a rat model to assess the activity of the vestibular system. Here we show that IR - evoked responses are reduced when the neurotransmission between vestibular hair cells and the afferent neurons is impaired upstream after the local perfusion of Neomycin (100 mM) and downstream with AMPA/kainate receptor antagonist CNQX (100 μM). Further modulation of the eye movement evoked by the IR is observed when Kv7 conductance, specific of calyx - ending afferents, is targeted with low doses of KCNQ antagonist XE991 (1mg/Kg). In accordance with previous studies performed in in vitro SGNs we report the colocalization of the temperature-dependent Transient Receptor Potential (TRPV4) and the endoplasmic reticulum. Furthermore, we recorded significant decrease in the IR - evoked eye movement after inactivation of the temperature-dependent Transient Receptor Potential (TRPV4). IR-responses reduced significantly when the temperature of the vestibular

neuroepithelium has been lowered below TRPV4 activation threshold (<26°C) with the local perfusion of temperature-controlled artificial perilymph as well as after the perfusion of TRPV4 channel blockers (GSK2193874 and HC067047) at different concentrations.Altogether, our findings show that IR stimulation modulates the activity of type I vestibular hair cells and we demonstrate the pivotal role of the endoplasmic TRPV4 in the IR activation of the vestibulo-ocular motor pathway in vivo.

Disclosures: F.M. Raciti: None. **W. Jiang:** None. **S. Rajguru:** None.

Poster

400. Peripheral Vestibular System

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 400.08/O8

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD (R01DC012347)

Title: Modelling neurotransmission at the vestibular hair cell synapse

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Abstract: In vestibular sensory epithelia of amniotes, afferent neurons form large cup-shaped synaptic terminals (calyces) on type I hair cells and small bouton terminals on type II hair cells. Type I hair cells transmit to calyces by two mechanisms: release of glutamate from vesicles (**quantal - Q**) and flow of ions from the hair cell into the cleft and the postsynaptic calyx (**non-quantal - NQ**). Details of this complex transmission remain unknown, and the relevant compartments (cells and synaptic cleft) are hard to access, hindering the measurement of ion concentrations and electric potentials. As a complementary approach, **we are creating a mathematical model of the vestibular hair cell-calyx synapse (VHCC model) with the goal of predicting and accounting for both Q and NQ transmission modes and their interactions.**
The VHCC model is implemented as a 2D axisymmetric parametric surface in COMSOL with dimensions of hair cell and calyx geometry taken from electron micrographs. It incorporates specific locations and surface densities of hair cell and calyceal ion channels (MET, HCN, K_V, Ca_V, Na_V; as shown by immunocytochemistry and whole-cell recordings) and pumps (Na-K ATPase, KCC), membrane capacitance, measured or estimated channel conductances, and channel activation times. The input to the model is a step deflection of the hair bundle. To model

the dynamic behavior of the system, the VHCC model uses expressions for K^+ and Na^+ electrodiffusion in the cleft, simplified Hodgkin-Huxley-style ion currents based on whole-cell recordings, stochastic vesicle release, and the cable equation for voltage change along the fiber between the synapse and spike initiation zone.
The VHCC model can mimic firing patterns found in experimental voltage and current recordings. The model incorporates the distinctive low-voltage-activated conductance of type I hair cells ($g_{K,L}$) and postsynaptic HCN channels, which permit Na^+ and K^+ flow and are critical to NQ transmission (Contini et al. J Physiol 595:777, 2017). Model simulations confirm that removing either HCN channels or $g_{K,L}$ blocks NQ transmission. The model also predicts spatio-temporal gradients in K^+ and Na^+ within the synaptic cleft. Manipulations that increase maximal KCC-mediated K^+flow out of the synaptic cleft or reduce the calyx to the size of a bouton terminal reduce cleft K^+ accumulation, NQ transmission, and afferent spike rate. Thus, **the VHCC model supports findings and hypotheses implicating $g_{K,L}$ and g_{HCN}, K^+ accumulation in the cleft, and calyx morphology as factors in NQ transmission.**

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Poster

400. Peripheral Vestibular System

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Program #/Poster #: 400.09/O9

Topic: D.06. Auditory & Vestibular Systems

Support: M.J. Murdock Charitable Trust Research Start-Up Grant for New Science Faculty

Title: GABA and GABA receptors in zebrafish sensory hair cells and lateral line afferents

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Abstract: The neurotransmitter gamma-aminobutyric acid (GABA) plays complex and incompletely understood roles in the inner ear. Decades of experimentation have provided evidence of GABA and numerous GABA receptor subunits in the cochlea and vestibular labyrinth, but the source and target of GABA within these tissues is varied. In the cochlea, GABA is an autoinhibitory transmitter released from cholinergic efferent neurons early in development. In the vestibular labyrinth, some data suggest GABA is an efferent neurotransmitter, but uniquely, in tissues of multiple species, GABA is synthesized by a subset of hair cells, likely those that also release glutamate. The function of GABA release from hair cells has not yet been fully described. The zebrafish lateral line is an established model with which to study hair cell physiology in general, but neuromast hair cells are most closely related

to those of the vestibular system. Consistent with this similarity, early studies of the toadfish and *Xenopus* lateral line demonstrated peripheral synthesis of GABA and modulation of afferent activity by GABA. We have begun to characterize GABA circuitry in the larval zebrafish lateral line to determine if it is an appropriate model with which to better understand the role of GABA in the vestibular periphery. Using RT-PCR, we detected the expression of 22 out of 23 GABA receptor genes in 5 dpf larvae. 17 of those were expressed in both head and trunk tissue samples, consistent with the expected expression pattern of lateral line genes. We have begun to identify which of those 17 genes are expressed in the lateral line via *in situ* hybridization. No discernible lateral line expression of *gabra3, gabra4* or *gabra6b* was detected. By contrast, our data show expression of *gabrb2, gabrb3, gabrg2,* and *gabbr1a* by afferent neurons in the lateral line ganglia. These genes code for ionotropic $GABA_AR$ and G protein coupled $GABA_BR$ subunits, suggesting complex sensitivity of afferent neurons to GABA. With immunohistochemistry, we confirmed that $GABA_BR$-expressing cells innervate neuromasts, and have terminals that co-localize with postsynaptic densities. Further, neuromast hair cells appear to be the local source of GABA: antibodies that bind Gad1/2 and GABA localize to them. Our data are consistent with hair cell modulation of afferent activity via GABA release. The zebrafish lateral line thus appears to be a suitable tissue with which to model GABA signaling by vestibular sensory hair cells.

Disclosures: **J. O'Keefe:** None. **E. Bache:** None. **M. Schwehr:** None. **C.P. Toro:** None.

Poster

400. Peripheral Vestibular System

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ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: D.06. Auditory & Vestibular Systems

Support: NASA Grant NNX13AL99G

Title: Targets of cholinergic terminals within vestibular sensory epithelia

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Abstract: Efferent vestibular neurons represent a centrifugal feedback circuit projecting to labyrinthine sensory epithelia. Analyses of efferent projection loci provide insight into the likelihood of potential cellular interactions of this cholinergic feedback. For example, we recently found that the density of efferent terminals was greater in the region of utricular epithelia harboring enhanced calyceal KCNQ4 expression. This suggested that if cholinergic

input was mediated through muscarinic receptors it could represent a mechanism to tune the m-current and modulate sensory input from type I hair cells. However, we found a relative paucity of efferent terminal projections directly onto afferent calyces, indicating that the circuit elements supporting such a tuning mechanism did not dominate the efferent landscape. Through this analysis we observed that efferent terminals aggregated in intraepithelial space below the base of afferent calyces. The objective of the present study was to investigate the cellular targets of the non-calyceal projection loci. This was achieved by exploring the projection loci onto calyces, afferent projections, and type II hair cells using immunohistochemistry and high-resolution microscopy. Presynaptic cholinergic terminals were labeled with antibodies targeting vesicular acetylcholine transporter (VAChT), while postsynaptic structures were labeled with antibodies for β3-tubulin (calyces and parent dendrites) and otoferlin (type II hair cells). Utricles and cristae from C57BL/6 mice were immunostained, mounted intact, and interrogated with confocal microscopy to determine the spatial relationships between pre- and postsynaptic components of efferent circuitry. While VAChT-positive projections terminated near type II hair cells, afferent calyces, and parent dendrites, most were associated with type II hair cells, including their basolateral extensions. VAChT terminals also targeted parent dendrites below the calyx bases. These findings indicate that the principal targets of centrifugal feedback are likely type II hair cells and signal integration sites of afferent neurons. This circuit would modify direct inputs from type II hair cells onto afferent boutons, or the inputs from type II hair cells onto the lateral face of the afferent calyx.

Disclosures: **J.J. Saldate:** None. **F.E. Schweizer:** None. **L.F. Hoffman:** None.

Poster

400. Peripheral Vestibular System

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Program #/Poster #: 400.11/O11

Topic: D.06. Auditory & Vestibular Systems

Support: VA RR&D 1I01RX001986
NIH R21DC015097
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NIH F32DC017063

Title: Intense noise exposure impacts rats' balance beam performance

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Abstract: The vestibular system plays a critical role in detection of head movements and orientation with respect to gravity, and is essential for normal postural control. Due to their anatomical proximity to the cochlea, the otolith organs are exposed to sound pressure and are at risk for noise overstimulation. Irregularly discharging vestibular afferents are sound-sensitive (Murofushi & Curthoys, 1997) and important components of descending vestibulo-spinal pathways (Bilotto et al., 1982). The vestibular short-latency evoked potential (VsEP) reflects the activity of irregular afferents (Jones et al., 2011) and noise-induced attenuation of this response is associated with a reduction in calyx-only afferent terminals in the vestibular periphery (Stewart et al., 2018). The goal of the current study is to correlate changes in VsEP responses caused by noise exposure with changes in rats' ability to cross a narrow balance beam. Adult male and female Long-Evans rats (400-500g) were exposed to 120dB SPL noise (0.5 - 4 kHz) for six hours on a single day. Motor performance was assessed before and up to 28 days after noise exposure with a balance beam crossing task. Balance beam crossings were analyzed for crossing time on each trial and for variability of crossing time across trials on each day. Twenty-eight days after noise exposure, VsEP responses were obtained in response to head jerks ranging from 0.32 - 5.5 g/ms. Rats were then euthanized for tissue analysis. Post exposure VsEP data were compared with control VsEP responses obtained prior to noise exposure.One day after noise exposure, animals were able to perform the balance beam task successfully. However, day-by-day and within day balance beam crossing times exhibited greater variability after noise exposure when compared to control data. Furthermore, across the tested population, there was a tendency for mean crossing time to gradually increase. Crossing times were longest 7-14 days post noise exposure and remained elongated until sacrifice 28 days post exposure. These data suggest a subtle vestibular-mediated motor impairment after noise-induced injury to the vestibular periphery. *Supported by VA RR&D 1I01RX001986, NIDCD R21 DC015097, T32 DC000011, F32 DC017063-01.*

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Poster

400. Peripheral Vestibular System

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Topic: D.06. Auditory & Vestibular Systems

Support: VA RR&D 1I01RX001986
NIDCD R21 DC015097
T32 DC000011
F32 DC017063-01

Title: Noise-induced injury to saccular affrents is associated with decreased vestibular nerve activity as assessed by the VsEP

Authors: *C. E. STEWART[1], A. KANICKI[1], D. BAUER[1], H. HAQUE[2], R. A. ALTSCHULER[1], W. KING[1];
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Abstract: While it is well accepted that the cochlea is sensitive to sound and susceptible to acoustic over-exposure, vulnerability of the vestibular portion of the inner ear to noise is less established. Previous work identified a reduction in striolar calyx endings in the sacculus, associated with abolishment of vestibular short-latency evoked potential (VsEP) responses to small head-jerk stimuli 21 days after noise exposure (Stewart et al., 2018). The current studies extended these findings by measuring VsEP responses to larger head jerk stimuli 28 days after noise exposure, and by examining regions of interest containing the entire width of the striola, identified as the region of the sacculus containing calretinin-immunopositive calyceal endings. Adult male and female Long-Evans rats were exposed to 120 dB SPL band-limited noise (0.5 - 4 kHz) for six hours on a single day. Before and for up to 28 days after noise exposure, VsEP responses to small (0.32 g/ms), moderate (2.2 g/ms), and large (5.5 g/ms) head-jerk stimuli were evaluated. Ears were then dissected and end organs were collected for immunostaining with a calretinin antibody to evaluate noise-induced changes in the sacculus. After noise exposure, VsEP responses to weak (0.32 g/ms) head jerk stimuli were abolished and responses to larger (2.2 and 5.5 g/ms) head jerk stimuli were attenuated. This attenuation was correlated with a reduction in the number of calyx-only afferent endings in the sacculus. Noise exposure was also associated with reduced calretinin immunostaining of striolar calyces. Across all sacculi, the number of calretinin-immunopositive calyceal endings decreased with increasing distance from the striolar mid-line, suggesting that this population of afferent terminals was not evenly distributed across the central region of the sacculus. This finding may be related to the apparent narrowing of the striolar region of the sacculus observed in noise exposed sacculi. These data build upon our previous work showing not only fewer calyceal terminals within the striolar region of the sacculus, but also a narrowing of the striolar region of the sacculus which correlates with reductions in VsEP amplitude.

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Poster

401. Auditory Processing: Vocalizations and Natural Sounds

Location: Hall A

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Topic: D.06. Auditory & Vestibular Systems

Support: Leon Levy Neuroscience Fellowship
NARSAD Young Investigator Award
Fyssen Foundation Postdoctoral Fellowship

Title: Responses to infant vocalizations in mouse oxytocin neurons

Authors: *S. VALTCHEVA[1], R. C. FROEMKE[2];
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Abstract: Healthy maternal sensitivity is characterized by the ability to reliably interpret and respond to infant signals, thus initiating appropriate caregiving responses. Motherhood is a dramatic natural experience but little is known about the specific circuits and neural mechanisms supporting the recognition of different infant cues. Recent studies from our lab (Marlin et al., 2015; Mitre et al., 2016) showed that the neurohormone oxytocin promotes long-term plasticity of neural responses to infant sounds in mouse auditory cortex in vivo. Release of oxytocin from the paraventricular nucleus (PVN) of the hypothalamus might help induce recognition of different infant cues such as cries. However, it remains unknown if infant vocalizations can activate oxytocin neurons. Here we performed in vivo cell-attached and whole-cell recordings from PVN oxytocin neurons in awake mice to investigate their responses to auditory stimuli. We used channelrhodopsin-assisted patching (Munoz et al. 2014) to record from optically-identified oxytocin neurons in maternal mice. We found that oxytocin neurons reliably respond to pup calls, but not to behaviorally-irrelevant pure tones. Interestingly, repeated presentation of pup calls specifically induced a gradual increase in tonic firing of individual oxytocin neurons but not of other PVN neurons. Using cell-type specific rabies virus tracing, we identified thalamic inputs which may drive auditory responses in oxytocin neurons. We further tested the strength of these connections by channelrhodopsin-assisted circuit mapping (Petreanu et al., 2007). We describe a novel noncanonical auditory pathway potentially relaying acoustic information about social sounds to PVN oxytocin neurons. Finally, we mapped populations of PVN neurons that are activated by pup calls or suckling via the immediate early gene c-fos and if these neurons were magno- or parvocellular. Our data suggest that oxytocin neurons differentially integrate auditory and somatosensory information which may be critical for the recognition of different infant cues, and for mediating peripheral and central oxytocin release.

Disclosures: S. Valtcheva: None. **R.C. Froemke:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

Location: Hall A

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC014299
Triological Society Clinician-Scientist Development Award

Title: Role of top-down inputs in auditory cortex during vocalization in marmoset monkeys

Authors: J. TSUNADA, ***S. J. ELIADES**;
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Abstract: Human speech is a sensory-motor process involving auditory self-monitoring to control vocal production and ensure accurate communication. Monitoring auditory feedback during vocal production allows one to quickly adjust speech to compensate for perceived changes in vocal output, a control behavior shared with many animal species. The auditory cortex has been implicated in this self-monitoring process based upon previous studies showing both a suppression of the auditory cortex during vocal production as well as sensitivity to changes in vocal feedback. However, the mechanisms of this vocalization-induced suppression in auditory cortex remain poorly studied. We recorded neural activity from the auditory cortex of marmoset monkeys while they produced self-initiated vocalizations, analyzing both spiking activity and local field potentials. We found that previously-demonstrated pre-vocal suppression of neural firing is associated with an increase in low-frequency theta-band activity. We further show that, for many neurons, the magnitude of pre-vocal spiking suppression correlates with the acoustics of the subsequent vocalization. These findings suggest that this pre-vocal input to the auditory cortex, presumably a top-down signal, contains specific information predicting the expected sound of a vocalization, consistent with current models of feedback vocal control. Additionally, we found that gamma-band oscillation activity increases during vocalization, in contrast to suppression at single- and multi-unit levels. This dissociation between spiking activity and local field potentials further implicates local processing within the auditory cortex as a possible mechanism of vocalization-induced suppression.

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Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: DC-013174 to DV

Title: Variant-independent representation of zebra finch songs in the auditory forebrain

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Abstract: Invariant neural representation of a given natural sound that is produced with variations is important for identifying specific signals used in vocal communication and individual recognition. For example, the same word spoken in different situations can be acoustically different. However, listeners still perceive it correctly. To investigate how variant-independent representation emerges in the auditory system, we recorded neural responses from multiple sites bilaterally in both the primary thalamo-recipient auditory area (Field L2) and a secondary area (caudo-medial nidopallium, NCM) of awake zebra finches while presenting naturally-produced variants of the songs of individual male zebra finches. The response temporal profiles for different variants at individual recording sites were more similar to each other in NCM than in Field L2. Moreover, in NCM, the response temporal profiles for different variants converged with passive exposure to repeated playbacks, but changed little in Field L2. In addition, a population-level analysis of response magnitude also showed that responses to different variants in NCM were more similar to each other than in Field L2. Together, these results suggest that invariant representation of zebra finch song variants emerges hierarchically in the auditory forebrain. Because variant-independent representation can be essential for word recognition, findings in the zebra finch model may provide insights into basic neural mechanisms that serve speech perception.

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Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC016535
NIH Grant DC002260

Title: Selective decoding of communication sounds in lateral belt auditory cortex

Authors: *J. D. DOWNER, C. E. SCHREINER, B. J. MALONE;
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Abstract: Anatomical differences among primate auditory cortical fields are well established but a comprehensive understanding of functional differences lags behind. To analyze functional differences between auditory cortical fields, we presented 4 stimulus sets, including both

synthetic and communication sounds, while recording single unit activity in core and lateral belt auditory cortex of 2 awake squirrel monkeys. Synthetic sounds were sinusoidally amplitude modulated broadband noise (AM) and sinusoidal frequency-modulated (FM) sweeps, each presented across a range of modulation frequencies (2-96 Hz). Communication sounds were squirrel monkey vocalizations (MV), with 2 exemplars for each of 13 vocalization classes, and human speech (HS) (10 repeated sentences from the TIMIT corpus). For each recorded unit, we measured trial-by-trial stimulus decoding accuracy separately for each stimulus set. Across stimulus sets, units in core fields exhibit significantly greater average decoding accuracy across all stimuli within the set relative to units the lateral belt. However, while a unit's overall decoding accuracy for a stimulus set may be low, it may still selectively decode individual stimuli within the set at high accuracy. Therefore, we separately analyzed decoding accuracy of units with the highest single-stimulus decoding accuracy, irrespective of average decoding accuracy. We then compared decoding accuracy for each stimulus set between the best-decoded 5 stimuli for these selective sub-populations. For synthetic stimuli (AM and FM), the selective sub-population of core units' average decoding accuracy significantly exceeded that of the lateral belt, as it did for overall decoding. However, for communication stimuli (MV and HS), the sub-population of selective units in the lateral belt exhibited significantly greater average decoding accuracy relative to core. These results indicate a key functional difference between core and lateral belt auditory cortex: whereas core units carry more overall stimulus information, sub-populations of lateral belt units selectively carry high information for communication sounds.

Disclosures: J.D. Downer: None. **C.E. Schreiner:** None. **B.J. Malone:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH DC016783
NSF GRFP

Title: Auditory memories for communication calls in zebra finches

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Abstract: In the zebra finch vocal repertoire, songs and distance calls are highly idiosyncratic vocalizations that are used to identify behaviorally relevant conspecifics, such as parents, siblings, or mates. However, it is unknown whether zebra finches are capable of memorizing the songs and calls produced by the many individuals in a colony, and what brain networks are

involved in this process. We hypothesized that the higher auditory areas CM and NCM play an important role in forming and storing these general auditory memories. In this study, we first performed behavioral experiments to assess the memory capacity of zebra finches for conspecific songs and distance calls. We then performed lesion experiments to determine the role of CM and NCM in the formation and storage of these auditory memories.
To test the memory capacity of zebra finches, we used an operant conditioning paradigm in which birds are trained to recognize non-rewarded vs rewarded stimuli consisting of conspecific songs and distance calls. We found that zebra finches have an extraordinary memory capacity; at least 16 songs and 12 distance calls from distinct individuals could be recognized in this task. To localize the brain regions involved in the storage, recall, and formation of these memories, we performed bilateral neurotoxic lesions in secondary auditory areas CM or NCM either before or after operant conditioning. Preliminary results suggest that CM is not critical for the recall or formation of these auditory memories; NCM lesions are ongoing. These experiments will reveal whether these secondary auditory regions are required for auditory memory in the context of vocal communication. Further work is needed to assess the possibility that these structures are individually sufficient for this and other auditory memory related tasks.

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Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01NS082179-06

Title: Genetic and physiological segregation of excitatory vs inhibitory cell types in songbird auditory cortex

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Abstract: Cortical circuits are highly organized to process complex sensory inputs. In mammals, inhibition quickly quenches excitatory transmission to achieve highly time-locked responses to sensory stimuli. This type of feed-forward inhibition circuit motif relies on distinct classes of

cortical neurons, mainly narrow-spiking, inhibitory interneurons and broad-spiking, excitatory projection neurons. In other vertebrates such as songbirds, the organization of cortex appears highly dissimilar to mammals and yet has a similar capacity for processing complex sensory stimuli. For example in the caudomedial nidopallium (NCM), neurons are highly selective for particular features of learned conspecific vocalizations (i.e., song). Based on developmental and functional studies, NCM is considered analogous to mammalian secondary auditory cortex. However, the circuit mechanisms by which NCM processes complex ethologically-relevant auditory stimuli remain unknown. To test predictions from mammalian auditory cortex, we employed AAV viral constructs to target calmodulin-dependent protein kinase II (CAMKII) and glutamate decarboxylase (GAD) selective promoters in NCM to express channelrhodopsin within putative excitatory and inhibitory neurons, respectively. Using extracellular optrode recordings we then photoidentified infected single units *in vivo*, and obtained their waveform and auditory response properties to song. We found a striking segregation of broad vs. narrow waveforms in CAMKII+ and GAD+ single units respectively, suggesting that, as in mammalian cortex, an important component of NCM circuit architecture may be feed-forward inhibition to rapidly quench excitation. Whole cell patch clamp recordings of infected cells revealed a similar segregation of phasic vs tonic responses in CAMKII+ and GAD+ neurons, respectively. Stimulus selectivity, physiological properties, and comparisons with mammalian auditory cortex in these distinct populations of NCM neurons will be discussed. Support from NINDS.

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Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Title: Degraded auditory and visual speech affects theta synchronization and alpha power differently

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Abstract: Understanding speech can pose a challenge, especially when speech is perceived as degraded, for example when using a hearing aid. Findings on brain dynamics involved in

degraded speech comprehension are mixed. We therefore investigated the effects of degraded continuous speech on three measures: intelligibility, theta synchronization, and alpha power. Additionally, we tested another commonly experienced degradation, namely that of blurred vision during visual speech perception (lip reading). Participants listened to unimodal auditory speech and watched unimodal visual speech with three different levels of degradation in a behavioural and an MEG experiment. In the auditory condition, intelligibility declined with declining clarity, implemented by fewer vocoding channels. Theta speech-brain synchronization increased with lower clarity in left auditory regions, while alpha power showed a widespread decrease. We assume that listening effort, which should be strongest for challenging conditions, led to both effects. The idea of a common process driving both measures is also consistent with the finding that increased synchronization (for stronger degradation) was associated with lower alpha power, mainly in right temporal regions. In the visual condition, intelligibility declined with increasing blurriness of the speaker's face. Theta lip-brain synchronization in bilateral visual areas decreased with degradation, while alpha power did not change, indicating that the blurry visual stimulus could not be compensated for by neural mechanisms. Together, these findings illustrate multi-layered neural mechanisms of degraded auditory and visual speech comprehension and suggest fundamental differences between both modalities.

Disclosures: **A. Hauswald:** None. **A. Keitel:** None. **S. Rösch:** None. **N. Weisz:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 401.08/O20

Topic: D.06. Auditory & Vestibular Systems

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German Research council (OG 105/1 to YO)
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Title: Speech encoding from simultaneous ECoG recordings of human temporal plane and lateral temporal cortex

Authors: *L. S. HAMILTON[1,2,3], Y. OGANIAN[1], E. F. CHANG[1];
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Abstract: Speech processing involves the transformation of information from acoustic to phonetic and higher-order linguistic categories. Intracranial recordings have contributed significantly to understanding this transformation, however limitations in surgical exposure have precluded simultaneous high-density sampling from core and surrounding auditory cortices. Our previous work has demonstrated encoding for acoustic-phonetic features, spectrotemporal modulation, and intonational pitch tuning in superior temporal gyrus (STG), as well as a posterior region of the STG that is specialized for detecting acoustic amplitude onsets, but how this relates to processing in core auditory cortex is less well understood. Here, we present a functional characterization of the entire human auditory cortex, including the core and surrounding belt/parabelt areas. We overcame previous limitations by using direct recordings from the surface of temporal plane after surgical microdissection of the deep recesses of the Sylvian fissure when indicated for clinical purposes (peri-Sylvian epilepsy or insular tumors). We obtained simultaneous high-density recordings from patients with high-density electrode grids over the temporal plane (including Heschl's gyrus, planum temporale, and planum polare) and also over the lateral superior temporal gyrus in the left hemisphere. We then recorded neural responses while participants listened to natural speech sentences and non-speech pure tone stimuli. We fit models combining acoustic and linguistic stimulus representations to predict neural responses. We also compared latencies across different auditory fields and functional areas. Speech activated much of the temporal plane and STG, including belt and core areas. Heschl's gyrus and planum temporale exhibited strong, narrow-bandwidth tone responses, and strong responses to speech, while phonetic feature representations dominated in mid-STG. This study provides a comprehensive characterization of feature encoding for speech across the human auditory cortex at high spatiotemporal resolution.

Disclosures: L.S. Hamilton: None. **Y. Oganian:** None. **E.F. Chang:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 401.09/O21

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC009810
NSF Grant IOS-1656825

Title: Neural representations of birdsongs across distinct processing regions of auditory cortex

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Abstract: Vocal communicators rely on auditory perception to extract social information from the environment. For example, songbirds recognize conspecifics, tutors, and mates based on the acoustic features of songs. The encoding of these vocalizations in auditory cortex is hierarchical; spike rates in each successive processing stage are progressively more selective for salient sounds. However, it is unknown to what extent changes in auditory coding across regions impact the neural representation of vocal sounds. Moreover, it is unclear if coding specializations in auditory cortex extend to the specific properties of learned stimuli, such as the tutor song. A common approach to quantifying coding specializations is to measure neural tuning for specific acoustic features in vocal sounds, but this method suffers from a bias toward parameters selected *a priori* and failure to isolate correlated features. Here, we used a stimulus reconstruction method that eliminates these limitations to determine how well song features can be recovered from evoked auditory cortical responses. In zebra finches (*Taeniopygia guttata*), we recorded the spiking activity of single neurons in the four major regions of auditory cortex (intermediate, superficial, deep, secondary) in response to 15 songs from 3 species. First, we clustered single-neuron response patterns according to their temporal profiles and found that the number of cell clusters increased in successively higher areas of the network hierarchy. Second, we examined whether neural responses could reconstruct conspecific songs more accurately than the songs of two closely related species. Third, we tested whether the neural representation of tutor song differed from those of other conspecific songs in each region. Reconstruction of individual songs from cortical activity revealed circuit-level transformations in coding specificity for species and tutor song in the auditory cortex.

Disclosures: J.M. Moore: None. **S.A. Shamma:** None. **S.M. Woolley:** None.

Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: NSF Grant IOS-1656825
NIH Grant R01 DC009810

Title: Cell types and connectivity of a vocalization-selective auditory cortical region in the songbird

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Abstract: Neural encoding of behaviorally relevant sounds, such as speech or other vocalizations, results from successive processing stages as signal information travels from the

periphery throughout the cortex. In songbirds, that learn and produce complex vocalizations for social communication, neurons in the deep layers of the auditory cortex respond selectively to acoustic features of species-specific vocalizations. Deep cortical neurons may therefore occupy a key position in the synaptic pathway through which social information is extracted from auditory cues. However, the cell types and connectivity of the deep region of the auditory cortex in songbirds remain poorly characterized. We used nanoinjections of the bidirectional tracer cholera toxin subunit B, and an adeno-associated viral vector driving fluorescent protein expression, to visualize deep cortical neurons and their projections in adult male zebra finches. We find that the deep region of the primary auditory cortex (Field L3) receives the majority of its afferents from superficial (Field L1 and caudal mesopallium, CM) cortical layers, suggesting that response selectivity for conspecific song arises after at least six synaptic processing stages, bypassing a direct connection with the anatomically adjacent thalamorecipient region, L2. We further show that Field L3 neurons cluster into distinct morphological types, which may map to a known distribution of electrophysiological signatures of putative interneurons and projection neurons. While we find that Field L3 projects widely to the caudal medial pallium, terminal fields are densest in two regions: the "shelf" of HVC and the "cup" of the robust nucleus of the arcopallium (RA). Results suggest that the neural representation of birdsong that originates in the deep regions of primary auditory cortex undergoes a superficial cortical "pre-processing" step, and then diverges into three pathways, two of which could influence singing behavior by virtue of their close spatial proximity to song pre-motor nuclei.

Disclosures: J.A. Edwards: None. **S.M. Woolley:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 401.11/O23

Topic: D.06. Auditory & Vestibular Systems

Support: Simons Collaboration on the Global Brain 542977SPI
NSF GRFP

Title: Characterization of neural dynamics during tutoring in the zebra finch

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Abstract: In the zebra finch, young males learn to produce a robust imitation of an adult male tutor song, a process dependent on exposure to the tutor during an early sensitive period. Generating a stable and persistent tutor song memory during this period is central to the imitation process because birds eventually learn to precisely reproduce the tutor song’s acoustic structure.

The production and refinement of this imitation is thought to be controlled by a combination of basal ganglia dependent reinforcement learning and the growth and splitting of chains of neural activity in the motor system. Splitting of the motor representation is a fundamental part of song learning because it provides a neural substrate for each target syllable, ensuring that there is a one-to-one correspondence between syllables in the tutor song and the developing imitation. While this correspondence is necessary for song learning, it remains unclear where information about the tutor song is stored and how this information is coupled to the motor system. Likewise, while reinforcement learning has been a powerful framework for understanding the gradual refinement of song syllables, it is unknown how neural circuits for song evaluation are set-up during tutoring. To address these questions, we used a miniaturized head-mounted microscope to record the population activity of auditory-motor song regions in juvenile zebra finches throughout the tutoring process. By tracking individual neurons responses to tutoring across days we characterize how the dynamics of neural populations represent the tutor song and how these dynamics change as a function of experience.

Disclosures: **A.H. Bahle:** None. **E.L. Mackevicius:** None. **N. Denissenko:** None. **M.S. Fee:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01-DC04290
K12 NINDS-CNS Getch Scholar Career Development Award
Iowa Neuroscience Institute Carver Trust Junior Research Program of Excellence

Title: Intracranially recorded neural activity in auditory cortex elicited by simple sounds and during naturalistic verbal interactions in children

Authors: *A. E. RHONE[1], M. STEINSCHNEIDER[2], B. F. SNOAD[1], K. V. NOURSKI[1], C. K. KOVACH[1], B. J. DLOUHY[1];
[1]Neurosurg., Univ. of Iowa, Iowa City, IA; [2]Albert Einstein Col. of Med., Bronx, NY

Abstract: Neurosurgical interventions for remediation of medically intractable epilepsy in children have become a viable clinical option in pediatric patients. A companion presentation (Steinschneider et al., SFN 2019) examines basic response properties of auditory cortex on Heschl's gyrus. Here, we examine neural activity recorded from lateral superior temporal gyrus (STG) and other fronto-temporo-parietal areas. All studies were approved by the NIH and the University of Iowa Institutional Review Board. Informed consent was provided by the children's

parents or legal guardians. Verbal or written assent was obtained from the children. Subdural grid electrodes were placed over the lateral STG in three right-handed subjects (ages 9, 13, and 14). One subject had left and two had right hemisphere coverage. Analysis focused on local field potentials and event-related high gamma power (70-150 Hz). Stimuli included a wide variety of sounds including tones, click trains, speech and environmental sounds. Additionally, children engaged in a question-answer dialog based on that used previously (Nourski et al, 2016, Front Hum Neurosci 10:202) and a structured version of the children's card game "Go Fish." Neural activity elicited by sounds was maximal on the lateral STG near the transverse temporal sulcus. Activity was seen for all sound types, though speech generally elicited more widespread responses than pure tones and click trains. Responses to human vocalizations and environmental sounds (Belin et al, Nature 2000, 403:309-12) were similar in magnitude. Consonant-vowel syllables elicited local field potentials that differed between voiced and unvoiced consonants (5 ms and 40 ms voice onset time, respectively). Naturalistic interactions were characterized by a widespread activation within and beyond auditory cortex, including prominent responses in parietal areas when the subjects heard number words.We conclude that responses to isolated word and nonword stimuli on the lateral STG are similar to those seen in adults. Furthermore, preliminary results provide proof of concept that naturalistic interactive tasks are feasible in children undergoing invasive monitoring for treatment of their medically intractable epilepsy and can be used to probe auditory cortical function.

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Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: CONACYT CB-256767
PAPIIT IN207919

Title: Perceptual constancy of naturalistic sounds in macaques

Authors: *L. LEMUS, J. MELCHOR, I. MORÁN, T. FIGUEROA;
Cognitive Neurosci., Univ. Nacional Autonoma De Mexico, Mexico, Mexico

Abstract: Perceptual constancy, of say, a word, is the ability to recognize it regardless of physical variations produced by different speakers. However, the acoustic attributes holding up of the perceptual constancy of naturalistic sounds are not well understood. We trained two rhesus monkeys to recognize many naturalistic sounds as a target or as a nontarget sounds. After

training, we presented versions of the learned sounds that were not previously heard by the monkeys. The monkeys were able to recognize such sounds consistently to their putative categories. Our results not just validate their capacity to perform in complex acoustic tasks, setting a promising perceptual model to study the neural bases of sound perception, but more importantly, suggest that the most salient spectrotemporal metrics of sounds uphold for the perceptual constancy of naturalistic sounds.

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Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: ERC Grant 646696 (Audadapt) to J.O.

Title: Encoding of natural sounds in the aging human auditory cortex

Authors: *J. ERB, L.-M. SCHMITT, J. OBLESER;
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Abstract: Recent advances in computational modelling of fMRI data have furthered our understanding of how the spectro-temporal modulations in natural sounds become represented in the human auditory system. Here, we ask whether and how cortical responses to these acoustic features change over life span. We acquired 3T-fMRI data while a group of young (n = 33, aged 18 - 32) and older adults (n = 21, aged 51 - 74) listened to a story presented against a competing stream of resynthesized natural sounds (0 dB SNR). We modelled the fMRI responses in auditory cortex as a function of the spectro-temporal modulations contained in the sound mixture, that is, temporal rate, spectral scale and frequency. In a fourfold cross-validation procedure, we used ridge regression to derive single- and multi-voxel modulation transfer functions (fMRI encoding and decoding) and compared those across age groups. Results from the encoding model indicate that topographical best feature maps are preserved in the aging auditory cortex: Tonotopic maps showed the typical mirror-symmetric frequency gradients along Heschl's gyrus in both age groups. Decoding sound features from auditory cortex yielded highest accuracies (obtained as Pearson's *r* between predicted and actual acoustic features) at frequencies of 230 - 580 Hz (median $r = 0.52$), scales of 0.25 cyc/oct ($r = 0.5$), and rates of 4 - 8 Hz ($r = 0.42$), irrespective of age. Although average decoding accuracies were comparable, we found age differences in tuning width: Tuning to temporal rate was sharper in young than in older participants. These results indicate that tuning to temporal modulations broadens in the aging auditory cortex.

Disclosures: **J. Erb:** None. **L. Schmitt:** None. **J. Obleser:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01-DC04290
K12 NINDS-CNS Getch Scholar Career Development Award
Iowa Neuroscience Institute Carver Trust Junior Research Program of Excellence

Title: Fundamental response properties of auditory cortical activity in Heschl's gyrus of children as observed from direct intracranial recordings

Authors: *M. STEINSCHNEIDER[1], A. E. RHONE[2], K. NOURSKI[2], C. KOVACH[2], B. DLOUHY[2];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]NEUROSURGERY, Univ. of Iowa, Iowa City, IA

Abstract: Improvement in technology and techniques has led to a greater use of neurosurgical interventions for remediation of medically intractable epilepsy in children. To clarify developmental features of audition, investigations into fundamental functional properties of auditory cortex in children have been initiated. These studies follow on the heels of previous investigations in adult patient-subjects (reviewed in Nourski, Laryngoscope Investig Otolaryngol 2017, 2:147-56), permitting comparisons between auditory cortical physiology in children and adults. Seven children were studied (3-18 years of age). All studies were approved by the NIH and University of Iowa Institutional Review Board. Informed consent was provided by the children's parents or legal guardians. Verbal assent was obtained from children ages 5-9, and written assent was obtained for older children. The 3-year-old child was studied only with passive presentation of stimuli and in the presence of a parent who could terminate research activities at any time. The 18-year-old provided his own informed consent. Depth electrodes targeting posterior insular cortex for seizure monitoring were placed in all subjects, permitting recordings from Heschl's gyrus or its immediate vicinity (4 left hemisphere, 5 right hemisphere). Click trains (repetition rates 25-200 Hz), pure tones (frequencies 0.25-8 kHz, and stop consonant-vowel syllables were presented. Analyses focused on local field potentials and event-related high gamma power. In common with adult subjects, response latencies progressively increased and high gamma power progressively decreased from posteromedial towards anterolateral Heschl's gyrus. Individual cortical sites were generally broadly tuned but did exhibit spectral preferences within the frequency range of the tones. Similar to adults, frequency-following responses (FFR) elicited by click trains were generally observed for rates up to 50-100 Hz and were maximal at the most posteromedial sites. FFR to the fundamental frequency of the

speech syllables (~100 Hz) was especially prominent in posteromedial Heschl's gyrus. We conclude that many fundamental properties of neural activity recorded from Heschl's gyrus are similar to those seen in adult subjects from early childhood onward.

Disclosures: M. Steinschneider: None. **A.E. Rhone:** None. **K. Nourski:** None. **C. Kovach:** None. **B. Dlouhy:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 401.16/O28

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD DC012557
NSF DGE-1137475

Title: Left auditory cortex learns the statistics of infant distress sounds

Authors: *J. SCHIAVO[1], S. VALTCHEVA[2], S. C. SONG[3], R. C. FROEMKE[4];
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Abstract: Sensitivity to variability in low-level features enables generalization across sounds that vary across individuals, such as vocalizations. Statistical learning enables a sensitivity to feature regularities and distributions. While the neural correlates of statistical learning have been well studied, the mechanisms underlying the sensitization of the auditory cortex to relevant statistics are poorly understood. Here, we use behavioral approaches in mice, together with *in vivo* 2-photon imaging and whole-cell recordings, to examine how the maternal brain learns the statistics of infant vocalizations. While these calls have distinct acoustic features from adult calls, they are variable across individuals. We therefore hypothesize that retrieving females are sensitive to key statistics for generalization. Importantly, since pup-naïve virgins do not behaviorally respond to calls, but will retrieve following maternal experience, we can utilize this behavior to assess the plasticity mechanisms underlying sensitization of virgin auditory cortex to vocalization statistics. We assessed the categorical boundary of pup calls in the temporal domain by speeding or slowing call rate. We first assessed the behavioral response boundary in maternal animals in a Y-maze. Retrieving females preferred to approach speakers playing pup calls temporally modulated at the ethological rate compared to morphs outside this range. Using *in vivo* two-photon imaging, we found that excitatory neurons in core auditory cortex of experienced virgins responded invariantly to morphs within the natural range, but do not generalize across statistics in naïve virgins. Inhibitory neurons in naïve cortex were broadly

tuned, resulting in a mismatch in excitatory and inhibitory tuning. *In vivo* whole-cell recordings showed strong excitatory drive across prototypical calls in both groups, but a drop in excitatory drive across morphs in naïve females. *In vitro*, inhibition more strongly adapted in left auditory cortex of females at repetition rates around the natural call rate, indicating an intrinsic sensitivity to stimuli at these timings. We then tracked excitatory and inhibitory populations using 2-photon imaging during cohousing, and found that excitatory and inhibitory populations matched 24 hours post-retrieval. Finally, to determine if females generalized based on feature distributions experienced during learning, we manipulated the sensory environment during co-housing by playing out-of-category calls. Both behavioral responses and cortical responses generalized to these statistics post-retrieval, indicative of experience-dependent statistical learning.

Disclosures: **J. Schiavo:** None. **S. Valtcheva:** None. **S.C. Song:** None. **R.C. Froemke:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 401.17/O29

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R01DC015138

Title: Neural correlation codes for sound identification and categorization: The contribution of sound spectrum and envelope correlation structure

Authors: *X. ZHAI[1], M. SADEGHI[1], F. KHATAMI[4], F. HE[2], D. PEDRICK[3], H. L. READ[3], I. STEVENSON[3], M. A. ESCABI[3];
[1]Electrical and Computer Engin., [2]Biomed. Engin., [3]Univ. of Connecticut, Storrs, CT; [4]Neurolog. surgery, Univ. of California, San Francisco, CA

Abstract: In natural sounds, spectro-temporal modulations are highly structured and varied, and the envelopes are correlated both across frequencies and time. Here we tested how the sound spectrum and correlation structure of natural sounds is reflected in the firing rates and correlation structure of neural ensembles, respectively, and how each of the neural representations (place rate code vs. neural correlations) contribute to a sound identification and categorization task. Five texture sounds (crackling fire, bird chorus, crowd noise, running water and rattling snake) were delivered to unanesthetized rabbits and neural activity was obtained from the auditory midbrain (the inferior colliculus, IC) using multi-channel neural recording arrays. We demonstrate that the correlated firing between frequency organized recording sites in the IC are modulated by each of the tested sounds and these neural correlations can be used to decode and identify sound textures. Specifically, stimulus-driven spectro-temporal correlations were measured across the frequency organized recording array and a naïve Bayes classifier was

applied to the ensemble correlation activities to identify the delivered sounds. The classifier was able to decode and identify these original sounds approaching near perfect accuracy (~90%). Furthermore, control tests indicate that the neural response correlation is largely invariant to changes in the sound spectrum. Preserving the modulation content of each sound while removing the sound spectrum (equalizing all sounds for 1/f power spectrum) did not have a major effect on the neural correlations or the classifier performance. However, firing rates changed substantially and the firing rate classifier performance was substantially reduced. On the other hand, removing the modulation content while preserving the original power spectrum led to a reduction in the classification accuracy for the correlation classifier. Next, we tested the neural correlation classifier on a three-sound categorization task (fire, water, and speech) with multiple exemplars (6 exemplars per category). The neural correlation structure for each sound was more similar within a category than across categories such that the classifier was able to decode and categorize the sounds with very high accuracy (>90%). These findings suggest that the coordinated firing in auditory midbrain ensembles is largely invariant to changes in the sound spectrum and thus can serve as a highly informative neural signal for identification and categorization.

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Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 401.18/O30

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R01DC015138

Title: Encoding vocalizations in background sounds at the cocktail party and in the woods

Authors: *D. PEDRICK[1], X. ZHAI[2], F. HE[3], I. STEVENSON[3], M. A. ESCABI[3];
[1]Biomed. Engin., [2]Electrical & Computer Engin., [3]Univ. of Connecticut, Storrs, CT

Abstract: Separating foreground sounds from background noise has long been the focus of the cocktail party conundrum. The goal of this study is to determine how the spectrum and modulation content of a background sound mask the neural encoding of a foreground vocalization. To evaluate the ability of certain sounds to mask vocalizations, natural and man-made background sounds were used as maskers (speech babble, construction noise, multi bird calls, babbling water, etc). Neural responses were recorded from the un-anaesthetized, head fixed Dutch Belt rabbit, in response to foreground vocalizations (speech and bird song) in the presences of the original and perturbed background sounds. To isolate the masking effects due to

spectrum content, background sounds were *phase randomized*, which preserves the original sound spectrum but removes the modulation content. Alternatively, the modulation masking effect was isolated by *spectrum matching* all the background sounds to pink noise (1/f spectrum). This manipulation preserves much of the modulation content. Single neuron spiking data from the Inferior Colliculus in response to vocalizations paired with original, spectrum matched and phase randomized background sounds was analyzed to determine how the encoding of the foreground sound was affected by different background conditions. Shuffled correlogram procedures were used to isolate the background driven and vocalization driven components of the neural activity. Background driven activity was obtained by shuffling correlograms across frozen background sound segments with unfrozen foreground vocalizations (same background sound excerpt, different foreground excerpts). Alternately, foreground driven activity was obtained by shuffling correlograms across frozen foreground sound segments with unfrozen backgrounds (same foreground sound excerpts, different background excerpts from the same recording). Preliminary results show that spectrum of the background sound has a strong modulatory effect on the response firing rates. On the other hand, the modulation content of the background sound affects the reliability and precision of firing of both the foreground and background response. Thus, the sound modulation spectrum can influence the detailed temporal firing pattern to foreground sounds beyond what is expected for pure spectral masking. The findings suggest that high-order modulation content of sounds is affecting the neural representation of vocalizations in a variety of natural and man-made background sounds and thus is a critical acoustic cue for detecting signals in the presence of noise.

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Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 401.19/O31

Topic: D.06. Auditory & Vestibular Systems

Support: DFG Grant 341673897

Title: The auditory dorsal pathway mirrors the semantic hierarchy of speech prediction

Authors: *L.-M. SCHMITT[1], J. ERB[1], S. TUNE[1], A. RYSOP[2], G. HARTWIGSEN[2], J. OBLESER[1];
[1]Dept. of Psychology, Univ. of Lübeck, Lübeck, Germany; [2]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: When poor acoustics challenge speech comprehension, listeners are thought to increasingly draw on semantic context to predict upcoming speech. However, previous research focused mostly on predictions derived from isolated speech material with short timescales of context (e.g., sentences). We here ask how the human brain builds up predictions when confronted with a multitude of timescales characteristic of natural speech. In a 3-T fMRI study, healthy participants (N=60, 18-74 years) listened to a one-hour natural narrative embedded in a competing stream (0 dB SNR) of resynthesized natural sounds. To model semantic predictability at five timescales corresponding to a logarithmic increase in context length (i.e., 1-24 content words), we computed the similarity between the word2vec embedding of each content word in the story and each timescale's average word embedding. In an initial analysis of data from 30 younger participants, we mapped the timescales of semantic predictability onto the BOLD signal using voxel-wise ridge regression within a fourfold cross-validation scheme. We found that the timescales of semantic prediction are organized along an auditory dorsal processing hierarchy: increased activity in the posterior portion of superior temporal gyrus is tightly coupled to short informative timescales, whereas parietal regions like the temporo-parietal junction and angular gyrus are most responsive to long informative timescales. Furthermore, brain areas most responsive to long timescales largely overlap with the dorsal default mode network. Next, we will use a measure of semantic predictability fine-tuned to the unique hierarchical structure underlying the context of each word by incorporating a deep neural network trained to determine the probability of an upcoming word given the semantics of a timescale.

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Poster

401. Auditory Processing: Vocalizations and Natural Sounds

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Program #/Poster #: 401.20/O32

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R01DC015138

Title: The contribution of stimulus-driven and noise correlation for neural decoding and identification of texture sounds

Authors: X. ZHAI[1], I. STEVENSON[2], ***M. A. ESCABI**[2];
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Abstract: The amount of information conveyed by neural ensemble about a sensory stimulus is critically dependent on the correlation structure of its neural activity. On the one hand, stimulus driven correlations between neurons are often thought of as form of redundant encoding with

limited encoding capacity and reduced efficiency. Alternately noise correlations, i. e., coordinated firing due to network activity unrelated to the sensory stimulus are often thought of as a form of noise that limit the classification accuracy of neural population codes. Using multi-channel neural recording arrays to record neural responses to natural sound textures in unanesthetized rabbits we demonstrate that correlated firing between frequency organized neural ensembles in the auditory midbrain (inferior colliculus) can be used to recognize sounds. To explore the contribution of stimulus driven and noise correlations for decoding the sound texture identity, we developed a noise-less and a single-trial classifier. The noiseless classifier excludes the noise correlations and thus sets an upper bound on the classification accuracy provided by the stimulus driven correlation structure. The single trial classifier, by comparison, requires that both the noise and stimulus driven correlations be taken into account. Unlike noise correlations, which are mostly unstructured (diagonalized) and vary little with the sound, stimulus driven correlations are highly stimulus dependent and their time-frequency structure was quite diverse and informative for the classification task. The noiseless classifier approached near perfect identification accuracy (average 90%) using stimulus-driven correlations only and sets an upper bound on the classification accuracy. Although the average single-trial classification performance was on average lower (70%), individual penetrations were found that approached near perfect accuracy (90%). This reduction in the classification accuracy was accurately accounted for by the noise correlations. When noise correlations are not included as part of the single-trial classifier model, single trial performance drops to near chance (20%). Furthermore, performance was highly correlated with the signal-to-noise ratio (SNR) of the neural correlations: classifier performance was higher for recording locations with higher SNR. Thus, unlike previous studies which have proposed that correlated firing is generally detrimental, these findings suggest that stimulus-driven correlations can be quite informative for sound identification whereas noise correlations limit the classification performance (supported by NIDCD R01DC015138).

Disclosures: X. Zhai: None. **I. Stevenson:** None. **M.A. Escabi:** None.

Poster

401. Auditory Processing: Vocalizations and Natural Sounds

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 401.21/O33

Topic: D.06. Auditory & Vestibular Systems

Support: LSRF Postdoctoral Fellowship to SNH
NSF GRFP to LL
Finding A Cure for Epilepsy and Seizures

Title: Temporal context invariance reveals neural processing timescales in human auditory cortex

Authors: *S. V. NORMAN-HAIGNERE[1], L. K. LONG[2], O. DEVINSKY[6], W. DOYLE[7], G. M. MCKHANN[3], C. A. SCHEVON[4], A. FLINKER[6], N. MESGARANI[5];
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Abstract: Natural sounds like speech and music are structured at many timescales, but it remains unclear how these diverse timescales are cortically represented. Do processing timescales increase along the putative cortical hierarchy? What timescales are used to code speech and music? Is there hemispheric or anatomical specialization for processing particular timescales? Answering these questions has been challenging because there is no general method for estimating integration periods: the time window within which stimulus features alter the neural response. Here, we introduce a simple experimental paradigm (the "temporal context invariance" paradigm) for inferring the integration period of any time-varying response. We present sequences of natural sound segments in which the same segment occurs in two different contexts. We then measure how long the segments need to be for the response to become invariant to the context. By applying this paradigm to human electrocorticography data from epilepsy patients (broadband gamma power), we map neural processing throughout primary (near Heschl's Gyrus) and secondary regions (superior temporal gyrus) of human auditory cortex. This map reveals a clear gradient in which integration periods grow from ~100 ms in primary auditory cortex to ~300/400 ms in secondary auditory cortex. Using a separate dataset of responses to a diverse set of natural sounds, we then test what information can be decoded from populations of electrodes with different integration periods. We find that spectral information is best decoded from short integration period electrodes (<200 ms) while sound categories (speech & music) are best decoded at longer timescales (>200 ms). These results provide support for hierarchical models, demonstrate the timescale at which speech and music selectivity first emerge, and validate our method.

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Poster

402. Cellular Mechanisms of Vestibular Control

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 402.01/O34

Topic: D.06. Auditory & Vestibular Systems

Support: MEXT KAKENHI Grant Number JP15K10742

Title: Electrophysiological responses of the medial vestibular neurons to serotonin application

Authors: *M. SHINO, O. NIKKUNI, Y. TAKAYASU, K. CHIKAMATSU;
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Abstract: The medial vestibular nucleus (MVN) located at the brainstem is involved in the velocity- position integration for horizontal eye movement. MVN plays an important role in occurring nystagmus as a center of vestibulo-ocular reflex (VOR) in cooperation with the prepositus hypoglossi nucleus (PHN). Serotonin synthesized in the raphe nucleus of the brainstem is a monoamine neurotransmitter and is widely distributed in the central nerves systems. As serotonin modulates emotion, sleep, and pain, its agonists are used for depression or migraine, and abrupt withdrawal from serotonergic drugs, such as selective serotonin reuptake inhibitor (SSRI) causes vertigo and dizziness frequently. Serotonin is also involved in vestibular compensation after unilateral vestibular dysfunction. In this study, to elucidate the neuronal mechanism between vertigo and serotonin, we investigated electrophysiological membrane properties of the MVN neurons to serotonin administration by whole-cell patch-clamp recordings in the young Wistar rats. In response to bath application of 40 microM serotonin, frequency modulation of spontaneous firing of the MVN neurons was classified into three types, decreased, increased, and stable type. Next, three parameters of the spike, the half-width of the spike, the time to peak afterhyperpolarization (AHP), and the amplitude of AHP were measured. Half-width was extended in firing decreased type, shortened in firing increased type, and no significant change in firing stable type. The time to peak AHP was a similar change in each firing types, extended in firing decreased type, shortened in firing increased type, and no change in firing stable type. The amplitude of AHP was shallowed in both firing decreased and increased type, and no change in firing stable type. Spontaneous inhibitory postsynaptic current (sIPSC) examined in voltage-clamp mode, was significantly increased by serotonin administration and completely abolished by additional application of picrotoxin and strychnine. These results indicated the probability that serotonin modulates the membrane properties of MVN neurons resulting in some effect on the vestibular system.

Disclosures: M. Shino: None. **Y. Takayasu:** None. **K. Chikamatsu:** None. **O. Nikkuni:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

Location: Hall A

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Program #/Poster #: 402.02/O35

Topic: D.06. Auditory & Vestibular Systems

Support: Departmental

Title: Atypical hair cell polarity in the canal cristae of the inner ear of the sea lamprey (Petromyzon marinus): Implications for vestibular system processing

Authors: K. TO[1], W. GAN[2], A. MAHMOUD[3], D. GIOVANNUCCI[4], T. ABDELHAMID[5], *A. A. MAKLAD[4,3];
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Abstract: In the current study, the inner ear of the sea lamprey was examined by scanning electron microscopy to elucidate some morphological findings of the ear in this species. The major emphasis of the study was to characterize the hair cell stereocilia bundle types and their morphological polarity in the canals' cristae. We revealed that there are three types of hair cells in the canal cristae according to their stereocilia bundle morphology. Type 1 hair cells, in which the kinocilium is slightly longer than the tallest stereocilia, was located along the medial banks of the crista. Type 1 kinocilia were of uniform polarity and pointing laterally (ampullipetal). Type 2 hair cells, in which kinocilia were much longer than the stereocilia, was most abundant in the central region of the crista with a fewer number scattered variably in the lateral zone. The polarity of Type 2 hair cells was variable and exhibited all orientations. Type 3 hair cells were found to have extremely long kinocilia (40-50 microns in length) with extremely short stereocilia. Type 3 hair cells were distributed along the peripheral, central and lateral zones of the crista. We demonstrated that the hair cell polarity in the canals' cristae of the lamprey is not uniform as previously described. Rather, a substantial population of hair cells have diverse polarity where all orientations are represented. The physiological significance of this diverse polarity and relationship to vestibular function will be discussed.

Disclosures: K. To: None. **W. Gan:** None. **A. Mahmoud:** None. **D. Giovannucci:** None. **T. Abdelhamid:** None. **A.A. Maklad:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH/NIDCD grant R01 DC008846 (GRH)

Title: Vestibular neurons with direct projections to the solitary nucleus in the rat

Authors: E. K. CHAPMAN, A. H. GAGLIUSO, G. P. MARTINELLI, ***G. R. HOLSTEIN**; Neurol., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Vestibular input to central autonomic nuclei includes projections influencing homeostatic regulatory functions such as gastrointestinal motility, respiration, heart rate and blood pressure. Two of these are direct pathways from caudal vestibular nuclear neurons to the rostral and caudal ventrolateral medullary regions, key relays in the baroreflex pathway. These pathways are comprised of distinct glutamatergic and GABAergic components, some of which co-localize imidazoleacetic acid ribotide (IAA-RP), an endogenous neuromodulator that binds to I1 imidazoline receptors and modulates blood pressure. The solitary nucleus (SolN) is also important for autonomic function, receiving general visceral afferent input that terminates with high topographic specificity in the intermedio-caudal region, a critical area for control of homeostatic activity. SolN also receives input from the vestibular system, providing an additional vestibulo-autonomic route for altering autonomic activity in response to changes in posture and head movements. The goal of this study was to visualize the chemical anatomy of vestibular nuclear neurons with direct projections to SolN.
Anterograde (PhaL) and retrograde (FluoroGold) tract tracing were combined with neurotransmitter and modulator immunolabeling in 12 adult male Long-Evans rats. Direct, sparsely branched but highly varicose axonal projections from neurons in the caudal vestibular nuclei to SolN were observed. The vestibular neurons giving rise to these projections were primarily located in ipsilateral medial vestibular nucleus. The cell bodies were intensely glutamate immunofluorescent and their axonal processes contained vesicular glutamate transporter 2, indicative of glutamatergic neurotransmission. IAA-RP co-existed in the glutamate-immunofluorescent, retrogradely-filled vestibular cells. The vestibulo-solitary neurons were encapsulated by axo-somatic GABAergic terminals suggesting that they are under tight inhibitory control. The results establish a chemoanatomical basis for transient vestibular activation of the output pathways from the caudal and intermediate regions of SolN. In this way, changes in static head position and movement of the head in space may directly influence heart rate, blood pressure and respiration, as well as GI motility. This would provide one anatomical explanation for the synchronous heart rate and blood pressure responses observed following peripheral vestibular activation, disorders such as neurogenic orthostatic hypotension, and the nausea and vomiting associated with motion sickness.
E. K. C. & A. H. G. contributed equally to this work.

Disclosures: **E.K. Chapman:** None. **A.H. Gagliuso:** None. **G.P. Martinelli:** None. **G.R. Holstein:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

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Program #/Poster #: 402.04/O37

Topic: D.06. Auditory & Vestibular Systems

Support: Wellcome
Gatsby Charitable Foundation

Title: An intersectional viral-genetic strategy for selective targeting of vestibular efferent neurons in awake behaving mice

Authors: *V. W. K. TUNG, A. J. MURRAY;
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Abstract: The brain learns about both the external environment and its own body movement through the activation of sensory receptors in the periphery. However, information from these peripheral sensors can be modified by the actions of central circuits through the actions of efferent pathways. In the vestibular system, the efferent vestibular nucleus (EVN) projects to the periphery and can modify the firing of sensory hair cells and afferents. However, the logic and by which the brain recruits the EVN to modify incoming vestibular signals is still unclear.
In order to probe the function of the EVN in awake-behaving animals we developed an intersectional viral strategy for selective targeting of these neurons. Mouse EVN neurons are cholinergic and co-express the neuropeptide calcitonin gene-related peptide (CGRP). We developed and adeno-associated virus construct that utilises a fragment of the CGRP-promoter to drive expression only in CGRP-positive cells. We combined this promoter with the cre-dependent expression of the recombinase FLPo. Stereotaxic injection of this AAV into the brainstem of ChAT::Cre mice results in the selective expression of FLPo in the EVN. Co-injection of flp-dependent AAV constructs allows for the selective visualisation and manipulation of EVN activity.
By combining viral injections with the injection of the retrograde tracer fluorogold into posterior or horizontal canal, we show that viral expression is restricted to the EVN. This strategy was sufficient to direct the expression of GCaMP6s to EVN neurons for fiber photometry recordings during mouse behaviour. Ongoing experiments are aimed at probing the behavioural effect of EVN perturbation through the selective expression of tetanus toxin light chain blocking the synaptic transmission of EVN neurons.

Disclosures: V.W.K. Tung: None. **A.J. Murray:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

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Topic: D.06. Auditory & Vestibular Systems

Support: CIHR PJT-162285

NIH DC2390

Title: Gain scaling adaptation in vestibular thalamus

Authors: *G. MCALLISTER[1], J. CARRIOT[1], J. X. BROOKS[1], H. HOOSHANGNEJAD[2], K. E. CULLEN[2], M. J. CHACRON[1];
[1]Physiol., McGill Univ., Montreal, QC, Canada; [2]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Growing evidence shows that thalamus sensory neurons dynamically adapt their response functions to efficiently encode natural sensory stimuli with changing properties. In particular, natural stimuli tend to contain low-frequency changes in amplitude, which often induce adaptive gain scaling in thalamus neurons. This gain scaling increases the dynamic range of neural responses and increases neural coding accuracy. Gain control has been demonstrated for many sensory systems including visual contrast, visual motion, and somatosensory responses, but not clearly for the vestibular system. Previous studies have found that vestibular stimulation resulting from natural self-motion display highly variable amplitude ranging up to 450 deg/s and 4 G. However, whether (and if so, how) central vestibular neurons adapt in order to encode such a large dynamic range remains unknown to date. Here we investigated how neurons within the ventral posterior lateral thalamus adapt to changes in stimulus amplitude. We recorded extracellular single-unit neural responses within the ventral posterior lateral thalamus of rhesus macaque monkeys (2 male, 1 female) during whole-body yaw rotations. Stimuli were sinusoidal carrier rotations at 1-8 Hz, modulated with square-wave (0.1 Hz) or triangle-wave (0.033 Hz) envelopes. We calculated the time-dependent neural gain using a sliding window linear regression, averaged across envelope cycles. To describe the net effect of stimulus amplitude on gain, we measured the rate of gain change as the amplitude increases linearly (for the triangle-wave envelope functions). Moreover, to detect gain adaptation, we measured the gradual gain change following the amplitude step (for the square-wave envelope functions). We found that neural responses to amplitude steps were strongly nonlinear and that neural gain adapted significantly following the amplitude step. Gain scaled inversely with peak amplitude; this scaling consisted of an initial rapid component followed by a gradual adaptive decrease over 2-5 seconds. Moreover, neural responses to linearly increasing amplitudes between 5 and 100 deg/s were also nonlinear as the gain was decreased three-fold. Specifically, gain decreased exponentially with increasing peak amplitude, and with a greater time constant at higher rotation frequencies. We hypothesize that the adaptation uncovered helps to increase the dynamic range of ventral posterior lateral thalamus neurons in order to more efficiently encode natural self-motion stimuli with variable amplitude.

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Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH/NIDCD grant R01 DC01379801 (SMR)
NIH/NIDCD grant R01 DC008846 (GRH).

Title: Focused infrared radiation of vestibular end-organs evokes changes in heart rate and blood pressure

Authors: *D. RICE[1], S. RAJGURU[2], G. R. HOLSTEIN[3], G. P. MARTINELLI[4];
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Abstract: In the present study, we have developed the application of pulsed infrared neural stimulation (INS) focused on the vertical semicircular canals as well as the otolith organs *in vivo* in a rodent model and characterized the resultant physiological modulation of heart rate (HR), blood pressure (BP) and heart rate variability (HRV). Long wavelength pulsed IR (1863nm) unilaterally targeted the posterior canal or utricle macula of anesthetized rats and cardiovascular responses evoked via the activation of the vestibulo-sympathetic reflex (VSR) were measured by a small animal single-pressure implantable device (DSI pressure sensing technologies, HD-S10) inserted into the femoral artery. Eye movements were simultaneously recorded using a custom-modified video-oculography system (ISCAN Inc.) to orient the optical fiber in the correct position. Post mortem micro-computed tomography was used to confirm the site of stimulation, the distance of the fiber from target structures, and orientation of the beam *in vivo*. The results were compared to contemporary electrical stimulation activation of the VSR. Stimulation of the posterior semicircular canals using frequency-modulated IR resulted in significant cardiovascular responses. Overall, the decrease in BP ranged from 3.5 to 19 mmHg and the decrease in HR ranged from 7.5 to 70 bpm (n=17). In at least half of these animals, the high to low frequency ratio of heart rate variability (HRV) increased during IR stimulation, suggesting activation of the sympathetic nervous pathway. IR directed unilaterally at the utricular macula also evoked characteristic vestibular-ocular reflex (VOR) eye movements. However, contrasting the posterior canal results, frequency modulated IR stimulation of the utricular macula failed to evoked significant changes in BP, HR, and HRV.

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Poster

402. Cellular Mechanisms of Vestibular Control

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Topic: D.06. Auditory & Vestibular Systems

Support: French Ministry of Higher Education and Research Grant for F. Pasquier PhD

Title: Effect of vestibular stimulation by rotation on motor activity rhythm

Authors: *F. PASQUIER[1], N. BESSOT[1], T. MARTIN[1], A. GAUTHIER[1], J. BULLA[2,3], P. DENISE[1], G. QUARCK[1];
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Abstract: Background: All mammal organisms display a daily cyclic variation in a number of physiological functions. While light represents the main stimulus for entraining mammalian circadian rhythms, recent literature raises the hypothesis that vestibular afferents could influence the biological rhythms. Animal and human studies have revealed anatomical and functional links between the vestibular system and the central biological clock generating biological rhythms. Moreover, animal data suggest that vestibular stimulation has an impact on biological rhythms synchronization, but this hypothesis was never tested in human subjects. The present study aims to test the impact of the vestibular stimulation induced by a rotary chair on the motor activity rhythm of young healthy adults. Method: Thirty-four healthy adults (aged 23,32 ± 2.57 years ; 13 women and 21 men) were recruited. Each subject underwent an Off Vertical Axis Rotation (20 min, 60°/s, 15° axis tilt) session and a sham session in a counterbalanced order; all in the late afternoon, on the same day of the week, and at the same hour to avoid any weekly or diurnal variations. Subjects wore an actigraph on the non-dominant hand during all the protocol. Actigraphy measurements started one week before the first session to have a baseline, and stopped a week after the second session. Results: Statistical analysis demonstrated a significant decrease of activity level in the evening following vestibular stimulation (161.71 ± 45.73 mvts/min) compared to sham (207.97 ± 86.86 mvts/min) and baseline (194.67 ± 98.00 mvts/min) conditions. Also, the peak time of the motor activity rhythm presented a significant advance in phase (-1.15 h) two days after the stimulation session ($p < 0.005$) compared to sham and baseline conditions. Discussion: This research reinforces the hypothesis of the implication of vestibular afferents on the motor activity rhythm in human. It corroborates the findings of alteration in circadian rhythms in vestibular-deficient patients. These results suggest the use of vestibular stimulation as a new method in circadian rhythms rehabilitation.

Disclosures: **F. Pasquier:** None. **N. Bessot:** None. **T. Martin:** None. **A. Gauthier:** None. **J. Bulla:** None. **P. Denise:** None. **G. Quarck:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

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Topic: D.06. Auditory & Vestibular Systems

Support: CIHR PJT-162285
NIH DC2390

Title: Vestibular perceptual neuronal substrate coding strategies for representing natural self-motion

Authors: *J. CARRIOT[1], G. MCALLISTER[1], H. HOOSHANGNEJAD[2], I. MACKROUS[1], J. X. BROOKS[1], K. E. CULLEN[2], M. J. CHACRON[1];
[1]McGill Univ., Montreal, QC, Canada; [2]The Johns Hopkins Univ., Baltimore, MD

Abstract: Self-motion is sensed by the vestibular system, contributing to automatic reflexes and spatial perception. While it is generally accepted that sensory systems have adapted their coding strategies to the statistics of natural signals, how the vestibular system processes natural self-motion is largely unknown because artificial (e.g., sinusoidal) stimuli have been typically used to date. Natural stimuli frequently display complex spatiotemporal characteristics. It is commonly assumed that, through both evolutionary and developmental processes, sensory neurons are adapted to the statistical properties of the stimuli to which they are exposed. This has led to the proposal that sensory systems optimally process natural stimuli by removing redundancy which is commonly referred to as whitening as the neural response then contains equal power at all frequencies (i.e., is “white”). While we have shown that VN neurons optimally encode natural self-motion through temporal whitening, how this information is decoded remains poorly understood. Here, we investigated how neurons within the ventral posterior lateral (VPL) Thalamus, which receive direct input from neurons within the vestibular nuclei (VN) and project to cortical structures respond to natural self-motion stimuli. Our results show that vestibular Thalamic neurons, contrary to VN neurons, do not display whitening. Indeed, their response power spectra were not constant as a function of frequency and instead resemble those of afferents. Our results therefore suggest that VPL neurons do not act as mere relays information relays. Rather, they show that information as to the head motion’s detailed time course is actively decoded in VPL before being transmitted to cortical structures. We hypothesize that such decoding is required for accurate self-motion perception.

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Poster

402. Cellular Mechanisms of Vestibular Control

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Program #/Poster #: 402.09/O42

Topic: D.06. Auditory & Vestibular Systems

Support: French Ministry of Higher Education and Research Grant for F. Pasquier PhD

Title: Effect of galvanic vestibular stimulation on motor activity rhythm in human

Authors: ***G. QUARCK**, F. C. PASQUIER, N. BESSOT, A. GAUTHIER, P. DENISE;
Univ. of Caen- COMETE-INSERM, Caen, France

Abstract: Background: Light is the dominant synchronizer to entrain circadian rhythms to 24h period. Also, non-photic stimuli play a role in biological rhythmicity. Human and animal studies demonstrated anatomical and functional links between the central biological clock and the vestibular system. Hypergravity negatively impacts temperature and locomotion rhythms of rats. In human, swinging protocols have demonstrated a positive effect in the wake-sleep transition. However, while literature demonstrates the impact of vestibular information on biological rhythmicity, the effect of vestibular stimulation on motor activity rhythm was never tested. Galvanic Vestibular Stimulation (GVS) is a non invasive, safe and useful tool to stimulate vestibular afferents. The present study targets the impact of GVS on the motor activity rhythm human. Method: 25 subjects participated in this study (11 women, 14 men aged 35,08 ± 9,78). Each subject underwent 2 sessions: a sham and a vestibular stimulation session, in a counterbalanced order. The sessions took place at midday, and were scheduled on the same day of the week (2 weeks in between), and at the same hour to avoid any diurnal or weekly variations. During the GVS a direct current (1mA) was applied on the mastoid processes for twenty minutes. Activity level was registered by actigraphy during all the protocol. Subjects continuously wore the actigraph on the non-dominant hand, starting one week before the first test session (baseline) and stopped a week after the second session.Results: Statistical analysis did not reveal significant differences in motor activity between the three conditions (sham, vestibular stimulation, baseline). Also, we did not observe any modifications of activity level during hours following the vestibular stimulation compared to sham or baseline conditions. Discussion: The expected results were a phase change in circadian rhythms and, in particular, the rhythm of motor activity, in the same way that afternoon physical activity practice can induce a significant phase advance on melatonin secretion. Our study demonstrated that daytime GVS did not induce phase shift in motor activity, but another study in our laboratory showed that afternoon vestibular stimulation using a rotatory chair did lead to a phase advance. We have now to explore if the

GVS is not efficient to induce biological rhythms modifications or if the protocol used is not suitable. Further research is necessary to test the impact of GVS on biological rhythms using a modified protocol.

Disclosures: **G. Quarck:** None. **F.C. Pasquier:** None. **N. Bessot:** None. **A. Gauthier:** None. **P. Denise:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

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Program #/Poster #: 402.10/O43

Topic: D.06. Auditory & Vestibular Systems

Title: Stochastic noise differentially affects neuronal subtypes within the medial vestibular nucleus *in vitro*

Authors: S. STEFANI[1], P. BREEN[2], J. SERRADOR[3], M. SCHUBERT[4], *A. J. CAMP[1];
[1]Univ. of Sydney, Sydney, Australia; [2]Marcs Institute, Western Sydney Univ., Sydney, Australia; [3]Rutgers New Jersey Med. Sch., Newark, NJ; [4]Johns Hopkins Med., Baltimore, MD

Abstract: Background: Stochastic resonance is a phenomenon whereby sensitivity to sub-threshold signals is modified via low amplitude noise application. Application of stochastic noise has been shown to improve visual, auditory, vestibular and cardiovascular functions within humans.
Objective: Here we aim to determine how stochastic noise effects the gain and phase dynamics of individual medial vestibular nucleus neurons *in vitro*.
Methods: All experimental materials and procedures were approved by the University of Sydney Animal Ethics Committee. All experiments were performed in 3 - 5 week-old male and female C57BL/6 mice. Whole-cell current-clamp recordings of individual MVN neurons were made at room temperature from 200 μm transverse tissue slices. Recordings were made in response to a suite of depolarising current steps (6 steps, 10 pA/step) with stochastic, sinusoidal or stochastic + sinusoidal noise or without noise (control). Spike rate vs current plots were produced and the slope of the line of best fit used to quantify neuronal gain. Neuronal type was characterised objectively based on their afterhyperpolarisation (AHP), and blind to the experimenter. Phase dynamics of neuronal firing was determined by analysing the timing of action potentials in accordance with the sinusoidal stimulus peaks.
Results: In 25/45 MVN neurons stochastic noise produced a significant alteration in neuronal gain when compared with the no noise control condition. In 12 of these neurons this difference was expressed as an increase in neuronal gain and in 13 of the cells, neuronal gain was reduced. Type A ($n = 12$) and C ($n = 6$) MVN neurons increased in gain (A; $p < 0.05$, C; $p = 0.06$), whilst type B ($n = 20$) were unchanged. Phase dynamics of MVN neuron was unaltered in all neuron

subtypes.
Conclusion: The sensitivity of MVN neurons can be influenced by the application of stochastic noise. Importantly this data suggests that the impact of stochastic noise is differential - that is, type A and C neurons appear most sensitive to stochastic noise. This differential may provide a "normalisation" mechanism to modulate the overall sensitivity of the vestibular system and as such may be useful in the development of therapeutic devices to treat those suffering from balance dysfunction.

Disclosures: S. Stefani: None. **P. Breen:** None. **J. Serrador:** None. **M. Schubert:** None. **A.J. Camp:** None.

Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: DFG Grant GR 988/25-1

Title: Structural connectivity patterns of visual-vestibular brain areas PIVC and PIC

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[1]Inst. für Psychologie, Univ. Regensburg, Regensburg, Germany; [2]Dept. of Cognitive, Linguistic & Psychological Sci., Brown Univ., Providence, RI

Abstract: Visual-vestibular integration is a key feature of human self-motion perception. The parieto-insular vestibular cortex (PIVC) and the posterior insular cortex (PIC) are relevant brain areas of the visual-vestibular network. Here, we examined the white matter connectivity of PIVC and PIC with intra-hemispheric cortical areas by probabilistic tractography based on diffusion-weighted magnetic resonance imaging in 20 human brains. PIVC and PIC were defined functionally using stimulation with caloric vestibular and visual motion cues in individual subjects during functional MRI. Our results showed high track probabilities for connections with PIVC in the lateral sulcus including the anterior insula, the inferior frontal cortex, the central sulcus, the superior frontal cortex, the cingulum, the intraparietal sulcus, the parieto-occipital sulcus, and parts of the calcarine sulcus. For connections with PIC high track probabilities were found in the supramarginal gyrus, superior temporal sulcus, inferior parts of the central sulcus, the intraparietal sulcus, the parieto-occipital sulcus, and also in parts of the calcarine sulcus. These findings replicate and extend previous results from our group showing that PIC and PIVC share connectivity with several cortical areas, whereby PIC exhibits stronger connections to posterior and PIVC to anterior parts of the cortex. Such connectivity could support multisensory integration for self-motion perception.

Disclosures: **A.L. Beer:** None. **M. Becker:** None. **S.M. Frank:** None. **M.W. Greenlee:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 402.12/P1

Topic: D.06. Auditory & Vestibular Systems

Support: CIHR PJT-162285
NIH DC2390

Title: Encoding strategies of natural self-motion in the early vestibular pathway

Authors: ***I. MACKROUS**[1], J. CARRIOT[2], K. E. CULLEN[3], M. J. CHACRON[1];
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Abstract: The vestibular system generates reflexes that are vital for gaze and posture stabilization, as well as for accurate spatial perception and motor control. Yet, most previous studies of self-motion processing by vestibular neurons have used artificial (e.g., sinusoidal) stimuli whose properties fundamentally differ from those experienced during natural everyday activities. Here we investigated how neurons in the vestibular nuclei (VN) encode naturalistic self-motion. Prior studies have shown that different cell classes within the VN project to different downstream areas and mediate reflexive versus perceptual behavioral responses. Specifically, vestibular-only (VO) neurons project to the ventral posterolateral (VPL) nucleus of the Thalamus, thereby mediating self-motion perception, as well as to the spinal cord, mediating vestibulo-spinal reflexes. In contrast, position-vestibular-pause (PVP) as well eye-head (EH) neurons project to eye motoneurons within the abducens nucleus and mediate reflexive behaviors such as the vestibulo-occular reflex (VOR). We found that many neurons responses were matched to natural stimulus statistics such as to optimally encode them through temporal whitening. Interestingly however, a subpopulation of VN neurons, identified for each cell type, showed response power spectra that that did not whitened, but instead followed the statistics of natural self-motion. Neuronal variability predicted the degree of optimal coding in that, more irregular neurons displayed greater whitened responses. Further, we found that the tuning properties of PVP neurons were sufficient to account for temporal whitening while this was not the case for EH and VO neurons (Mitchell et al. 2018). For these 2 latter neuron types, both neuronal variability and tuning were required to account for the different observed degrees of optimized coding. Overall, our results show that not all neurons within VN perform optimally encode of natural self-motion stimuli. We hypothesize that the output of neurons that do not temporally whiten enables the proper decoding of the optimized information carried by temporally whitened responses by downstream brain areas. As such, our results have important

implications for understanding how optimized coding is achieved by early vestibular pathways. Specifically, they suggest that similar coding strategies are used to transmit information about self-motion for both vital reflexes as well as self-motion perception.

Disclosures: I. Mackrous: None. **J. Carriot:** None. **K.E. Cullen:** None. **M.J. Chacron:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 402.13/P2

Topic: D.06. Auditory & Vestibular Systems

Support: FRM DEQ20170336764

Title: Development of membrane properties of vestibular neurons in perinatal mice

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Abstract: The neuronal vestibular network is involved in postural reflexes by integrating sensory vestibular inputs into *ad hoc* fast corrective motor actions. While most studies have focused on features of the mature vestibular network, the acquisition of the electrophysiological properties required for proper integration of vestibular inputs during early development remained unknown. Moreover, the ontogeny of these properties on distinct vestibulo-motor pathways had yet to be determined. Here we describe the membrane properties of vestibular neurons from embryonic stage E14.5 to postnatal day 5 in mice. We focused on two functionally distinct groups: the vestibular neurons that project to the contralateral vestibular nucleus (commissural neurons) and those projecting to the spinal cord *via* the Lateral VestibuloSpinal Tract (LVST neurons). At E14.5, vestibular neurons from both groups were unable to generate spike trains, even upon injection of depolarizing currents, suggesting an immature phenotype. At E15.5, more than 80% of the neurons still exhibited immature features. From E16.5 to P5, both commissural and LVST neurons progressively acquired the ability to sustain spike trains in response to depolarizing currents and could be classified similarly to mature preparations as type A or type B neurons according to the absence or the presence of a double after hyperpolarization, respectively. In the LVST population the proportion of type A neurons progressively increased to reach about 70% by P2-P5 whereas this proportion remained stable at 60% in the commissural group from E16.5 to P5. For type B neurons, their proportion remained stable (around 30%) from E16.5 to P5 in the LVST, but were detected only after birth in the commissural group. Functionally, most of the type A neurons of both groups and commissural type B neurons were spontaneously active at rest, while LVST type B neurons were all silent. Morphologically, only

type B neurons of both groups exhibited developmental changes with an increase in the number of neurites after birth. Altogether, our results show that vestibular neurons acquire their electrophysiological identity after E14.5 onwards and that the maturation of their membrane properties is not uniform across the different functional subgroups of vestibular neurons.

Disclosures: C.J. Dubois: None. **L. Cardoit:** None. **F.M. Lambert:** None. **M. Thoby-brisson:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

Location: Hall A

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Program #/Poster #: 402.14/P3

Topic: D.06. Auditory & Vestibular Systems

Support: Fondation pour la recherche médicale
CNRS

Title: Characterization of intrinsic membrane properties of vestibulospinal neurons through xenopus development

Authors: *A. OLECHOWSKI-BESSAGUET[1], L. CARDOIT[2], M. THOBY BRISSON[3], F. M. LAMBERT[4];
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Abstract: Central vestibular neurons (2°OVN) involved in vestibulo-motor pathways present distinct intrinsic membrane properties in relation to specific vestibular functions in a given sensory-motor reference frame. Neuronal and behavioral remodeling that occur during amphibian metamorphosis represent a relevant plasticity model to investigate this functional vestibular organization. In adult terrestrial frog, two groups of 2°OVN were described according to their discharge dynamic: Phasic neurons exhibiting a high-frequency burst of 1-3 spikes, with monophasic AHP and tonic neurons firing continuously spikes with a biphasic AHP. These 2°OVN sub-populations act as band-pass and low-pass neuronal filters, respectively. Nonetheless this characterization was established on unspecified 2°OVN and so far nothing is known about the maturation of their features trough the frog metamorphosis.
This study aims to investigate 1/ intrinsic membrane properties of 2°OVN from identified vestibular pathways and dedicated to a precise vestibulo-motor command and 2/ their developmental maturation when the posturo-locomotor system is completely re-organized. The lateral vestibulo-spinal tract (LVST) produces vestibulospinal reflexes involved in postural control. Intrinsic membrane properties of LVST 2°OVN were characterized in larval (stage 53-

55) and juvenile *Xenopus laevis* aquatic frog. Rhodamine dextran (RDA) crystals were applied in the rostral hemi-cord to label ipsilateral brainstem vestibulo-spinal neurons by retrograde tracing. Patch-clamp recordings of RDA^+ LVST neurons were performed on 300µm transversal brainstem slices. Discharges dynamics were obtained in response to positive current step injections (+50pA to +300pA). Both developmental stages exhibited classical phasic (1-3 spikes) and tonic (continuous firing) LVST neurons. A third class of neurons was found with intermediate firing pattern (>3 spikes but not continuous) and varying discharge dynamics. Most of the recorded neurons presented a biphasic AHP and action potentials duration was larger in larvae than in juvenile.
This study depicts a functional link between 2°OVN neuronal properties and sensory-motor transformation produced by a given vestibular circuit and its developmental maturation.

Disclosures: A. Olechowski-Bessaguet: None. **L. Cardoit:** None. **M. Thoby Brisson:** None. **F.M. Lambert:** None.

Poster

402. Cellular Mechanisms of Vestibular Control

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 402.15/P4

Topic: B.09. Network interactions

Support: ISF Grant 1496_2016

Title: On the organization of electrical coupling between inferior olive neurons, and its influence on synchronized rhythmicity

Authors: *N. VRIELER[1], M. Y. UUSISAARI[2], Y. YAROM[1];
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Abstract: The synchronicity in the rhythmic activity patterns produced by the inferior olive (IO) network is thought to be highly relevant to the cerebellar computation. While this synchronicity is known to depend on the dendro-dendritic gap junction (GJ)-coupling between IO neurons, it is unclear how this electrical coupling can allow specific subpopulations of neurons to synchronize their rhythmic subthreshold oscillations (STOs), rather than causing the entire network to act together. In the work presented here, we explore the possibility that spatio-temporal synchronicity patterns are inherent to the organization of the electrical coupling between IO neurons. Using a combination of morphological and electrophysiological data from mouse IO neurons collected in *in vitro* patch clamp experiments, we characterize the relationship between electrical dendro-dendritic coupling and intrinsic physiological response properties. Our results suggest that subsets of neurons with densely overlapping dendrites form anatomical clusters in the IO network, while other neurons form sparser connections across clusters. The morphological

and electrophysiological data together support the intriguing possibility that the networks' anatomically clustered neurons are wired to maintain steady rhythmic activity. Meanwhile, large-scale synchronicity patterns in the network may be mediated by the neurons connecting across clusters.

Disclosures: **N. Vrieler:** None. **M.Y. Uusisaari:** None. **Y. Yarom:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.01/P5

Topic: D.07. Vision

Support: NSERC

Title: Orientation-dependent bias on connectomes in cat's secondary visual cortex

Authors: ***R. LUSSIEZ**[1], A. OUELHAZI[2], S. MOLOTCHNIKOFF[3];
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Abstract: As previously demonstrated in numerous studies, the cat's visual cortices V1 and V2 are functionally organized, following an orientation column pattern. However, the stimulus-evoked response does not exclusively rely on this orientation selectivity, but also on the complex interplay between orientation columns and cortical layers. This complex interplay can be revealed by examining the neuronal circuits, also called connectomes. It has been shown that for a same cell-assembly in V1, the connectome (functional connections between neurons) changes depending on the visual orientation, presented as a sine-wave grating (Bharmauria, 2015). As V2 shares the same organization than V1, our aim in this study is to investigate the stimulus-dependent connectomes in V2. Using electrophysiological multi-unit recordings in V2, we recorded the neurons' electrical activity in both supra- and infragranular cortical layers of an adult head-fixed anesthetized cat. Spike-trains cross-correlations were computed to study functional connections between simultaneously recorded neurons. Similarly to V1, we observed a change in network connections depending on the presented orientation. Moreover, and quite interestingly, oblique orientations close to cardinal orientations (22.5°, 67.5°, 112.5°, 157.5°) generate a higher number of functional connections. We hypothesize that while cardinal orientations (0° and 90°) are overrepresented in the visual cortex, these oblique orientations require an additional emergence of functional connections to be effectively processed in relation to dominant cardinal orientations.
Sup: NSERC

Disclosures: **R. Lussiez:** None. **A. Ouelhazi:** None. **S. Molotchnikoff:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.02/P6

Topic: D.07. Vision

Support: NS-088906
P30 EY17039

Title: A comparison of columnar and laminar neuronal distribution in human and macaque V1

Authors: *V. GARCIA-MARIN, J. G. KELLY, M. J. HAWKEN;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Neuronal density is thought to be critical to understanding the architecture of the cortex. One question that is central to the generality of cortical structure is the number of neurons in a cortical column through the depth of cortex. In the current study we compared the neuronal numbers in an ocular dominance column (ODC) in monkey and human primary visual cortex (V1). In human V1 previous studies have reported different neuronal densities; ranging from 18 to 123 x 10^3 neurons/mm^3. First we reevaluated the neuronal density in human V1 using immunohistochemistry and an automatic quantification method that we previously used in monkey V1 (Kelly & Hawken, 2017). Using antibodies to uniquely identify all the neurons (anti-NeuN) is a more specific approach than using Nissl staining. Using immunofluorescence confocal microscopy for NeuN and DAPI (a general cell marker), followed by post-imaging automatic segmentation, we quantified the distribution of neurons in 7 humans (14 columns). The average density across layers was 75 x 10^3 neurons/mm^3 and 159 x 10^3 DAPI cells/mm^3. Obtaining accurate neuronal density is an important step in establishing a link between processing modules in different species. In V1, the ODC is an important module that contains the processing for the full range of orientations at a range of spatial scales for one eye. Using the data obtained from our current study along with our data from macaque V1 (Kelly & Hawken, 2017; Garcia-Marin et al., 2019) and the ODC size in human and macaque, we estimated there were 159 x 10^3 neurons in one human ODC compared to 69 x 10^3 in one macaque ODC. Further, we estimated that were 1261 minicolumns per ODC for human and 625 minicolumns per ODC for macaque. Using the total number of neurons per ODC, we estimated that there were 127 neurons per minicolumn in human and 111 neurons per minicolumn in macaque. These estimates suggest that at the finest columnar scale - the minicolumn - there is a close match in the number of neurons between human and macaque. Yet at the meso-scale the human cortex has about 2.3 times the number of neurons per ODC than in the macaque even though the density of neurons in human cortex is about 1/3 of the density in macaque.

Disclosures: **V. Garcia-Marin:** None. **J.G. Kelly:** None. **M.J. Hawken:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.03/P7

Topic: D.07. Vision

Support: NSERC
FRQNT (Quebec)

Title: Cortico-cortical axons of the visual cortex are not myelinated

Authors: *J. ROY, E.-M. FRIGON, D. BOIRE;
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Abstract: Myelin ensheathment increases transmission speed and could also improve synchronicity of signals despite differences in length of axonal paths to reach a specific target. Myelin distribution along the length of single axons of the cerebral cortex is poorly know. Although both myelinated and unmyelinated axons are present in both grey and white matter, the identity of the myelinated fibers at the single axon level is widely ignored. Moreover, myelin sheath can vary in length and thickness along a same axon. Typically, it is believed that in the CNS, axons connecting distant brain areas are wrapped in myelin. In addition, there are observations of myelinated local and recurrent axon collaterals of cortical pyramidal neurons. A recent study has reported that most myelinated fibers of the rodent cortex are axons of parvalbumin positive GABAergic interneurons. The objective of this study is to see the myelination pattern of visual cortex cortico-cortical projection axons in the mouse. Iontophoretic injections of the anterograde tracer, *Phaseolus vulgaris* leucoagglutinin (Phal), were performed in the visual cortex of C57BL/6J mice. After one-week survival, brains were harvested and cut. Myelin (myelin binding protein MBP) and Phal were revealed by immunohistochemistry. Confocal microscopic analyses of colocalization (proximity) of the axonal and MBP labeling showed that most of the visual cortex efferent axons are not myelinated. Most projections to ipsilateral cortices travelled through the gray matter whereas those to contralateral cortices travelled through the white matter. No myelinated grey matter axons were observed. Moreover, it was also noted that the orientation and morphology of the Phal labeled axons didn't correspond to the myelin labeling patterns. Myelin labeling in the mouse cortex appears as a regular columnar radial pattern of axonal bundles crossed by tangential horizontal fibers, while the Phal labeled axons had oblique orientations and travelled up and down between the myelinated fibers. The lack of colocalization in addition to morphology, suggest that most ipsilateral corticocortical projections in the mouse are through unmyelinated fibers.

Disclosures: **J. Roy:** None. **E. Frigon:** None. **D. Boire:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.04/P8

Topic: D.07. Vision

Support: HBP/ 785907-SGA2

Title: Complete synaptic coverage of dendrites of calcium-binding protein expressing GABAergic interneurons in mouse primary visual cortex

Authors: *P. TALAPKA[1], Z. KOCSIS[2], L. D. MARSI[1], V. E. SZARVAS[1], Z. F. KISVARDAY[1];
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Abstract: Despite a vast number of well-organized datasets concerning the morphological, electrophysiological and synaptic properties of GABAergic inhibitory interneurons (INs), quantitative data on single cells as to their synaptic connectivity with other members of the neural network are quite rudimentary. Our major goal is to generate a quantitative electron microscopic (EM) database of the complete synaptic coverage of major subtypes of INs in the mouse primary somatosensory (S1) and visual (V1) cortices. Here we report our newly-developed immunohistochemistry-correlated EM method proved successful in allowing of tracing long dendrite segments originating from the parent soma. This method generates utmost ultrastructural quality which is free of deficits caused by the immunohistochemical procedure. 60 μm thick coronal vibratome sections were collected from tissue blocks containing cortical areas S1 and V1. Adjoining sections were reserved for EM analyses ("non-labelled") and stained for a particular GABAergic subtype marker ("labelled"), respectively. Thereafter, we utilized the "mirror" technique which rests on the precise identification of "mirror" cells which are cut in half by the sectioning plane of adjoining sections. Calbindin-D_{28K} (CB), calretinin (CR) and parvalbumin (PV) immunopositive INs were chosen from different layers of mouse V1, identified in the "non-labelled" sections and embedded in resin blocks. 50 nm ultrathin serial sections covering the whole thickness of the vibratome sections (~1200) were collected, processed for transmission EM analyses and documented typically at x30 k magnification. Section loss was less than 3%. Three dendrites, belonging to CB, CR or PV containing INs were traced (photographed) and reconstructed in 3D. We were able to determine the main synaptic parameters: distribution and exact distance of excitatory and inhibitory synapses from soma location, surface area and volume of the presynaptic boutons, morphometric parameters of vesicles, surface extent of the active zones. Here we provide the first complete quantitative

analyses of synaptic coverage of dendrites belonging the calcium-binding protein containing IN subtypes in the mouse neocortex. Supported by the Human Brain Project (785907-SGA2).

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Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.05/P9

Topic: D.07. Vision

Support: MH105982
EY023173
MH114830
EPFL-BBP funding

Title: Whole-brain reconstruction and classification of single spiny neurons in the neocortex of mice

Authors: *Y. WANG[1,2], P. XIE[1], X. KUANG[2], Y. LI[2], E. SHEN[1], P. LESNAR[1], A. FEINER[1,3], Y. WANG[3], H. KUO[1], Y. YU[1], Z. ZHOU[1,4], H. GONG[4], Q. LUO[4], H. PENG[1], J. HARRIS[1], H. ZENG[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Wenzhou Med. Univ., Wenzhou, China; [3]Shanghai Univ., Shanghai, China; [4]Huazhong Univ. of Sci. and Technol., Wuhan, China

Abstract: Excitatory spiny neurons are the majority in neocortex , which have been intensively studied and defined into many types in different layers using brain slices with structures especially axons severed. The full neuronal morphologies are still unknown for most neuron types. To describe and classify these neurons based on complete morphologies, we performed computation-assisted morphological reconstructions to obtain full dendrites and axons of single neurons across all layers of multiple cortical regions using NL360 and Vaa3D-TeraVR from a high resolution whole brain image stack (>10K images, 0.2 x 0.2 x 1 micron at XYZ axis) acquired with a two-photon fluorescence micro-optical sectioning tomography system (2p-fMOST). We sparsely labeled neurons using tamoxifen-inducible Cre driver lines (Gnb4-CreERT2 and Cux2-CreERT2) crossed to a bright GFP reporter (Ai139, Ai140 and Ai166). Based on whole brain reconstruction, especially long axonal projections, we describe several preliminary results for neurons across cortical layers. (1) Ipsilateral-projecting pyramidal cells (PCs) typically target multiple cortical and sub-cortical regions; those of the same type have mostly shared target regions. (2) Contralateral-projecting PCs typically form an axon cluster around soma and also project to the homotypic region of contralateral hemisphere, forming

another axonal cluster. (3) Most PC types have ipsi- and bi-lateral projection subtypes, but only bilateral subtypes were found in some regions (such as L3TPCs in MOp, L6bPCs in AIv, L2/3TPCs and L4SSCs in AUD). (4) Axonal morphologies of L4 neurons, especially long projections, vary according to soma location and cell type. L4SSCs typically have only a local axon in the SSp but often form ipsi- and contralateral projections in VIS and AUD. L4TPCs and L4UPCs have projections to both neighboring and distal regions. (5) Like TPCs in other layers, ipsilateral L2TPCs typically have an apical dendrite, but bilateral L2TPCs commonly have 2 to 3 apical dendrites. (6) Clustering analyses based on dendrosomatic and axonal features with a large sample of L6bPCs, revealed multiple groups (i.e., objectively classified types) that were consistent with those simply grouped based on soma locations. Taken together, morphological features of fully reconstructed neurons suggest that axonal and dendritic morphologies, especially long axonal projections, vary according to soma locations, indicative of a topographic organizational principle in cortical network. These results demonstrate the potential of large-scale whole brain single neuron reconstructions for revealing general organizational rules of connectivity.

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Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.06/P10

Topic: D.07. Vision

Title: Morphological and transcriptomic diversity of somatostatin neurons in mouse visual cortex labeled *in vitro* and *in vivo*

Authors: *R. DALLEY, S. KEBEDE, A. MUKORA, G. WILLIAMS, D. SANDMAN, R. DE FRATES, E. SHEN, K. LINK, C. GAMLIN, Z. ZHOU, H. KUO, Y. YU, P. XIE, F. BAFTIZADEH BAGHAL, N. GOUWENS, B. LEE, J. MILLER, R. NICOVICH, Y. WANG, H. PENG, K. SMITH, B. TASIC, Z. YAO, E. LEIN, J. BERG, G. MURPHY, H. ZENG, S. SORENSEN;
Allen Institue for Brain Sci., Seattle, WA

Abstract: There are a multitude of characteristics that define a cell type. Morphology is one of the most traditional and can be used in combination with other cellular properties, such as gene expression and electrophysiology, to identify robust cell types. One of the most distinct inhibitory cell types are Somatostatin (Sst) - expressing Martinotti cells, which are characterized by extensive layer 1 innervation. Other Sst-expressing cells lack extensive layer 1 innervation

and/or have unique inhibitory, long-range projection axons. Previously, we have described six morpho-electric (ME) types of the Sst subclass in mouse, primary visual cortex (Gouwens et al., 2018) that align, and further divide, the above-mentioned types. We now have a large dataset of reconstructed Sst neurons (>300 neurons) in mouse visual cortex that facilitates a more in-depth understanding of the diversity of this population. Here we describe the relationship between morphological types and ME-types and for a subset of cells we can directly relate morphological properties and types to transcriptomic cell types described in another recent paper (Tasic et al., 2018). With new advances in sparse genetic labeling and high-resolution whole brain fluorescence micro-optical sectioning tomography (fMOST) imaging, we extended this dataset to include some of the first whole brain reconstructed Sst neurons. We are further establishing the correspondence between the *in vitro*-labeled Sst neurons within and across transcriptomic types and the Sst neurons labeled *in vivo.*

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Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.07/P11

Topic: D.07. Vision

Title: Morphological diversity within and across transcriptomically-defined interneuron types in mouse primary visual cortex

Authors: *S. A. SORENSEN[1], R. DALLEY[1], C. LEE[1], S. KEBEDE[1], A. MUKORA[1], G. WILLIAMS[1], D. SANDMAN[1], K. E. LINK[1], C. GAMLIN[1], J. BERG[1], N. W. GOUWENS[1], B. R. LEE[1], J. A. MILLER[1], R. NICOVICH[1], H. PENG[1], K. SMITH[1], B. TASIC[2], Z. YAO[1], J. T. TING[3], G. J. MURPHY[1], E. LEIN[3], C. KOCH[1], H. ZENG[4];
[2]Cell and Circuit Genet., [3]Human Cell Types, [4]Structured Sci., [1]Allen Inst. for Brain Sci., Seattle, WA

Abstract: There is a major effort in the field of neuroscience to understand and describe the diversity of cell types in the brain. Recently, single cell transcriptomics has massively accelerated this effort, and transformed the way that we approach cell type classification. Instead of relying on cellular properties such as morphology, physiology and/or function to define cell types, transcriptomic studies rely largely on gene expression pattern. Much less work has been

done to understand the relationship between gene-defined cell types and these other important cellular properties.
The 'Patch-Seq' method is a modified patch clamp protocol that was developed to directly access gene expression, morphology, and electrophysiology from the same cell. We used this method on acute mouse brain slices to perform a systematic, multiple modality characterization of hundreds of inhibitory neurons across multiple molecular subclasses (e.g., Sst, Pvalb and Vip) in the adult primary visual cortex. Using transcriptomic data, we mapped each cell to a previously described transcriptome-based cell type taxonomy (Tasic et al., 2018), and reconstructed a representative set of neurons from multiple inhibitory transcriptomic types. From these data, we derived a quantitative morphological signature for each transcriptomic type. We also performed clustering analyses based on morphology alone, and described the relationship between morphological types and transcriptomic types. These analyses revealed a complex relationship between morphological properties and transcriptomic cell types. For some transcriptomic types, there is a clear consistency with morphological types, particularly within a layer. For other transcriptomic types, the morphology varies in terms of features such as axon extent, while other features, such as axon distribution around the soma are consistent within a type. To gain a better understanding of the genes that underlie morphological differences within an inhibitory subclass, we also investigated how gene expression differs as a function of anatomical location, laminar branch distribution and morphology. This work provides the basis for understanding the relationship between morphological and transcriptomic properties of cortical neurons.

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Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.08/P12

Topic: D.07. Vision

Title: Electro-transcriptional diversity of interneurons in the mouse visual cortex revealed through standardized patch-seq data collection

Authors: *A. BUDZILLO[1], F. BAFTIZADEH[1], J. BERG[1], A. BUCHIN[1], O. FONG[1], J. GOLDY[1], N. W. GOUWENS[1], B. E. KALMBACH[2], T. K. KIM[3], B. R. LEE[1], J. MILLER[1], S. MOK[1], K. SMITH[1], B. TASIC[4], Z. YAO[1], E. LEIN[2], J. T. TING[2], S. A. SORENSEN[1], G. J. MURPHY[1], H. ZENG[5];

[2]Human Cell Types, [3]R & D, [4]Cell and Circuit Genet., [5]Structured Sci., [1]Allen Inst. for Brain Sci., Seattle, WA

Abstract: A critical component of the mouse neocortex is its rich variety of inhibitory interneuron populations, with well-described differences in transcriptomic, electrical, and morphological properties. Understanding how these properties relate within and across these subsets of neurons can provide the foundation for elucidating their ultimate function.
We provide a global approach to addressing how well electrical and transcriptional signatures of inhibitory interneurons correspond via standardized pipeline generation of 'Patch-Seq' data from thousands of neurons from the mouse visual cortex. The electrophysiological properties of single cells are derived from automated feature extraction of recordings collected with the whole cell patch clamp technique. At the end of a recording, the cell's nucleus is harvested for transcriptional profiling via RNA-seq; transcriptomic class is assigned by mapping the cell to the Allen Institute's previously defined transcriptomic tree. We see strong correspondence between the electrophysiological and transcriptional profiles in the 4 major classes of interneurons - Pvalb, Sst, Vip, and Lamp5. Within each class we observe cases in which individual transcriptomically defined subsets of cells have distinct and overlapping electrophysiological features. These data are a valuable tool in the interpretation of transcriptomic-based classification schemes.

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Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.09/P13

Topic: D.07. Vision

Title: Correspondences between transcriptomic, electrophysiological, and morphological characteristics of interneurons in mouse visual cortex

Authors: *N. W. GOUWENS, F. BAFTIZADEH, A. BUDZILLO, R. DALLEY, J. MILLER, S. A. SORENSEN, B. TASIC, Z. YAO, A. ARKHIPOV, G. J. MURPHY, C. KOCH, H. ZENG; Allen Inst. for Brain Sci., Seattle, WA

Abstract: Addressing the complexity of neuronal circuits by classifying cellular components into meaningful types is an ongoing challenge. Recent single-cell transcriptomic studies have

provided high-dimensional, high-throughput classifications of neocortical neurons. While the broad subclasses identified by these studies have been shown to be consistent with types defined by electrophysiological and morphological properties, it is unclear if these properties remain consistent at the level of individual transcriptomic types, which would have implications for the roles of those types in cortical circuits. To investigate this, we made whole-cell Patch-Seq recordings from mouse visual cortical interneurons labeled by transgenic lines, primarily from the Sst and Pvalb subclasses. The neurons' intrinsic electrophysiological properties were characterized by a standardized stimulus protocol, and the neurons were filled with biocytin for morphological reconstruction. After recording, the nuclear contents of the cell were extracted to profile the transcriptome by RNA-seq. This data set, with electrophysiological, morphological, and transcriptomic all collected from the same cells, enabled us to assess directly the correspondences between transcriptomic types (or t-types, identified by mapping to a benchmark data set of FACS-collected cells) and their electrophysiological and morphological properties, which were characterized in a low-dimensional feature space. We also compared the t-types to a new set of types identified by unsupervised clustering based on combining transcriptomic data with electrophysiological and morphological features. We found that, in agreement with earlier studies, all three properties were consistent at the subclass level; for example, cells with Pvalb t-types exhibited joint electrophysiological and morphological characteristics that were largely distinct from those of cells with Sst t-types. We also identified additional consistency at the level of individual transcriptomic types; however, the strength of the correspondences varied across types and across interneuron subclasses. Our analyses here inform cell type classification by characterizing patterns of cell-by-cell co-variation in gene expression, local morphology, and electrophysiological properties, as well as by estimating the extent to which intrinsic electrophysiological and morphological properties can be inferred if the transcriptomic type of an interneuron is known.

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Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.10/P14

Topic: D.07. Vision

Support: This work was supported by the generosity of Paul G. Allen

Title: Distribution and strength of interlaminar synaptic connectivity in mouse primary visual cortex revealed by two-photon optogenetic stimulation

Authors: *T. A. HAGE, A. BOSMA-MOODY, C. A. BAKER, M. B. KRATZ, L. CAMPAGNOLA, S. C. SEEMAN, T. JARSKY, G. J. MURPHY;
Allen Inst. for Brain Sci., Seattle, WA

Abstract: An initial step towards understanding how cortical neurons transform and process information is to thoroughly characterize the connectivity within and between classes and types of neurons. Our approach to do so employs targeted 2-photon optogenetic stimulation at cellular resolution to measure connectivity in the primary visual cortex of adult (>P40) mice. To restrict expression of the ChrimsonR opsin to cell classes of interest, we used both a Cre-dependent transgenic effector line (Ai167) and a combinatorial approach where an AAV-FLEX ChrimsonR (soma-targeted) was injected into selected Cre lines. The results described here utilized the excitatory Scnn1a-, Rorb-, Tlx3- and Ntsr1-Cre lines, and the inhibitory Sst-Cre line. We photostimulated putative presynaptic neurons by rapid galvo-based-spiral scanning of the soma. We measured synaptic inputs via simultaneous whole cell patch clamp of 1-4 postsynaptic cells. Initial data collection focused on L2/3 pyramidal neurons as a postsynaptic class. We characterized canonical feed-forward inputs from L4 excitatory neurons and feedback inputs from L5 and L6. Additionally, we explored within- and between- layer inhibitory connections from somatostatin neurons onto L2/3 pyramidal neurons. Across this study, we identified more than 250 connections from over 4500 probed. The highest rates of connectivity onto L2/3 pyramidal neurons were observed from L4 excitatory neurons and local Sst-neurons within L2/3. Furthermore, we found infrequent feedback connections from excitatory and Sst-neurons in L5 onto L2/3 pyramidal neurons, notably, with PSP amplitudes that were often larger than the more canonical feed-forward inputs. By combining simultaneous patch clamp recordings with photostimulation of dozens of individual putative presynaptic cells, we have measured >25 instances of divergence of a single presynaptic neuron to 2-4 postsynaptic cells. Similarly, we have observed >50 examples of convergence of 2-12 presynaptic neurons onto individual postsynaptic neurons. Future analyses will describe heterogeneity in the strength of afferent and efferent connections of single cells as well as variability in the spatial profiles of connection probability and strength measured within and between cell classes.

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Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.11/P15

Topic: D.07. Vision

Title: Extraction of distinct cell types from within a genetically continuous population

Authors: *E. J. KIM[1,2], Z. ZHANG[1], L. HUANG[1], T. ITO[1], M. W. JACOBS[1,2], A. L. JUAVINETT[1,3], G. SENTURK[1,3], M. HU[1], M. KU[1], J. R. NERY[1], J. R. ECKER[1,4], E. M. CALLAWAY[1];
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Abstract: The brain is composed of millions of diverse neurons that differ systematically in the genes that they express. Analyses of single cell gene expression data reveal clusters which correspond to different cell types that differ in their connectivity and function. Can neurons from within a single genetic cluster be further extracted based on connectional properties, and if so do they differ systematically in the genes that they express? For example, most recent studies of single cell transcriptome analyses suggest only two or three distinct genetic cell type clusters in layer 2/3 (L2/3) pyramidal neurons of mouse primary visual cortex (V1). In contrast, L2/3 neurons in V1 are known to project more than 10 different higher cortical areas, resulting in far more than two or three cell types with distinct connectivity. Here we address these questions by combining retrograde labeling, single cell gene expression, and rabies-based analyses of connectivity to assess cortical-cortical projection neurons in the mouse V1 projecting to higher visual areas called AL (anterolateral) or PM (posteromedial). We find that pyramidal neurons projecting to different cortical targets, AL or PM, and with known functional differences differ systematically in their gene expression despite forming only a single genetic cluster with continuous variability. In addition, these two different neuronal types receive preferential feedback inputs from the higher visual areas they project to, suggesting like-to-like circuit organization as feedback to feedforward connectivity mode. These observations demonstrate that single cell gene expression analysis in isolation is insufficient to distinguish all neuron types.

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Poster

403. Visual Cortex: Circuits

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Support: EMBO
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Title: Inter-areal coordination and dendritic integration in visual cortex

Authors: *D. HERRMANN, M. FISEK, A. EGEA-WEISS, M. CLOVES, M. HAUSSER; UCL, London, United Kingdom

Abstract: A striking feature of cortex is the morphologically elaborate and electrically excitable dendritic arbors of pyramidal neurons. Dendrites can sample inputs selectively and act as functional subunits, providing a powerful substrate for computation. However, principles governing the relationship between dendritic recruitment and information flow through long-range cortical networks remain unclear. To examine this relationship, we performed *in vivo* two-photon imaging experiments in two visual cortical areas in the mouse: primary visual cortex (V1) and the higher visual area LM. We investigated how dendritic recruitment and cortical coordination depend on sensory stimulation and behavioral state in awake head-fixed mice running spontaneously on wheels without reward, focusing specifically on cortico-cortically projecting layer 5 neurons. We measured dendritic activation patterns using simultaneous dual-color volume imaging in apical and basal dendritic domains by co-expressing the green neurotransmitter indicators SF-iGluSnFR for glutamate or iGABA-SnFR for GABA in combination with the red calcium indicator jRGECO1a. We examined stimulus and locomotion-evoked changes in input (glutamate or GABA) and output (calcium) signals recorded across large fields of view, providing cell-type specific population level input-output relationships. Next, we measured somatic and dendritic calcium signals in individual neurons. At both the population level and in individual neurons we find that dendritic recruitment depends on visual stimulation and locomotor state and predicts increased functional coupling between cortical areas during locomotion. To directly test this prediction, we used patterned two-photon optogenetics to activate small populations in V1 or LM while monitoring responses in the recipient area. We find feedforward and feedback influences are differentially organized topographically and differentially modulated by behavioral state. Separately, we measured correlated variability in activity across cortical areas to relate dendritic recruitment and functional connectivity to correlations in large cortical populations. In summary, our results suggest that there exists an intimate relationship between dendritic processing and cortico-cortical coordination.

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Poster

403. Visual Cortex: Circuits

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Title: The feedback receptive field of neurons in the mammalian primary visual cortex

Authors: *A. KELLER[1], M. M. ROTH[1], M. SCANZIANI[2];
[1]UCSF, San Francisco, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: We sense our environment through a chain of connections linking the sensory organs to neurons in the brain. In the visual system, this feedforward pathway defines the classical receptive field (cRF), the visual space where a stimulus excites the neuron. The response of neurons to stimuli inside the cRF can be inhibited by stimuli outside the cRF when the stimuli inside and outside share similar features. This inhibition implements an efficient coding strategy through which neurons compare the inside with the outside of the cRF to only report differences. Here we show that in mouse primary visual cortex those same inhibitory outside stimuli become strongly excitatory when the stimulus inside the cRF is absent. Excitation by outside stimuli is slower and delayed compared to excitation by inside stimuli and is preferentially reduced by anesthesia or by silencing higher visual areas, indicating that it originates from feedback projections. Furthermore, neurons excited by outside stimuli are selectively located in cortical layers receiving feedback projections. By responding to stimuli inside or outside the cRF but not to both together, these neurons compute an exclusive-or function (XOR). Thus, we have identified an excitatory feedback receptive field that, by complementing the feedforward cRF, enables neurons to capture differences between stimulus features across visual space independently if the excitatory stimulus is inside or outside the cRF. This generalization may contribute to efficient coding strategies like predictive processing.

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Poster

403. Visual Cortex: Circuits

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Topic: D.07. Vision

Support: Howard Hughes Medical Institute
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Title: Eye movement information in V1 is carried through feedback projections

Authors: *S. K. MIURA[1], M. SCANZIANI[2];
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Abstract: Saccades have a profound impact on vision, both perceptually and physiologically. Using mouse primary visual cortex (V1) as a model system, we have begun to dissect the circuit that carries saccade information to cortex.
Similar to previous reports in cats and monkeys, we find that saccades modulate activity in mouse V1. This activity change is caused by input of extra-retinal origin that persists even under suppression of all neuronal activity in the eyes. Further, we show that it contains information about the direction of eye movement. Indeed, saccade direction can be predicted based on V1 activity, even before the onset of saccades.
In order to determine the source of the saccade information in V1, we took a candidate approach in which we silenced different brain regions while recording saccade-related activity in V1. Our first candidate was the dorsal lateral geniculate nucleus (dLGN) in thalamus, as the dLGN activity is also modulated by saccades. However, silencing the dLGN did not eliminate the modulation in V1. In contrast, silencing V1 eliminated saccade-related activity in the dLGN, suggesting that V1 is the source of saccade-information input in the dLGN and not vice-versa.
Upon further exploration, we identified the latero-posterior (LP) nucleus in the thalamus as the source of saccade information in V1. Consistent with studies in monkeys, neurons in mouse LP are also modulated by saccades. When LP was silenced pharmacologically, V1 was no longer modulated in response to saccades.
From our study, we suggest that saccade information flows from LP through V1 to the dLGN through the feedback pathway. The identification of the source of saccade information in V1 now enables us to explore the interaction of saccade information with visual information, and how this affects perception of the animals.

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Poster

403. Visual Cortex: Circuits

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.15/P19

Topic: D.07. Vision

Support: NIH Grant NS097287

Title: Behavioral state and movement strongly modulate mesoscale pan-cortical neural activity

Authors: *E. D. VICKERS, S. JO, E. MCCARTHY, D. A. MCCORMICK;
Inst. of Neurosci., Univ. of Oregon, Eugene, OR

Abstract: Recent groundbreaking work has shown that nearly one-third of widespread brain activity during both spontaneous and task-driven behavioral epochs can be predicted by taking into account fluctuations of an assortment of externally observable arousal state measures such as walking, pupil diameter, whisker pad activity, and other small body movements. However, it is not clear that such a relationship exists for all brain areas to the same degree, or whether correlations between activity across widespread cortical subnetworks involved in sensory processing and decision making are also dependent on behavioral state. In order to address these outstanding questions, we developed in-vivo preparations in the mouse that allow us to perform both 1-photon widefield and 2-photon mesoscale calcium imaging, at different times in a single mouse, of either the entire dorsal cortical surface, including bilateral visual, somatosensory, motor, and premotor areas (upright), or a similarly large area of tempero-parietal cortex that also contains auditory cortical areas (left-rotated). Thus, we are able to simultaneously record the activity of large numbers of individual neurons across the entire cortical surface at up to 5 Hz (2-photon), and then, in the same mouse, to examine spatially down-sampled activity at up to 50 Hz (widefield). We examined behavioral state and movement-dependent patterns of widespread activity in large, overlapping subsets of excitatory neurons, as well as in parvalbumin- (PV), somatostatin- (SOM), and vasoactive intestinal peptide- (VIP) containing cortical interneurons. We focused either on differences in the state-dependence of spontaneous activity in bilateral visual, somatosensory, and premotor cortices (M2) in our upright preparation, or in unilateral auditory, somatosensory, visual, and premotor in our left-rotated preparation. Preliminary findings suggest that the bulk activity of both excitatory and inhibitory neurons across many brain areas correlates robustly to movement, pupil diameter fluctuations, and bilateral whisker motion energy. Despite these broad trends, we also observe considerable heterogeneity in degree of state and movement dependence, and in overall sparsity of activity, both across neighboring individual neurons and distant brain regions. Finally, we are developing a go / no-go audiovisual detection task that will allow us to examine changes in subnetwork activity and functional connectivity (both spontaneous and task-related) at both the widefield and single neuron level during task acquisition and learning in mice that are trained on auditory vs. visual target versions of the task.

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Poster

403. Visual Cortex: Circuits

Location: Hall A

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Program #/Poster #: 403.16/P20

Topic: D.07. Vision

Support: CIHR

Title: Orientation selectivity to synthetic natural patterns in a cortical-like model of the cat primary visual cortex

Authors: *H. LADRET[1], N. CORTES[2], L. PERRINET[3], C. F. CASANOVA[4];
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Abstract: A key property of the neurons in the primary visual cortex (V1) is their selectivity to oriented stimuli in the visual field. Orientation selectivity allows the segmentation of objects in natural visual scenes, which is the first step in building integrative representations from retinal inputs. As such, V1 has always been of central interest in creating artificial neural networks and the recent years have seen a growing interest in the creation of explainable yet robust and adaptive models of cortical visual processes, for fundamental or applied purposes. One notable challenge for those models is to behave reliably in generic natural environments, where information is usually hidden in noise, while most models are typically studied with oriented gratings. Here we show that a simple biologically inspired neural network accounts for orientation selectivity to natural-like textures in the cat's primary visual cortex. Our spiking neural network (SNN) is made of point neurons organized in recurrent and hierarchical layers based on the structure of cortical layers IV and II/III. We found that Spike-timing plasticity and synaptic recurrence allowed the SNN to self-organize its connections weights and reproduce the activity of neurons recorded with laminar probes in cortical areas 17 and 18 of cats, notably orientation tuning responses. After less than 5 seconds of stimulus presentation, the SNN displays narrow orientation selectivity (bandwidth = 10°) characteristic of sparse representations, removes noise from the input and learns the structure of natural pattern repetitions. Our results support the use of natural stimuli to study theoretical and experimental cortical dynamics. Furthermore, this model encourages using SNNs to reduce complexity in cortical networks as a method to understand the separate contribution of different components in the laminar organization of the cortex. From an applied perspective, the computations this network performs could also be used as an alternative to classical blackbox Deep Learning models used in artificial vision.

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Poster

403. Visual Cortex: Circuits

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Topic: D.07. Vision

Support: Sloan Foundation Summer Fellowship (PMB)

NIH R01 EY027023 (WB)

Title: Modeling the influence of surround suppression on size and contrast tuning in V1 and MT

Authors: *P. M. BAKER, W. BAIR;
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Abstract: The responses of many V1 neurons are suppressed by stimuli that extend beyond their classical receptive fields (RFs). This suppression has been proposed to contribute to pattern motion computation in MT neurons (Rust et al., 2006, Nat Neurosci; Majaj et al., 2007, J Neurosci; Tsui et al., 2010, J Neurophysiol). However these studies have not described how including iso-orientation surround suppression (IOSS) in V1 inputs shapes basic tuning for size and contrast in the recipient MT neurons. We have developed an image-computable modeling framework to build pattern-selective MT units (Baker and Bair, 2016, J Neurosci), and we extended this framework to include physiologically plausible V1 IOSS. We implemented commonly proposed phenomenological models of V1 IOSS that fit the range of suppression strengths found in macaque V1 (Sceniak et al., 2001, J Neurophysiol; Cavanaugh et al., 2002, J Neurosci). Our model MT units sum V1 inputs with RFs that are distributed across visual space to produce MT RFs ~5x the size of V1 RFs. We then tested tuning for stimulus size and contrast for both V1 and MT model units as we varied the strength of V1-level IOSS. We found that when IOSS was implemented as a V1 divisive normalization term, contrast response in both V1 and MT model units depended on stimulus size: the semi-saturation contrast (c50) obtained from fits to response vs. contrast curves decreased as size increased, both within and beyond the classical RF. We made a novel analysis of previously published data from 47 macaque V1 complex cells (Cavanaugh et al., 2002, J Neurosci) that supports this relationship between c50 and size. Our model shows that this effect may also contribute to a similar dependence of c50 on size that was previously described in macaque MT neurons (Sclar et al.,1990, Vision Res). As a consequence of this link between stimulus size and contrast tuning, the divisive form of V1 IOSS also offers a novel explanation for differences in c50 between V1 and MT, which in our model arises as a direct consequence of differences between V1 and MT units in spatial RF extent. We also found that a divisive form of V1 IOSS increased pattern index values obtained from responses to plaid stimuli in both component and pattern MT model units. Thus, V1 IOSS can be a physiologically plausible stand-in for the direction-tuned normalization that has been suggested to contribute to pattern sensitivity in MT. Beyond the dorsal pathway, this work also has broader significance in demonstrating how multiple normalization stages in filter-based, cascaded LN models may be instrumental for shaping response selectivities at higher levels of the circuit. We thank JA Movshon and J Cavanaugh for sharing data.

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Poster

403. Visual Cortex: Circuits

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Title: Top-down modulations of bottom-up signal processing in a microcircuit model involving PV, SOM and VIP inhibitory interneurons

Authors: *N. WAGATSUMA[1,2], S. NOBUKAWA[3], T. FUKAI[4,2];
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Abstract: Various types of inhibitory neurons contact each other for organizing a cortical network (Kawaguchi and Kubota, Cereb. Cortex, 1997; Markram et al., Nat. Rev. Neurosci., 2008). A majority of cortical inhibitory neurons expresses one of three genes, parvalbumin (PV), somatostatin (SOM) and vasoactive intestinal polypeptide (VIP) (Ruddy et al., Dev. Neurobiol., 2011), which play critical roles for regulating the activities of neuronal network. However, the detailed neuronal microcircuit consisting of these inhibitory neuron types and excitatory pyramidal (Pyr) neurons for processing external inputs such as feedforward sensory and feedback attentional signals remains largely unknown. Here, we developed a computational microcircuit model with biologically plausible structure and network of cortical visual layers 2 and 3 (Thomson et al., 2002; Binzegeer et al., 2004; Pfeffer et al., 2013). In our model, cooperation of Pyr neurons and three types of inhibitory interneurons determine the network activities. PV-positive inhibitory neurons are the largest inhibitory population in our model and mainly inhibited Pyr neurons and themselves. SOM-positive inhibitory neurons transmitted their signals to all other neuronal populations. VIP-positive inhibitory neurons suppressed the responses of SOM neurons and were activated by feedback signals. In addition, Pyr neurons were recurrently connected with a long-tailed, lognormal, distribution in their synaptic weights (Teramea et al., Sci. Rep., 2012). For testing the model responses, we carried out simulations of the model with various feedforward inputs representing the visual stimuli and feedback signals mediating selective attention. Interestingly, modulatory feedback signals to VIP neuronal populations more effectively activated our network via disinhibition of the Pyr neuron population compared to driving feedback inputs. Our proposed model might provide insights into the roles of inhibitory neuron types for regulating the activities of cortical microcircuit.

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Poster

403. Visual Cortex: Circuits

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Title: Characterizing optogenetic rebound effects to parvalbumin expressing interneuron photostimulation in mouse primary visual cortex

Authors: J. T. SHAPIRO, J. L. KING, ***N. A. CROWDER**;
Psychology and Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: Mouse primary visual cortex (V1) has served as a model brain region for studying cortical circuitry due to the many genetic tools available in this species. Specifically, optogenetic tools have been used to map local GABAergic interneuron circuits and investigate unique roles for specific cell subtypes. Most of this past work has focused on how neural activity or behavior is modified when the contribution of specific cell types within local V1 circuits are artificially increased or decreased, but little attention has been paid to potential rebound effects that may be produced when photostimulation is terminated and inhibition and excitation in the circuit rebalance. To explore these potential optogenetic rebound effects we paired optogenetic activation of parvalbumin-expressing interneurons (PV+) with several visual stimulus types while collecting *in vivo* electrophysiological recordings from putative pyramidal (Pyr) and PV+ cells during and after PV+ photostimulation. Preliminary analyses indicate that when photostimulation was paired with flashed stimuli nonspecific rebound effects were produced, including following bars flashed inside or outside of the classical receptive field or sinusoidal gratings of preferred or non-preferred orientations. Generally, Pyr neurons that produced after-responses to flashed stimuli had larger rebound effects. Optogenetic rebound effects following drifting gratings tended to have smaller amplitudes. When photostimulation was terminated prior to the visual stimulus disappearing, Pyr cells showed prolonged suppression followed by delayed low amplitude excitatory rebounds. Increasing understanding of how and why optogenetic rebound effects occur may provide insights into how excitation and inhibition are balanced in cortex. Furthermore, this understanding may aid in the design or interpretation of behavioral studies that use optogenetic modulation because behavior may be altered not only from optogenetic stimulation itself, but from the optogenetic rebound as well.

Disclosures: **J.T. Shapiro:** None. **J.L. King:** None. **N.A. Crowder:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.20/P24

Topic: D.07. Vision

Support: 2018-2019 UAB Faculty Development Grant program (FDGP)
NSF Grant Award #1539034 RII Track-2 FEC: Bridging Cognitive Science and Neuroscience Using Innovative Imaging Technologies

Title: Inhibitory neuronal responses in the primary visual cortex of the aging mouse

Authors: *C. V. DIENI[1], M. SAVAGE[1], L. SINCICH[2], P. GAMLIN[1];
[1]Ophthalmology and Visual Sci., [2]Optometry & Vision Sci., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Deteriorating vision is a common condition associated with aging. With age, the central visual pathways and the retina both manifest anatomical and physiological changes that impair function. Overall neuronal activity in the central visual areas is reduced due to neuronal degeneration, which includes synaptic and dendritic spine loss, accompanied by a reduction in dendritic and axonal size. How age affects the relationship between the excitatory and inhibitory populations in the visual cortex has not been clarified. Since the synchronicity and cooperative activity of excitation and inhibition in cortical circuits is important for processing information, we have investigated whether aging alters the GABAergic inhibitory circuitry in healthy mice. We hypothesize that different classes of inhibitory neurons play distinct functional roles in the dynamic regulation of primary visual cortex (V1) over the life span. Here we focus on cells expressing parvalbumin (PV), which account for ~35% of inhibitory neurons in mouse neocortex and are densely connected to neighboring excitatory pyramidal neurons.To study inhibitory neuronal populations in V1 we used a combination of *in vitro* electrophysiological recordings combined with *in vivo* two-photon (2P) imaging using using a PVCre specific calcium indicator (jRCamp1b), comparing neural activity between young and old mice, comparing neural activity between young and old mice. We used *in vitro* cell-attached patch recordings in V1 cortical slices from transgenic reporter mice expressing PVCre Channelrhodopsin-2 (PVCre CHR2) to study the role of PV interneurons on V1 local circuitry. We found that spontaneous inhibitory currents (IPSCs) in pyramidal neurons in layer 2/3 were larger in magnitude and more numerous in aged mice. When we light-activated the PVCre CHR2 interneurons and measured the evoked IPSCs in pyramidal neurons we saw a reduction in current amplitude in older mice, but this was not significant compared to younger mice in the small sample tested to date. To examine if PV neural activity was altered *in vivo*, we recorded calcium transients with 2P imaging while the animals were visually stimulated with sinusoidal gratings of various orientations at a spatial

frequency of 0.05 cycle per degree. Preliminary data suggest that PV interneurons in layer 2/3 of young cortex exhibit little orientation tuning, whereas older mice had many neurons that appeared to be well tuned for stimulus orientation. These results suggest that the role of inhibitory circuitry in V1 changes with age and that PV interneurons may alter orientation tuning profiles in aged mouse cortex.

Disclosures: C.V. Dieni: None. **M. Savage:** None. **L. Sincich:** None. **P. Gamlin:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.21/P25

Topic: D.07. Vision

Support: NIH NINDS R01NS107968

Title: Probing the role of cortical inhibitory neurons during visual spatial perception

Authors: *J. DEL ROSARIO[1,2], A. OTSUKI[1], B. WILLIAMS[1], L. BLANPAIN[2], B. HAIDER[1,2];
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Abstract: Many studies of visual processing have established that inhibitory neurons shape the representation of visual information, particularly in primary visual cortex (V1). The use of transgenic mice has allowed detailed investigation of specific interneuron subtypes, and their roles in visual responses. Several studies show that inhibitory subtypes play distinct roles in spatial integration of visual stimuli (Adesnik et al., 2012; Dipoppa et al., 2018). However, there are few studies of the role of inhibitory subtypes on visual spatial perception. It is unclear if parvalbumin-expressing (PV+) and somatostatin expressing (SST+) inhibitory interneurons in V1 play overlapping or distinct roles for spatial perception of stimuli during behavior.
To investigate this question, we developed a behavioral task where head-fixed mice detected visual stimuli (Gabor gratings) localized in one of two discrete portions of visual space (binocular or monocular visual fields, ~70° apart; Speed et al., 2019). Mice indicated detection of visual stimuli by licking for water rewards. Mice were stationary, minimizing potential interactions of perception and locomotion. We trained mice expressing Channelrhodopsin-2 in PV+ or SST+ interneurons, and stimulated these interneurons in monocular or binocular V1 during stimulus presentation. Stimulation of either PV+ or SST+ neurons in localized regions of V1 significantly impaired perceptual report of small, low contrast stimuli that appeared in the retinotopically matched portion of the visual field (SST+: 84% impairment, 4 mice; PV+: 86% impairment, 3 mice). Perceptual impairments varied with light intensity, stimulus contrast, and stimulus location. Surprisingly, SST+ activation caused greater impairments than PV+ activation

for perceptual report of stimuli in retinotopically distant regions of visual space (>40° away from the site of stimulation).
We next investigated neural circuit activity by performing high-density silicon probe recordings during optogenetic stimulation in awake mice, and isolated spiking activity of SST+ (n=6), PV+ (n=31), and regular-spiking (RS) putative excitatory (n=83) neurons. Stimulation of either SST+ or PV+ interneurons rapidly inhibited RS neuron firing (50% suppression within 4 ms, n=77). Furthermore, SST+ neurons responded more (26% more than PV+ and 71% more than RS neurons) to visual stimuli in distal regions of the visual field (>30° away from the center of the receptive field). These results suggest that the broad spatial responsiveness of SST+ inhibitory interneurons may directly shape the perception of visual stimuli appearing across large regions of visual space.

Disclosures: J. Del Rosario: None. **A. Otsuki:** None. **B. Williams:** None. **L. Blanpain:** None. **B. Haider:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.22/P26

Topic: D.07. Vision

Support: NRF-2016R1C1B2016039

Title: Long-range cortical connections for circuit optimization of hierarchical visual cortex

Authors: *S. BAEK[1], Y. PARK[1], W. CHOI[1,2], S.-B. PAIK[1,2];
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Abstract: Deep neural networks (DNN), inspired by the feedforward hierarchical structure of the brain, can perform image classification with human-level accuracy (He et al., 2015). However, DNNs with a number of layers as few as in the ventral stream of the human visual cortex (Krizhevsky et al 2012) cannot achieve human-level performance. Given this, what is the strategy of the brain to construct a network that performs more efficient visual information processing than DNNs can achieve? Here, we suggest that the long-range horizontal connectivity (LRC) observed in the early visual cortex of mammals (Bosking et al., 1997) might be a key factor for reducing structural costs in the visual system. We hypothesized that the LRC might reduce the average path length of a network so that visual perception is achieved with less wiring cost compared with feedforward (FF) networks. To validate our hypothesis, we designed a three-layer neural network model with convergent FF projections. Then, we designed a labeled-image set of various local- and global-correlations in the spatial organization. We compared the

classification performance of networks when LRCs were added to the model. We found that adding LRCs improved performance significantly more than just adding FFs with the same wiring cost. In addition, the enhanced performance with LRCs accompanied a noticeable reduction in the characteristic path length of the network. To confirm that the contribution of LRCs is selective for the global-correlation feature of images, we performed a pruning method that deleted unimportant connections for classification during training sessions. The remaining number of LRCs optimized for the images with global correlation was significantly larger than that optimized for images with local correlation only. Overall, our results suggest that LRCs can reduce the path length of the neural network and thereby enable more efficient visual information processing.

Disclosures: **S. Baek:** None. **Y. Park:** None. **S. Paik:** None. **W. Choi:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.23/P27

Topic: D.07. Vision

Support: National Eye Institute Grant EY02675
Princeton Neuroscience Institute Innovation Fund

Title: A long timescale stimulus history effect in the primary visual cortex

Authors: *H. KIM, J. HOMANN, D. W. TANK, M. J. BERRY, II;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Responses of neurons in the primary visual cortex (V1) are often understood as encoding the current visual stimulus. Yet, some studies indicate that temporal contingency effects exist in the responses of neurons in early sensory areas. We explored if the recent stimulus history would alter the response of V1 layer 2/3 pyramidal cells. We used two-photon calcium imaging in head-fixed awake mice during presentation of sequences of complex images. The activity of individual neurons was sparse, such that either one or none of the images in the sequence typically yielded a strong response. We then substituted an image preceding this primary image in order to determine if responses to the primary image were effected. We found that the amplitude of the neuron's response could be significantly altered by substitutions up to five images back from the primary image, even when the substituted image elicited virtually no response by itself. This stimulus history effect was heterogeneous across the population, with some cells showing facilitation and others suppression. For individual cells, the history effect was robust and reproducible across days. Our data show that responses of V1 neurons not only reflect the current stimulus but also encode, through their response amplitude, information about

multiple images previously presented as far as 1000 msec in the past. This might enable V1 to retain information about the extended trajectory of past stimuli and perform complex temporal computations that are as of yet not appreciated.

Disclosures: H. Kim: None. **J. Homann:** None. **D.W. Tank:** None. **M.J. Berry:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

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Topic: D.07. Vision

Support: NIH Grant 1R01MH116500
Whitehall foundation grant

Title: Cellular mechanism of visually evoked theta oscillation in mouse primary visual cortex

Authors: *M. GAO, A. A. CHUBYKIN;
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Abstract: The theta oscillation (4-8 Hz) of neural networks is a common phenomenon found throughout the mammalian central nervous system. It plays a critical role in information processing, communication of brain areas, learning and memory. Neuronal disorders, including autism spectrum disorder (ASD), Schizophrenia and Parkinson's disease among others, are often accompanied by abnormalities in theta oscillations. Understanding the mechanism of theta oscillation will provide us with insight into the computations in the brain and may help develop treatments for the related neuronal disorders. However, the physiological functions of theta oscillation in neocortex and the underlying mechanism remain unclear. Our group has recently discovered that familiar visual stimuli can robustly induce theta oscillations in the primary visual cortex (V1) of visually trained mice. To gain more mechanistic insight into this phenomenon, we utilized in vivo patch clamp recordings to dissect the activity of individual neurons during these oscillations. We recorded visual tuning of V1 neurons in untrained and trained mice to assess the effect of theta oscillation on the orientation (OS) and direction selectivity (DS). Using optogenetics, we measured the synaptic strength of specific projections in untrained and trained mice. Light-evoked excitatory postsynaptic currents (EPSCs) showed a significant increase in the strength of L5 projections to other layers of V1 in the trained mice. We have also discovered a decrease in the strength of the thalamocortical synapses following training. We found 4-8 Hz oscillations of membrane potential (Vm) and bursts of firing were evoked in single neurons in response to the familiar stimulus. Interestingly, the firing rate of visual responses was reduced after training. Although the firing rate of visual responses to all directions and orientations of sinusoidal drifting gratings was reduced, the direction selectivity index (DSI) was increased. This

increase was correlated with Vm theta power of the null direction. The results suggest that the theta oscillation can improve direction selectivity by modulating the stimulus-triggered spiking activity.

Disclosures: M. Gao: None. **A.A. Chubykin:** None.

Poster

403. Visual Cortex: Circuits

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.25/P29

Topic: D.07. Vision

Support: NIH Grant 5R01MH116500-02

Title: Visual experience-dependent oscillations and underlying circuit connectivity changes are impaired in V1 of fragile X mice

Authors: *S. T. KISSINGER[1], Q. WU[1], C. J. QUINN[2], A. A. CHUBYKIN[1];
[1]Dept. of Biol. Sci., [2]Dept. of Industrial Engin., Purdue Univ., West Lafayette, IN

Abstract: Fragile X syndrome (FXS) is the most common inherited form of autism. Several neurophysiological impairments have been identified in individual neurons of the mouse model of FXS (*Fmr1* KO) thus far, including enhanced hippocampal long-term depression (LTD), increased excitatory to inhibitory (E/I) ratios, and impaired functional connectivity. However, little is known about how experience dependent changes in neural activity are disrupted in FXS at the scale of circuits or neural ensembles. Previously, we demonstrated that perceptual training to visual stimuli over several days promotes the emergence of visually evoked low-frequency oscillations in the primary visual cortex (V1) of awake mice, reflecting familiarity with a visual stimulus. We used this simple paradigm to assess perceptual learning in *Fmr1* KO mice with a multifaceted approach; including extracellular recordings across all layers of V1, computational analysis of functional connectivity (directed information) between layers, and channelrhodopsin-2 assisted circuit mapping (CRACM) in acute brain slices. Extracellular recordings revealed that the visually evoked oscillations are shorter in duration and lower in frequency in *Fmr1* KO mice (particularly in layers 2/3 and 4), reflecting differences in the intrinsic properties of the neurons driving these oscillations. Using directed information analysis, we found that perceptual training causes a net increase in information flow from cortical layers 2/3, 4 and 5 onto layer 4 fast spiking cells, connections which are attenuated in *Fmr1* KO mice compared to WT controls. Whole cell patch-clamp recordings using CRACM revealed that perceptual training significantly increased the synaptic strength of L5 pyramidal onto L4 fast spiking neurons, connections which are also significantly weaker in *Fmr1* KO mice compared to WT controls. This study has achieved two primary goals. First, we have demonstrated increases in the synaptic strength and

functional connectivity of specific connections that likely drive the experience dependent oscillations in V1. Second, we discovered that the oscillations are impaired in *Fmr1* KO mice, granting us insights into how an experienced sensory stimulus is differentially encoded in autism.

Disclosures: S.T. Kissinger: None. **Q. Wu:** None. **C.J. Quinn:** None. **A.A. Chubykin:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.26/P30

Topic: D.07. Vision

Support: Rutgers University – Newark Chancellor's Seed Grant

Title: Neuropilin-2/plexin-A3 receptors contributes to the proper wiring and function of the mouse primary visual cortex

Authors: *H. KHDOUR[1], T. S. TRAN[2], P.-O. POLACK[3];
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Abstract: Information processing in the mammalian neocortex is supported by the precise connectivity in the neural circuits underpinning the function. The establishment of the cortical circuits during development is under the control of multiple signaling systems. Several studies have shown the importance of the secreted Semaphorin 3F (Sema3F) in the establishment of cortical connectivity. Previously, we demonstrated that Sema3F signaling through its obligate receptor Neuropilin-2 (Nrp2) and signal transducing co-receptor Plexin A3 (PlxnA3) regulates spine number and morphologies on the proximal shaft of apical dendrites in layer 5 (L5) pyramidal neurons from the somatosensory cortex (S1). Another study showed Sema3F null mice exhibited increased spine density of layer 4 (L4) cortical neurons in primary visual cortex (V1). The identity of the inputs on these supernumerary spines in S1 and V1 are not known. Anatomical tracing showed that in the absence of the Sema3F signaling during development thalamocortical axons from the somatosensory and motor thalamic relay nuclei misproject their axons to the visual cortex at P7. However, since these previous studies were performed during developmental stages, it is not known whether the aberrant thalamocortical wiring still persists in the adult brain and contributes to the supernumerary spines found in L4 and L5 cortical neurons. Our hypothesis is that Sema3F signaling plays a key role in the maturation of a functional network in V1 and S1 by pruning ectopic thalamocortical synapses. To test this hypothesis, we conducted a survey for spine density in V1, S1, M1 and mPFC in L2/3, L4 and L5 using the Golgi staining method. We are currently performing retrograde tracing in adult and P14 Nrp2-/-

and PlxnA3-/- mice to identify the inputs on the supernumerary spines of L4 and L5 V1 neuron. We found that: [1] Npn2-/- and PlxnA3-/- brain sections exhibit supernumerary synapses in V1 L5 and L4; and [2] the ectopic projections from non-visual thalamic nuclei found at P7 are corrected in the adult Npn2 and PlxnA3 null mice. To assess the functionality of the cortical networks in V1, we are testing visual processing in the different V1 layers of wild type and Npn2 KO mice using calcium imaging. We find deficits in the maturation of the computational properties of L4 and L5, but not L2/3, neurons. Altogether, our results provide novel insights to previously unappreciated functions of Sema3F signaling in the refinement of the thalamocortical connectivity of V1, and it's role in the functional maturation of V1 circuit.

Disclosures: **H. Khdour:** A. Employment/Salary (full or part-time):; RUTGERS UNIVERSITY. **T.S. Tran:** None. **P. Polack:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

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Topic: D.07. Vision

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NIH Grant K99 EY029374
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Research to Prevent Blindness

Title: Neural mechanisms for modulation of cortical variability

Authors: *L. NURMINEN[1], A. ANGELUCCI[2];
[1]Univ. of Utah, Salt Lake City, UT; [2]Ophthalmol, Moran Eye Inst., Univ. of Utah, Salt Lake Cty, UT

Abstract: Our goal was to provide a better understanding of the mechanisms underlying modulation of cortical variability. To achieve this, we recorded responses of single- and multiunits in primary visual cortex (V1) of sufentanil anesthetized marmoset monkeys, while optogenetically manipulating ArchT expressing axon terminals of V2-to-V1 feedback neurons. Injections of Cre-dependent and Cre-expressing AAV9 were made within 1 mm of the V1-V2 border, located *in vivo* using optical imaging, performed through a thinned skull. We (Nurminen et al. 2018) previously showed that this viral mixture results in near exclusive anterograde infection in primate cortex. We recorded responses of V1 neurons to high contrast grating stimuli of increasing size and computed fano-factor (FF) as a measure of variability. Our results replicate stimulus-induced decline in variability (Churchland et al. 2010) in marmoset V1. On average, baseline fano-factor was higher than the fano-factor measured while a grating stimulus

covered neurons' summation receptive field (sRF). However, in a significant proportion of neurons, presenting a small stimulus amplified variability. For stimulus sizes smaller than, and equal to the sRF, we found an inverse relationship between firing-rate (FR) and FF: as stimulus size increased up to the sRF diameter, firing-rate increase, and fano-factor decreased. The strength of the inverse relationship was the weakest in the granular (G) layer. When the stimulus covered the sRF, the most variable neurons were found in the G layer. When RF surround was stimulated, some neurons preserved the inverse relationship between FR and variability, while for others FR and variability became positively correlated. Our preliminary analyses revealed that neurons with weak network coupling showed the strongest positive relationship between FR and variability. To further investigate the mechanisms that modulate cortical variability, we optogenetically inactivated V2-to-V1 feedback connections. In all the neurons in which the optogenetic manipulation affected the FF vs. size functions, FFs were reduced when the stimulus size was small. In these neurons, the relationship between FF and FR became weaker when feedback connections were inactivated. Our results suggest that receptive field center and surround affect neural variability in different ways and that variability modulation in V1 depends on cortical layer and feedback connections.

Disclosures: L. Nurminen: None. **A. Angelucci:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

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Program #/Poster #: 403.28/P32

Topic: D.07. Vision

Support: NIH R01 MH111447
NSF # 1539034

Title: Three-photon imaging of neural and vascular responses in the visual cortex

Authors: *A. ROY, C. LIU, A. SIMONS, D. FARINELLA, P. KARA;
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Abstract: The mammalian neocortex is divided into six distinct anatomical layers, with feedforward inputs arriving primarily into layer 4, local computations shaping response selectivity in layers 2/3, and top-down influences from other regions arriving via layers 1 and 6. In addition, in the primary visual cortex of non-rodent mammals, neurons with similar functional properties such as orientation and direction selectivity cluster together radially along the cortical surface, thereby forming functional maps in cortical layers 2/3. To date, structural and functional two-photon imaging of neurons in these layers have unraveled a wide array of principles that govern how these functional columns are organized and how neurons therein carry out various

computations. In recent years, two-photon imaging of vascular dynamics in layers 2/3 has also begun to reveal how local neural activity is coupled to changes in blood flow in the local vasculature. The precision of the mapping of neural and vascular features in layers 4–6 remains unknown, particularly because the visual cortex in non-rodent mammals is considerably thicker (~ 2 mm) compared to mice (~ 0.74 mm) and these deeper layers have been inaccessible with conventional two-photon imaging methods. However, three-photon imaging, by virtue of decreased scattering of longer wavelength excitation light and enhanced non-linear confinement of the excitation volume, provides suitable access to such depths. We have set up a three-photon microscope capable of imaging green and red fluorophores and label-free third-harmonic-generation signals from depths of > 1 mm in the neocortex. We mapped out the range of three-photon excitation wavelengths for an assortment of commonly used green and red fluorophores. Then, using these fluorophores we carried out three-photon imaging experiments of neural and vascular responses. We will present data regarding organization of the neural maps across various cortical layers and how single-vessel responses (dilation, blood velocity) in these cortical layers correlate with local neural activity.

Disclosures: **A. Roy:** None. **C. Liu:** None. **A. Simons:** None. **D. Farinella:** None. **P. Kara:** None.

Poster

403. Visual Cortex: Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 403.29/P33

Topic: D.07. Vision

Support: NIH Grant NS064033

Title: Four-dimensional mapping of stimulation-induced cortico-cortical spectral responses across the visual areas

Authors: *A. SUGIURA[1], N. MIMURA[1], K. TANAKA[1], Y. NAKAI[1], H. MOTOI[1], E. ASANO[1,2];
[1]Dept. of Pediatrics, Children's Hosp. of Michigan, [2]Dept. of Neurology, Children's Hosp. of Michigan, Wayne State Univ., Detroit, MI

Abstract: We provided whole-brain level animations showing the dynamics of cortico-cortical spectral responses (CCSRs) elicited by single-pulse electrical stimulation to the lower- and higher-order visual areas. This study analyzed electrocorticography (ECoG) signals of 22 patients with focal epilepsy. As part of the pre-surgical evaluation, we delivered trains of electrical stimuli (pulse width: 0.3 ms; intensity: 5 mA; frequency: 1 Hz; train duration: 40 s) to a neighboring pair of subdural electrodes implanted at the vision-related areas (i.e., medical-

occipital, lateral-occipital, and fusiform gyri). We performed a time-frequency analysis at each electrode site to determine the stimulation-related modulations of gamma activity, as compared to the baseline period at 150 to 100 ms before the stimulus. On the three-dimensional FreeSurfer average surface image, we animated the dynamics of CCSRs at gamma band at all electrode sites showing significant amplitude modulations at 10-50 ms after the stimulation. Stimulation of the medical-occipital region elicited CCSRs at the lateral-occipital region. Stimulation of the lateral-occipital region elicited CCSRs at the fusiform, inferior-parietal, and inferior-temporal gyri, as well as at the frontal eye field. Stimulation of the fusiform region elicited CCSRs at the anterior part of the fusiform, parahippocampal, and entorhinal gyri. The present study demonstrated the capability of the aforementioned vision-related areas to deliver neural information directly via single axonal connectivity.

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Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 404.01/P34

Topic: D.08. Visual Sensory-motor Processing

Support: ARC Grant CE140100007
ARC Grant DE120102883
ARC Grant DP140101968
NHMRC Grant 1020839
NHMRC Grant 1082144
EU Grant FP7-ICT 217077- EYESHOTS
EU Grant FP7-PEOPLE-2011-IOF 300452

Title: Thalamic afferents to the precuneate cortex of the macaque monkey

Authors: *M. GAMBERINI[1], L. PASSARELLI[1], D. IMPIERI[1], P. FATTORI[1], C. GALLETTI[1], M. G. ROSA[2,3], S. BAKOLA[2,3];
[1]Biomed. and Neuromotor Sci., Univ. of Bologna, Bologna, Italy; [2]Physiol. and Biomedicine Discovery Inst., Monash Univ., Melbourne, Australia; [3]Australian Res. Council, Ctr. of Excellence for Integrative Brain Function, Monash Univ. Node, Melbourne, Australia

Abstract: The precuneate cortex of macaque brain hosts different cortical areas. The aim of the present study was to characterize the thalamic afferents to the macaque precuneate cortex, based on a set of tracer injections in areas PGm, 31, and in nearby area PEci. Fluorescent retrograde neuronal tracers were injected in the precuneate cortex within and adjacent to area PGm of four

hemispheres of four male adult monkeys (*Macaca fascicularis*). Tracers were injected into the cortical thickness using a micro-syringe, or placed as crystals on the mesial cortex of the hemispheres based on visual inspection of the brain after a portion of the posterior parietal cortex of the contralateral hemisphere was retracted. Cyto- and myelo-architectural analysis of histological material established that in six cases tracers were delivered within the limits of area PGm, in one case within those of area 31 and one within area PEci. The three areas here analyzed present many similarities in the regional distribution of thalamic afferents but the specific nuclear origin of thalamic input varies across areas. The precuneate cortex receives strong input from the posterior thalamus, in particular, from the lateral posterior and the pulvinar complex, and moderate input from the medial, lateral, and intralaminar thalamic regions. Area PGm presents strong connectivity with the associative medial and visual lateral divisions of the pulvinar, whereas areas 31 and PEci receive exclusive afferents from the oral division of the pulvinar. All areas receive input from the 'motor' division of lateral thalamus, in particular from the ventral lateral (VL) nucleus, while area PEci receives input from both the 'sensory' (ventral posterior lateral, VPL) and 'motor' domains of the lateral thalamus. Consistent thalamic input to all three areas arrived from the medial dorsal (MD) nucleus, while the anterior lateral dorsal (LD) nucleus is uniquely connected with area PGm. Finally, areas 31 and PEci receive moderate afferents from the intralaminar region, in particular from the central lateral (CL) nucleus, whereas area PGm receives minor afferents from different intralaminar nuclei. The pattern of afferents indicate that macaque area PGm integrates information from a set of association, motor, and limbic regions of the thalamus, and emphasizes links with visual and multimodal thalamic regions. With respect to the adjacent superior parietal areas, where the thalamic afferents from VPL to areas PE, PEc, PEci, and from the oral pulvinar to area 31 describe a sensorimotor function, area PGm shows a role mainly focused in the processing of spatial and attentional information useful for navigation in a complex environment.

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Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

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Program #/Poster #: 404.02/P35

Topic: D.08. Visual Sensory-motor Processing

Support: ERC grant WIRELESS (678307)
Italian MIUR grant GANGLIA (R16PWSFBPL)

Title: Sensory and motor properties of physiologically identified neuronal classes in multiple areas of the parieto-frontal grasping network

Authors: *C. G. FERRONI[1], M. MARANESI[1], A. LIVI[2], M. LANZILOTTO[1], D. ALBERTINI[1], L. BONINI[1];
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Abstract: The attribution of functional properties to specific neuronal classes (e.g. inhibitory interneurons, big or small pyramidal neurons, etc.) is crucial to better understand the neural machinery underlying sensory and motor processes. Indeed, several studies showed that cortical neurons can be identified by jointly considering a variety of features of their spike waveform and firing properties. However, the specific relation between physiologically-identified neuronal classes and their coding properties remains unclear. To address this issue, here we studied the features of extracellularly recorded spikes of 492 well-isolated single neurons. Neurons were sampled from 5 hemispheres of 3 macaque monkeys while they performed, or observed an experimenter performing, a reaching-grasping go/no-go task with three different objects as targets. Single neurons were recorded from anterior intraparietal area AIP (n=134), ventral premotor area F5 (n=135) and pre-supplementary motor area F6 (n=223). First, we performed an unsupervised clustering of spike waveforms that reliably dissociated 5 clusters across the investigated cortical regions. Then, we compared the response properties across cell class and found that visual responses to object presentation and action execution/observation differ across classes. Furthermore, we found functional specificities for cortical areas within each class. Interestingly, the number of neurons included in each cell class varied dramatically across areas, suggesting a possible link between physiologically identified classes and local cytoarchitectural specificities. Our findings shed light on the cellular mechanisms underlying local processing of sensorimotor information for planning and executing grasping actions and the observation of others' action.

Disclosures: C.G. Ferroni: None. **M. Maranesi:** None. **A. Livi:** None. **M. Lanzilotto:** None. **D. Albertini:** None. **L. Bonini:** None.

Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

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Program #/Poster #: 404.03/P36

Topic: D.08. Visual Sensory-motor Processing

Support: FP7/2007-2013 Grant 600925
European Research Council (ERC) Grant 678307

Title: View-dependent processing of observed manipulative action in the monkey anterior intraparietal area (AIP)

Authors: *M. LANZILOTTO[1], M. MARANESI[1], A. LIVI[2], C. G. FERRONI[1], L. BONINI[1], G. A. ORBAN[1];
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Abstract: The processing of observed manipulative actions (OMAs) is a fundamental prerequisite of social behavior in primates. Classical models assign a crucial role to temporal brain regions, which convey visual information to premotor cortex via inferior parietal areas. A recent our study (Lanzilotto et al. 2019) demonstrated that anterior intraparietal area (AIP) might be considered a crucial node of the monkey's action observation network, encoding the identity of a large variety of OMAs relative to those explored by previous studies. Nonetheless, whether and to what extent neuronal representation of OMAs is invariant to changes in the visual features of the stimuli, such as actor's body posture (standing or sitting) and visual perspective (frontal or side-view), is still unknown. To address this issue, we chronically recorded (multi- and single) unit activity with linear silicon probes from area AIP of two macaques (MK1 and MK2). During the recordings, the monkeys observed videos portraying 7 different manipulative action exemplars (drag, drop, grasp, push, roll, rotate and squeeze) performed on 2 objects of different colors (orange and magenta) by 2 actors (male and female) in 4 *formats* defined by combinations of two postures (sitting and standing) and two viewpoints (lateral and frontal). We recorded 297 units, of which 113 (38%) were action-selective in at least one format, as revealed by a 7x3 (actions x epochs) repeated measures ANOVA applied to each format ($p<0.05$), separately. Among action-selective units, more than half (n=63, 56%) exhibited OMAs selectivity only in one format (ranging from 10% for Standing-Frontal to 19% for Standing-Lateral format). Individual unit analysis, population and machine learning approaches confirmed that AIP neurons encode OMAs identity within each format, as recently demonstrated (Lanzilotto et al 2019), but also highlight that OMA representation depends on the visual features of each format, showing little evidence of visual invariance. These findings support the view that monkey's AIP constitutes an early node for the processing of OMA, representing their different instantiation by largely non-overlapping neuronal populations.

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Poster

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Topic: D.08. Visual Sensory-motor Processing

Support: ERC WIRELESS 678307

Title: Linkage of multiple sensory and motor properties in single neuron activity of the macaque anterior intraparietal area

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Abstract: The processing of sensory cues, observed objects, and self/others' actions is crucial for coordinating social interactions and relies on a network of interconnected frontal and parietal areas. Among these latter, the anterior intraparietal area (AIP) is considered a crucial node for many of these functions, such as the extraction of object affordances and the encoding and selection of hand postures for grasping, as well as the representation of observed manipulative actions. However, these properties have been demonstrated by different studies carried out with highly different tasks and methodologies, leaving unclear how AIP can underlie so many complex and heterogeneous processes. To address this issue, we recorded AIP single neuron activity from two macaques during 1) a visuomotor task in which they viewed and grasped (or refrained from grasping) objects of different size and shape (EXE), 2) an observation task in which a human performed the same task (OBS) in the monkey's peripersonal (OBSp) or extrapersonal (OBSe) space, 3) an observation task in which videos of grasping actions and static frames depicting objects or static images of grasping hands were presented (OBSv). Recordings were carried out with chronically-implanted linear multielectrode silicon probes. We isolated 134 single neurons with highly restrictive criteria. Then, we performed a hierarchical cluster analysis by computing the Mahalanobis distances among conditions of interest in all task contexts. These data show that AIP strongly encodes spatial information regarding the actual or expected position of objects relative to the monkey, as well as the type of target object, from target presentation epoch along the entire task unfolding period. Furthermore, AIP population activity also provides motor information as to whether and how the subject will act, and agent-related information about who is going to act in a specific context. Interestingly, AIP population activity differentiates the two extrapersonal contexts (OBSe and OBSv), but the type of object is not significantly represented in none of them. These results emphasized the role of AIP in the integration of sensory and motor properties for planning actions on reachable objects and agent-based representation of actions.

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Poster

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Title: Connectional gradients underlie functional transitions in monkey pre-supplementary motor area

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Abstract: The pre-supplementary motor area F6 is involved in a variety of functions in multiple domains, from planning/withholding goal-directed actions in space to rule-based cognitive processes and social interactions. Yet, the neural machinery underlying this functional heterogeneity remains unclear. Here, we recorded a total of 291 units by means of chronic linear multielectrode silicon probes in different rostro-caudal sites of cytoarchitectonically verified area F6 in two monkeys. We measured local neural population dynamics while the monkeys performed a Go/No-Go reach-to-grasp task and observed the same task as performed by an experimenter in both the monkey's peripersonal and extrapersonal space. At the end of the recordings, the two probes implanted in each animal were removed and an antero-retrograde neural tracer was injected at the center of the spot previously occupied by each explanted probe, allowing us to investigate both the cortical and striatal connectivity of each recorded site. Finally, we correlated multimodal populations tuning with local connectivity patterns in all the four functionally characterized sites. We found stronger tuning of rostral area F6 to spatial position of objects and agents relative to the caudal one, which in turn exhibits stronger tuning to self and other's (observed) action. Functional specificities were associated with a rostro-caudal transition in connectivity strength from lateral prefrontal cortex, pregenual anterior cingulate cortex and associative striatum (rostrally), to dorso-ventral premotor areas and the motor putamen (caudally). These findings suggest that the functional heterogeneity of the pre-supplementary area F6 is accounted for by gradual transitions in functional properties grounded on local cortico-cortical and cortico-striatal connectional specificities.

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Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

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Topic: D.08. Visual Sensory-motor Processing

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Title: Common reference frames for spatial and non-spatial motor responses in parietal area V6A

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Abstract: Goal-directed movements involve a series of neural computations that compare the sensory representations of goal location and effector position and transform them into motor commands. Neurons in posterior parietal cortex (PPC) control several effectors (e.g. eye, hand, foot) and encode goal location in a variety of spatial coordinate systems ranging from one relative to gaze (eye-centered reference frame) to coordinate systems centered on head, shoulder or hand. Despite many neurophysiological and imaging studies on effector specificity in PPC, there is little evidence on whether reference frames depend also on the selected effector and/or motor response. We addressed this issue in macaque PPC area V6A where we have previously reported that, during a fixate-to-reach task in 3D space with varied initial hand positions, most cells used mixed body- and hand-centered coordinates (Hadjidimitrakis et al. 2014). Here, we used singular value decomposition and gradient analyses to characterize the reference frames of V6A neurons (n=50) recorded from a monkey that fixated the same array of targets and after the Go cue, instead of reaching, performed a non-spatial motor response (button release). We found that most neurons used neither "pure" body-, nor hand-centered, but mixed body/hand coordinates. Interestingly, along the task progress, the effect of hand position on activity was stronger compared to that of target location, whereas activity more consistent with body-centered coding was present only in a subset of neurons active early in the task. Applying the same analyses to a larger population of V6A neurons recorded from the same monkey during reaches yielded similar results, indicating the use of consistent reference frames between spatial and non-spatial motor responses, thus highlighting V6A role in forming default movement plans. Moreover, our results suggest that single PPC neurons have stable reference frames across time and that the sensorimotor transformations most likely occur via the sequential recruitment of subpopulations with distinct reference frames.

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Poster

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Topic: D.08. Visual Sensory-motor Processing

Support: ANR-18-CE37-0022

Title: Functional and structural connectivity of the cingulate sulcus visual (CSv) area in macaque monkeys

Authors: V. DE CASTRO, C. LEGUEN, Y. HÉJJA-BRICHARD, P. AUDURIER, B. R. COTTEREAU, ***J.-B. DURAND**;
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Abstract: Recently, we described the cortical network involved in processing egomotion-compatible optic flow using functional MRI in 3 awake macaque monkeys (Cottereau et al, 2017). In all the animals, significant selectivity was observed in several areas previously associated with optic flow processing, and notably in MSTd, VIP, 7a/Opt, FEFsem and VPS. We also described a new region of the cingulate sulcus that may be a homologous of the cingulate sulcus visual (CSv) area evidenced in humans with the same protocol (Wall and Smith, 2008). To go further in the study of this potential homology, we aim at characterizing the connectivity of this monkey cingulate region, as recently achieved for the human CSv (Smith et al, 2017). Under slight anesthesia (Medetomidine: 10µg/kg, Ketamine: 10mg/kg), the same 3 monkeys underwent 3 sessions of resting-state fMRI (rs-fMRI) and 1 session of diffusion-tensor imaging (DTI) in order to assess the functional and structural connectivity of this cingulate region. In each of the 3 sessions of rs-fMRI, we recorded 2 runs of 20 minutes (GE-EPI sequences, 600 volumes/run, 32 axial slices, TR/TE: 2000/30ms, voxel size: 1.25x1.25x1.8 mm, SENSE factor: 2). For the DTI session, 3 repetitions were included in a sequence lasting 45 minutes (128 directions, b=1000s/mm2, 1 b0 volume with no diffusion weighting, TR/TE: 7132/61ms, voxel size: 0.89x0.89x1 mm, EPI factor: 55). Converging evidence from rs-fMRI and DTI indicates that the cingulate region previously documented in the same monkeys is connected to several visual (and vestibular) areas involved in optic flow processing, such as MSTd, VPS and 7a/Opt. Interestingly, we also observed connections with the foot and leg representations in the somatosensory and motor cortices. Altogether, this connectivity pattern echoes the one documented for human CSv, reinforcing the view of a strong functional homology. In both species, CSv may play an important role in linking sensory and motor systems for the online control of locomotion. (Ref: Cottereau BR, Smith AT, Rima S, Fize D, Héjja-Brichard Y, Renaud L, Lejards C, Vayssière N, Trotter Y, Durand JB. Processing of Egomotion-Consistent Optic Flow in the Rhesus Macaque Cortex. Cereb Cortex. 2017 Jan 1; 27(1):330-343. Smith AT,

Beer AL, Furlan M, Mars RB. Connectivity of the Cingulate Sulcus Visual Area (CSv) in the Human Cerebral Cortex. Cereb Cortex. 2018 Feb 1; 28(2):713-725. Wall MB, Smith AT. The representation of egomotion in the human brain. Curr Biol. 2008 Feb 12; 18(3):191-4.)

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Poster

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Topic: D.08. Visual Sensory-motor Processing

Support: Grant-in-Aid for Scientific Research(S)(#26221003)

Title: Activity of frontal network for performance of forced-choice manual response task in blindsight monkey

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Abstract: Patients with the damage to the primary visual cortex (V1) report loss of visual awareness in their affected visual field but show considerable residual visuo-motor functions, the phenomenon termed blindsight. Most of previous studies in animal model of blindsight have employed oculomotor tasks to assess their residual visuo-motor functions. However, because of its nature, in the oculomotor behavior, it is sometimes difficult to differentiate reflexive orienting response and cognitive response. In addition, neural basis of visuo-motor response involving effectors other than eyes in blindsight condition is still unclear. To clarify points shown above, we applied a two alternative forced choice manual response task (2AFCMR) in the macaque monkey. In this task, eye positions were kept at a central fixation point. Then, push- or pull-responses of a lever was instructed by the location of visual cue presented in either upper or lower part of the visual hemifield. The monkey was trained to push or pull the lever in response to the Go signal presented 500-1000ms after the cue. Bilateral electrocorticogram (ECoG) was recorded before and after unilateral lesion of V1 with chronically implanted electrode arrays (48ch each) from dorsolateral prefrontal cortex, frontal eye field, dorsal and ventral premotor cortex and primary motor cortex. Before the V1 lesion, the luminance contract of the cue was 0.075 (near threshold), and the success rate was 86%. After the V1 lesion, the animal was trained at the maximum luminance contrast (0.95) from Day 16 after the lesion and the success ratio became above the chance level around Day 50 after the V1 lesion and reached 65 -79 % until

Day 55. Before and after the V1 lesion, we adjusted luminance contrast of visual cue to make the success rate is comparable between conditions. Before the V1 lesion, the time-frequency analysis showed that low frequency activity (5-8Hz: theta, 8-13Hz: alpha) in the premotor regions was increased in the trials where the monkey successfully detected the stimuli (i.e. Hit trials) compared to the trials where the monkey failed to detect the presented target (i.e. Miss trials). This increment was observed either before or after the Go signal. After the V1 lesion, the increment of the low frequency activity before the Go signal did not change from before the V1 lesion, whereas the low frequency activity after the Go signal was greater in the Miss trials than the Hit trials. These results suggest that neural processes of visual stimuli without oculomotor response in the premotor regions were preserved before and after the V1 lesion, whereas those before visuomotor response onset were affected by the V1 lesion.

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Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

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Topic: E.04. Voluntary Movements

Support: Simons 543049

Title: Constraints shape shared vs. separate dynamics in multitask networks

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Abstract: Human thought is inherently flexible. Preliminary work has identified how both artificial and biological networks perform context dependent operations within the framework of a single task[1,2]. However, it is unknown how computations for many tasks interact within a single network. To advance our understanding of the flexibility of biological networks, we are interested in how computations for multiple tasks are implemented within a single network of neurons. We use recurrently connected artificial neural networks as a model system to address this question, because the architecture is inspired by biological neural systems and because these model systems are capable of learning many tasks. We train each recurrent neural network (RNN) to perform a set of 20 standard cognitive tasks that includes variants of memory guided response, perceptual decision making, context-dependent decision-making, multi-sensory

integration, parametric working memory, inhibitory control, delayed match-to-sample, and delayed match-to-category tasks[3]. After training on all tasks, the network connections are fixed and the changes in activity patterns are computed solely from the initial condition, $x(0)$, and the input, $u(t)$.
We study network activity during the performance of each task as a dynamical system, governed by the initial state and the inputs to the system[4]. At the beginning of each trial, the network is cued on which task to perform. This 'rule' input produces a stable fixed point that neural trajectories move toward. Collectively, these task rule dependent fixed points are organized in neural activity space according to task similarity. Following stimulus onset, hidden unit activity evolves in mostly separate subspaces for each task. The organization of these subspaces depends on the constraints on the network. Under certain constraints, the subspaces are aligned such that the same decoder can readout the stimulus identity or the motor plan in the same way across tasks. Training on multiple tasks compared to training on a single task changes the dimensionality of working memory tasks, suggesting multi-task training could change the algorithms that perform memory computations. We will further characterize the effects of various constraints in order to develop hypotheses for testing in biological cortical networks during the performance of multiple tasks.
References: [1] Mante, V., Sussillo, D., Shenoy, K. V. & Newsome, W. T. (2013). [2] Remington, E. D., Narain, D., Hosseini, E. A. & Jazayeri, M. (2018). [3] Yang, G. R., Song, H. F., Newsome, W. T. & Wang, X.-J. (2019). [4] Sussillo D., Barak O. (2013).

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Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

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Topic: E.04. Voluntary Movements

Support: Stanford Neurosciences Institute
ONR Grant N00014-18-1-2158
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Howard Hughes Medical Institute

Title: Joint modeling of neural population dynamics and behavior in single-trial perceptual decision making

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Abstract: An emerging hypothesis in systems neuroscience is that neural population dynamics implement the computations that process sensory cues into behavioral responses. If computation is indeed performed through these dynamics, understanding the dynamical motifs expressed by a neural population could provide a rich description of how that population solves a task, flexibly generalizes across tasks, and learns to solve new tasks.

Existing approaches to identifying dynamical motifs typically employ either "task-models" or "neural data models." Task-models rely on training a recurrent neural network (RNN) to solve a task. After training, the RNN's activity can then be compared neural activity recorded in animals performing the same task. However, there may be many qualitatively different dynamical motifs that can solve a task, and the particular motif learned by an RNN can depend dramatically on model design and task representation. On the other hand, neural data-models seek dynamical latent variables that accurately summarize recorded population activity. While these models provide powerful single-trial estimates of neural state, their complexity can render them difficult to interpret. Further, neural data models lack an explicit link to the task, behavior and underlying computation.

Here we propose Goal-Oriented Learning of Dynamics (GOLD), a joint neural-behavioral model that overcomes the aforementioned limitations. GOLD uses RNNs to learn latent dynamics capable of generating single-trial behavior (i.e. task modeling), but constrains those dynamics to be consistent with single-trial population recordings (i.e. neural-data modeling). GOLD thus provides access to an interpretable, data-driven link between task goals, observed behavior, underlying neural computation, and observed neural population dynamics.

We demonstrate GOLD on population recordings from dorsal premotor cortex in rhesus macaques performing perceptual decision making. Using trials that were held out during model fitting, we found that GOLD solves the task via dynamical motifs that are substantially more consistent with the neural recordings compared to task models. GOLD-identified dynamics also predicted previously elusive single-trial behavioral effects, such as extreme reaction times. Finally, we demonstrate the interpretability of GOLD by reverse engineering its RNN, revealing a structure of condition-dependent fixed points that govern the dynamics and solve the task. Moving forward, GOLD may provide a comprehensive description of how a single neural population flexibly re-configures its dynamics across a multitude of tasks.

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Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

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Program #/Poster #: 404.11/Q2

Topic: E.04. Voluntary Movements

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NIH Grant R01NS111982

Title: Discrete population states in motor cortex during different arm movement tasks

Authors: *C. SPONHEIM[1], V. PAPADOURAKIS[2], N. KADMON HARPAZ[4], N. G. HATSOPOULOS[3];
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Abstract: Despite being one of the longest-studied brain areas, we have yet to elucidate the role of primary motor cortex (M1) in the generation of movement. An essential part of understanding the role of M1 is the temporal structure of commands sent from its neural population to the spinal cord. Previous research indicates that although M1 activity continuously changes throughout a movement, there remains a latent structure with discrete states, corresponding to accelerative and decelerative kinematic components of movement (Kadmon Harpaz et al., 2018). Rhesus macaques performed either a random target pursuit task (RTP), or a standard eight direction center-out reaching task. We recorded from M1 and premotor cortex (PM) using chronically implanted, multi-electrode arrays while each monkey performed two alternating tasks, allowing us to evaluate activity from two tasks in the same population of neurons. We also recorded muscle activity during the tasks. We used a hidden Markov model to identify common latent states in M1 neural population activity. We then compared the timing and length of those latent states to the kinematics of arm movements. We were able to replicate previous findings, supporting the idea that latent states in M1 map to accelerative and decelerative components of motion. We are also in the process of identifying whether models trained on one task effectively identify consistent kinematic parameters in the other task. In addition, we compared the performance of models trained on one task (either RTP or center-out) with that trained on the other task. We also anticipate that the latent states observed in cortical activity will not be present during analysis of EMG activity. These results suggest that neural representations of arm movements may be constructed from elementary sets of building blocks. These elementary states, put together in different discrete combinations, create the vast array of movements of which animals are capable. This method can also be used to interrogate the differences in temporal structure between M1 and PM.

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Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

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Topic: E.04. Voluntary Movements

Support: NIH R01 104898-01

Title: Developing a motor task platform for freely-behaving common marmosets

Authors: ***M. SUNDIANG**, J. D. WALKER, D. D. MOORE, J. MACLEAN, N. HATSOPOULOS;
Univ. of Chicago, Chicago, IL

Abstract: Goal directed, skilled movements and their acquisition through learning have the potential to provide novel insights into motor control. Until recently, a common experimental paradigm for studying these behaviors has involved restrained subjects performing constrained reaching tasks. We have learned a lot from these tasks because it has allowed us to isolate specific movement patterns and perform trial averaging of neural activity. However this constrained approach to behavior may not capture the complexity of neural dynamics that accompany more naturalistic behaviors. Here, we present a novel motor task for studying skill acquisition in freely behaving marmosets. In their natural habitat, marmosets exhibit foraging behavior during which they stalk and pounce on prey which are usually insects such as beetles, crickets and locusts. We take advantage of the marmosets' natural tendency to forage for food and allow them to learn new prey hunting strategies. To do so, we use a correlated random walk to model the movement dynamics of a natural prey of the marmoset, specifically the *Tenebrio molitor* beetle. A moving target follows the generated beetle trajectory and is presented to the marmoset via a touch screen. Because the prey trajectories are created programmatically, the task is flexible: we can present the same trajectory multiple times to assess motor learning, and we can generate different prey dynamics to assess higher level learning, to name a few examples. The marmosets can demonstrate motor skill learning by increasing successful captures, decreasing their time to capture the prey, and performing captures using a more stereotyped and efficient arm trajectory. Using DeepLabCut, a pose estimation toolbox, we track upper limb kinematics without using markers that might interfere with the behavior. Previous work in our lab developed a platform for wirelessly recording from a multi-electrode array in marmoset M1 and neighboring cortical areas, allowing us to collect simultaneous neural and behavioral data from untethered, freely-behaving marmosets. Thus, our motor task platform opens an avenue for

studying complex motor behavior and a richer understanding of the neural dynamics that generate these behaviors.

Disclosures: M. Sundiang: None. **J.D. Walker:** None. **D.D. Moore:** None. **J. MacLean:** None. **N. Hatsopoulos:** None.

Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 404.13/Q4

Topic: E.04. Voluntary Movements

Support: NIH R01 104898-01

Title: Comparing pose estimation performance using DeepLabCut and marker-based tracking

Authors: *D. D. MOORE[1], J. D. WALKER[1], J. N. MACLEAN[2], N. G. HATSOPOULOS[1]; [1]Dept. of Organismal Biol. and Anat., [2]Neurobio., Univ. of Chicago, Chicago, IL

Abstract: To reveal the neurophysiological underpinnings of natural voluntary movement, neural recordings must be paired with accurate tracking of limbs and postures. DeepLabCut (Mathis et al, 2018), a recent machine learning based advance in markerless tracking technology, has been demonstrated to efficiently track body parts across a broad range of species, behaviors, and settings with accuracy comparable to that of human labeling. To our knowledge, however, DeepLabCut has not yet been compared to simultaneous high resolution marker-based tracking. Such a comparison would indicate the extent to which DeepLabCut reliably characterizes kinematics with accuracy similar to existing marker-based systems that are far more prevalent in non-human primate motor control research. A 3D x-ray video fluoroscopy system that tracks markers placed under the skin (XROMM) provides a well-established baseline for comparison to pose estimation performed with DeepLabCut on video recordings of behavior. To this end, we collect simultaneously recorded behavioral data using both XROMM and high speed video (two Blackfly S cameras at 200 frames per second) as common marmosets engage in naturalistic foraging, then reconstruct three-dimensional kinematics of reaching events in a shared coordinate system. By doing so, we directly compare the Cartesian position and velocity of the hand, wrist, arm and torso computed by each tool to determine the accuracy of DeepLabCut relative to the XROMM. We then evaluate whether this accuracy is sufficient to reconstruct joint angles from marker positions. If DeepLabCut provides comparable pose estimation accuracy as expected, its impressive efficiency and flexibility will enable the study of more naturalistic, unconstrained behaviors in many fields including non-human primate motor control.

Disclosures: **D.D. Moore:** None. **J.D. Walker:** None. **J.N. MacLean:** None. **N.G. Hatsopoulos:** None.

Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 404.14/Q5

Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI 15K01854
JSPS KAKENHI 17KK0140
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JSPS KAKENHI (Non-linear Neuro-oscillology) 15H05879
Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network)16H06276

Title: Three dimensional dynamics of β oscillation phase in the monkey motor cortex during a reaching task

Authors: *H. WATANABE[1,2], K. TAKAHASHI[2,3], H. MUSHIAKE[1];
[1]Grad. Sch. of Med., Tohoku Univ., Sendai, Japan; [2]Dept. of Organismal Biol. and Anat., The Univ. of Chicago, Chicago, IL; [3]The Univ. of Chicago, Research Computing Center, IL

Abstract: *β* oscillations (15-30 Hz) are ubiquitous in the motor cortex of the mammals. There have been a few attempts to analyze 3D spatiotemporal dynamics of neuronal activities at *β* band in the motor cortex. Previously we characterized horizontal dynamics of *β* oscillations from electrocorticogram (ECoG) recording by looking timings of phase locking to the instruction cues and movement onsets. Here, we implanted 3D electrode arrays into the primary motor cortex (M1) and the premotor cortex (PM) of a monkey and analyzed how site-dependent phase locking of *β* oscillations can be characterized in 3D and how the locking is related to behavioral events. The monkey was trained to use a single arm to perform a reaching. The monkey kept the hand at the resting position for 2 seconds after a visual instruction target-cue randomly indicating one of the two positions, then reached to the target after an acoustic go-cue. Two 128-channel electrode arrays (Matrix Array™, NeuroNexus, MI, US) were implanted in M1 and PM contralateral to the arm. Each array consisted of an ECoG grid (32 channels), and a 3D intracortical part (96 channels) having 12 needle probes horizontally spaced by 0.4mm with eight electrodes 0.2mm apart along each needle. A prominent *β* peak was identified at 22 Hz. To extract the amplitude and phase of the *β* band, a signal from each contact was bidirectionally filtered (20-24Hz), then Hilbert transformed. The Percent of Phase Locking (PPL) of *β* oscillations over trials was computed in relation to the instruction and go cues respectively.
The PPL transiently increased among most channels around the reaching onsets, while the *β*

amplitude decreased. The PPL in many channels occurred after instruction-cue as previously reported. Most channels showed statistically significant PPL after the instruction-cues and around reaching onsets. There was a temporal sequence of peak timing of the PPL was characterized to start from M1 then PM around the instruction cue and to transient back to M1 before the movement onset. Furthermore, the peak PPL timing patterns along the depth were different for the two positions. These results indicate that the temporal dynamics of the phase of β oscillation carry task-relevant information contained in instruction cue signals and about reaching.

Disclosures: H. Watanabe: None. **K. Takahashi:** Other; NeuroNexus. **H. Mushiake:** None.

Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 404.15/Q6

Topic: D.08. Visual Sensory-motor Processing

Support: CIHR
York Faculty of Health
CFREF

Title: Rapid visuomotor transformations and multiple gain field effects in primate ventral premotor (PMv) cortex during head-unrestrained reaches

Authors: *V. NACHER, H. K. ARORA, V. BHARMAURIA, X. YAN, S. SUN, H. WANG, J. D. CRAWFORD;
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Abstract: In natural conditions, reaching involves a coordinated sequence of gaze, head, and arm movements toward a visual stimulus. Several studies have examined eye-head-hand coordination in the human, but the underlying neural mechanisms, especially those controlling head motion, have not been studied. To this aim, two monkeys were trained in a reaching paradigm that allowed unencumbered head motion and reaching in depth. Gaze, head and motion were recorded using search coil and touch screen technology, respectively. Animals touched one of three central LEDs (different initial hand locations) at waist level while maintaining gaze on a central fixation dot (with a jitter of 7-10° from trial to trial) and were then rewarded if they touched a target appearing at one of 15 locations in a 40° x 20° (visual angle) array. Simultaneously, extracellular single unit activity was recorded from a prefrontal region that included ventral premotor cortex (PMv). Behavioral analysis showed the expected gaze-head-reach sequence, with enhanced gaze accuracy and increased head movement during and after gaze the gaze shift, compared to no-reach controls. Preliminary neurophysiological analysis

showed an assortment of target/stimulus, gaze, pre-reach and reach- related responses in PMv. We first tested for gaze, head and hand gain fields during the different neuronal responses, and found that 55% of target-aligned responses, 70% of gaze-aligned responses and all pre-reach-aligned responses were gain modulated by initial hand position. A small fraction of neurons showed a gain fields for both initial gaze and hand position during target-aligned responses (20%) and pre-hand-onset-aligned responses (10%). After removing the gain field effects, we fitted the residual data against various spatial models and found that the initial visual response best encoded the target relative to the eye (Te), whereas immediately afterwards, during gaze shifts until reach, neural responses preferentially coded displacement of the hand (dA). This Te-dA transformation occurred rapidly within a time window ranging from 150-200 ms. A more complete analysis will aim to describe the complete coding and distribution of gaze, head, and reach signals in this region.

Disclosures: **V. Nacher:** None. **H.K. Arora:** None. **V. Bharmauria:** None. **X. Yan:** None. **S. Sun:** None. **H. Wang:** None. **J.D. Crawford:** None.

Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 404.16/Q7

Topic: D.08. Visual Sensory-motor Processing

Title: Early life blindsight: The pulvinar versus lateral geniculate nucleus

Authors: *D. M. FOX, I.-C. MUNDINANO, J. A. BOURNE;
Australian Regenerative Med. Inst., Clayton, Australia

Abstract: Extensive damage to the primary visual cortex (V1) typically results in cortical blindness, eliminating conscious visual perception. However, some patients elicit remarkable preservation of unconscious visual behaviour within their blind field, such as accurately reaching to grasp for objects. This phenomenon, which has garnered much interest, is known as ‘blindsight’. The neural substrate affording such visual capacity is still debated, with the majority of the focus on the visual thalamic nuclei. In this study, we aimed to delineate the relative influence of two thalamic nuclei proposed to be involved in providing auxiliary visual information; the lateral geniculate nucleus (LGN) and the medial subdivision of the inferior pulvinar (PIm) following early-life injury to V1. We used a multi-faceted approach; detailing the anatomy, connectivity and behaviour in the common marmoset (*Callithrix jacchus*, n = 10). Using our MRI-guided approach, animals received a left uni-lateral lesion to V1 and either the LGN (n=4) or PIm (n=4) at postnatal day 14. Early anatomical changes were identified by structural and diffusion MRI analysis, revealing differences in higher-order dorsal stream areas, compared to the intact hemisphere. Eighteen months following injury, we investigated their

visually-guided behaviours under naturalistic and psychophysical settings to explore the presence and extent of their blindsight capacity. We found that both lesion groups exhibited blindsight-like symptoms all presenting with a defined scotoma. However, each cohort possessed different capacities in which they could respond to contrast- and motion-sensitive stimuli within the scotoma. This dissociation revealed that PIm/V1 lesion animals showed decreased motion sensitivities while LGN/V1 lesions animals exhibited decreased contrast sensitivities. Prehensile kinematics remained similar amongst both lesion cohorts yet differed to intact controls in reaching velocity and end-point errors. Coinciding with the dorsal stream changes observed in the diffusion MRI, neural tracing and immunohistochemical techniques demonstrated similar changes in the density of anatomical projections to dorsal stream areas and their cellular profile. These results suggest an extensive role for visual thalamic nuclei in facilitating blindsight-like properties from an architectonic to an awake behaving level. However, PIm showed a greater influence following early-life lesion of V1 confirming its key role in the development of higher-order visual areas. These results also support the notion of blindsight as a modular phenomenon comprising multiple areas rather than a unitary model.

Disclosures: **D.M. Fox:** None. **I. Mundinano:** None. **J.A. Bourne:** None.

Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 404.17/Q8

Topic: E.04. Voluntary Movements

Support: NIH Grant R01 NS045853

Title: Mesoscopic patterns in motor cortical excitability facilitate movement initiation

Authors: *K. BALASUBRAMANIAN[1], V. PAPADOURAKIS[1], W. LIANG[2], K. TAKAHASHI[1], M. BEST[1], A. J. SUMINSKI[3,4], N. G. HATSOPOULOS[1,2];
[1]Organismal Biol. and Anat., [2]Committee on Computat. Neurosci., Univ. of Chicago, Chicago, IL; [3]Dept. of Neurolog. Surgery, Univ. of Wisconsin-Madison, Madison, WI; [4]Biomed. Engin., Univ. of Wisconsin Madison, Madison, WI

Abstract: Motor cortical neurons (M1) modulate prior to movement initiation but also during movement planning, action observation and motor imagery where movement does not occur. We hypothesize that for movement initiation to occur, two conditions must be met. First, local desynchronization of M1 occurs prior to movement initiation as revealed by attenuation in the local field potential oscillation amplitude in the beta frequency range, which is considered a signature of enhanced motor cortical excitability. Second, the onset of beta attenuation is not synchronous across different sites in M1 but rather takes place at different latencies in an orderly

spatial fashion reflecting planar propagating patterns of excitability along a spatially oriented axis. We show that both conditions are met in the non-human primate arm area of M1 for reaching initiation and in orofacial M1 for initiation of tongue protrusion. Moreover, we show that a preponderance of functional connections among units in M1 are oriented along the propagating axis during reaching initiation but not during planning and preparation. By decoding muscle activity using an artificial neural network from beta amplitude profiles across a set of electrodes, we also show that spatial perturbations that disrupt the natural propagating pattern of attenuation are more effective in disturbing decoding performance. Finally, to provide more direct causal evidence that this propagating pattern is necessary for movement initiation, we delivered spatio-temporal patterns of electrical stimulation that were either congruent or incongruent with the natural propagating patterns for reaching movements and showed that movement initiation was significantly delayed for incongruent electrical stimulation patterns.

Disclosures: K. Balasubramanian: None. **V. Papadourakis:** None. **W. Liang:** None. **K. Takahashi:** None. **M. Best:** None. **A.J. Suminski:** None. **N.G. Hatsopoulos:** F. Consulting Fees (e.g., advisory boards); Consultant.

Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 404.18/Q9

Topic: E.04. Voluntary Movements

Support: NIH R01 NS045853
NIH R01 NS111982

Title: Shared and non shared neural subspaces between action execution and multi sensory action observation in the macaque primary motor cortex

Authors: *V. PAPADOURAKIS[1], A. J. SUMINSKI[2], K. TAKAHASHI[1], N. G. HATSOPOULOS[1];
[1]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [2]Dept. of Neurolog. Surgery, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Our ability to move and interact with our environment relies heavily on sensory information. Sensory input is used by the motor system to access information about important variables such as hand position or target location. Primary motor cortex (M1) is considered a major hub responsible for driving movement. M1 also responds in situations that don’t involve the generation of overt motor commands, such as passive movement of the limbs or action observation. The nature and significance of this activity is under investigation. These responses could simply reflect the feedback signals used during active movement, or they could serve a

non-movement related sensory process. Here, we compare the activity of populations of M1 neurons, recorded during the execution and multi- or uni-modal passive observation of upper limb movements. We take advantage of dimensionality reduction methods to explore the existence of and characterize shared and non-shared neural subspaces between these conditions. Two monkeys were trained to execute and observe a random target pursuit task. During execution, the animals moved a visual cursor aligned with their hand location using a two-link robotic exoskeleton. Immediately following a target hit, a new target appeared in a random location. During observation, the movements generated during execution were replayed to the monkey in three different observation variants. In the visual playback condition, cursor and targets were visible to the animal during playback while the animal maintained a static arm posture. In the proprioceptive playback condition, cursor and targets were invisible and the monkey's arm was moved by the robotic exoskeleton along the invisible cursor trajectory. In the visual + proprioceptive playback condition, cursor and targets were visible and the monkey's hand was moved by the exoskeleton. A 10x10 electrode array was used to record the spiking activity of single units in M1, while the monkeys performed the task. We were able to identify both shared and non-shared subspaces in almost all the across condition comparisons. Shared subspaces accounted for the majority of the population variance, indicating the existence of an underlying correlation structure of population activity that is preserved across conditions. In alignment with our single neuron results, population activity during action execution was more related to multimodal than to unimodal action observation. We also found condition-exclusive subspaces in all conditions. These unique population patterns could represent variables that differ between action execution and observation such as movement commands or feedforward sensory predictions.

Disclosures: V. Papadourakis: None. **A.J. Suminski:** None. **K. Takahashi:** None. **N.G. Hatsopoulos:** None.

Poster

404. Cortical Planning and Execution: Neurophysiology in Nonhuman Primates II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 404.19/Q10

Topic: E.04. Voluntary Movements

Support: NIH Grant DE023816-01

Title: Cortical representation of bite force and gape in macaques

Authors: F. I. ARCE-MCSHANE[1], B. J. SESSLE[3], N. G. HATSOPOULOS[2], ***C. F. R. ROSS**[2]; [1]Dept. of Organismal Biol. and Anat., [2]Univ. of Chicago, Chicago, IL; [3]Univ. Toronto, Toronto, ON, Canada

Abstract: The precise control of bite force at varying degrees of mouth opening is vital for human mastication. Yet, the cortical control of bite force and gape is still poorly understood. To elucidate how neurons differentially encode bite force and gape, we examined neuronal spiking activity recorded with microelectrode arrays implanted chronically in the primary motor (MIo), primary somatosensory (SIo), and cortical masticatory (CMA) areas of the orofacial sensorimotor cortex in rhesus macaques. We used generalized linear models (GLM) to predict the time-varying spiking activity of each neuron as a function of bite force, gape, and spike history of a neuron. Across all areas and animals, bite force accounted for most of the information used to predict spiking: the performance of encoding models degraded significantly when bite force was removed but not when gape was removed. Encoding models in MIo performed better than in SIo but not any better in CMA. Across all cortical areas, the proportion of force-related neurons was higher than gape-related neurons and was highest in MIo. Lastly, the population of neurons in MIo, SIo, and CMA exhibited differences in preferred temporal lags of bite force in predicting spiking activity. Overall, the results suggest that while single-unit and population responses in all three areas play a role in encoding bite force and gape, the relative importance of these predictors are different across MIo, SIo and CMA.

Disclosures: C.F.R. Ross: None. **F.I. Arce-Mcshane:** None. **N.G. Hatsopoulos:** None. **B.J. Sessle:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.01/Q11

Topic: E.02. Cerebellum

Title: Sex differences in purkinje cell density in the cerebellar vermis of mice

Authors: *W. E. GRISHAM[1], **W. R. TOMITA**[2];
[1]Psychology, [2]Neurosci., UCLA, Los Angeles, CA

Abstract: Previously, MRI analyses of the cerebellar vermis revealed sex differences in mice (Corre et al. 2016). We examined these differences with a fine-grained, cellular approach, scrutinizing the vermis in cerebellar lobules 4 through 8 in XX female and XY^- -Sry male mice. Mice were gonadectomized at 73-77 days old and sacrificed at approximately 3 months. Brains were sectioned at 40-microns, mounted, and stained with thionin. Purkinje cell counts, area of granular and Purkinje cell layers, density of Purkinje cells, and mean thickness of the granular and Purkinje cell layers were measured at 10x in lobules 4 through 8.
Female mice showed greater Purkinje cell density than male mice across all lobules. No other measures, however, displayed a significant sex difference, though Purkinje cell counts showed a strong trend ($p = 0.059$). The differences in Purkinje cell density may reflect differences in

number because the cell layer areas did not show significant sex differences ($p = 0.719$). This sex difference in Purkinje cell density may be a result of organizational effects, changes at puberty, sex chromosome complement, or due to neurosteroids. Androgen receptors have been detected in Purkinje cells. Thus, androgens could affect change either in development or possibly at puberty. Sex chromosomes are another possible explanation; a difference in calbindin, which is only present in the cerebellum in Purkinje cells, is different due to sex chromosomes (XX > XY—regardless of gonad) (Abel et al. 2011). Neurosteroids may also play a role in these differences; the cerebellar Purkinje cells produce large amounts of neurosteroids (Tsutsui et al. 2011). These neurosteroids have different functions and are produced during neonatal life (Tsutsui et al. 2011). While allopregnanolone promotes Purkinje and granule cell survival, estradiol and progesterone promote Purkinje cell dendritic growth, spinogenesis, and synaptogenesis (Tsutsui et al. 2011). These neurosteroid effects could help explain the sex differences in Purkinje cell density.

Disclosures: W.E. Grisham: None. **W.R. Tomita:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.02/Q12

Topic: E.02. Cerebellum

Support: I01BX003666

Title: Impact of Purkinje cell simple spike synchrony on signal transmission from flocculus

Authors: *J. S. STAHL, A. KETTING-OLIVIER;
Neurol. Service, Cleveland Dept. of Veterans Affairs Med. Ctr., Cleveland, OH

Abstract: The degree to which Purkinje cells (PCs) ramifying on a single deep cerebellar nucleus (DCN) neuron fire in synchrony has been demonstrated to influence the DCN neuron's mean activity and response to excitation, prompting speculations that PCs carry a "synchrony code" in their simple spike (SS) activity. In this view, PCs exercise a gating function, with greater synchrony permitting greater transfer of excitatory signals through DCN neurons. However, PCs in the flocculus are not simply gates, as they are known to encode eye movement commands in their simple spike firing rates. It is unknown whether flocculus SS synchrony has any impact on the transfer of that rate-coded signal to the PCs' synaptic targets in the vestibular nuclei. An impact of SS synchrony on signal transfer might explain why disruptions in PC rhythmicity - if they also disrupt PC-PC synchrony - lead to cerebellar eye movement deficits in ataxic mice carrying mutations of genes for P/Q (CaV2.1) calcium channels. To determine if PC-PC synchrony impacts transfer of rate-coded signals, we exploited a property of optogenetics;

pulsed photostimuli engender stimulus-locked spiking that should synchronize PCs, whereas continuous stimulation engenders spikes temporally independent of the stimulus. We implanted a fiber optic cannula near the flocculus and recorded flocculus PCs in mice that express channelrhodopsin-2 specifically in PCs (F1 crosses of Ai32 and L7Cre-2 homozygotes). We stimulated the flocculus using either a fixed-amplitude, pulsed photostimulus whose 4ms pulses varied on a 0.4 Hz cycle between 14-73 pulse/s, or a photostimulus whose intensity varied continuously, also at 0.4 Hz. Both stimuli evoked sinusoidal variations of eye position with a diagonal trajectory. Tetrode recordings of 7 pairs of PCs proved that the pulsed stimulus evoked greater PC-PC synchrony, defined as the two PCs firing within 1.5 ms of each other. Single PC activity and the concomitant eye movements were processed by Fourier analysis to extract the amplitudes of firing rate modulation (|F|) and eye position (|E|), respectively. The |F|/|E| ratio was marginally higher during the pulsed than the continuous stimulus, indicating that the rate-coded signal associated with higher PC-PC synchrony was not transferred to downstream circuitry better than the signal associated with lower synchrony. The results cast doubt on whether PC synchrony plays any role in moment-to-moment regulation of eye movements driven by the flocculus (e.g., the optokinetic reflex), and whether alterations in synchrony provide a link between irregular firing in PCs of P/Q mutants and the mutants' vestibulocerebellar deficits.

Disclosures: J.S. Stahl: None. **A. Ketting-Olivier:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.03/Q13

Topic: E.02. Cerebellum

Title: Purkinje cell firing rate changes in response to alternating current stimulation

Authors: H. CHI, S. CHERIAN, M.-E. FALDAS, *H. LU;
PCOM - Georgia Campus, Suwanee, GA

Abstract: Transcranial electric stimulation (tES) is an effective treatment method for cerebellar ataxia. The underlying mechanism is not fully understood. To understand the mechanism of tES to the cerebellar Purkinje cells (PCs), we have previously used the direct current stimulation (DCS) with whole-cell patch-clamp recordings. There was no significant increase in firing rate under either positive or negative DCS. In this project, in vitro experiments were conducted to quantitatively measure the effects of alternating current stimulation (ACS) on Purkinje cells as they are the sole output neurons of the cerebellar cortex. Spontaneous activity was examined first to detect the direct response to ACS with bias current (-0.45 nA to -0.25 nA, n=3). In general, the firing rate of the Purkinje cells increased (5 Hz to 15 Hz) during ACS. Basic property was tested using current injections (-0.5 nA to +0.5 nA) to compare three states: before, during, and after

ACS. Student's t-test was used to examine the frequency changes from ACS. No significant changes were observed between pre- and post- stimulations (p=0.87, n=9) or between pre- and during- stimulations (p=0.90, n=8). In consistence with this result, no significant change was found with input resistance between pre- and post- stimulations (p=0.79, n=9). To further our understanding of the frequency change in spontaneous activity, tests to include cell orientations and stimulation intensities will be considered.

Disclosures: H. Chi: None. **S. Cherian:** None. **M. Faldas:** None. **H. Lu:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.04/Q14

Topic: E.02. Cerebellum

Title: The hypergravity induced disruption of cerebellar motor coordination

Authors: *W. NOH[1], M. LEE[1], K.-S. KIM[3], H. KIM[3], S. YANG[2];
[2]Nano-Bioengineering, [1]Incheon Natl. Univ., Incheon, Korea, Republic of; [3]Inha Univ., Incheon, Korea, Republic of

Abstract: The cerebellum coordinates voluntary movements to gain smooth and balanced motor activity. It remains unknown how gravity is associated with cerebellum-dependent behaviors and Purkinje cell's activities. In order to investigate a causal relationship between the cerebellum physiology and gravity, we measured the AMPA-mediated fast currents and mGluR1-mediated slow currents, and cerebellum-dependent behaviors such as foot print and irregular ladder under a hypergravity condition(4G). We found abnormal foot print and irregular ladder in response to hypergravity which are correlated with decreased AMPA/mGluR1-mediated currents in Purkinje cells. These results indicate that hypergravity could severely disrupt the activity of Purkinje cells in Cerebellum.

Disclosures: W. Noh: None. **M. Lee:** None. **K. Kim:** None. **H. Kim:** None. **S. Yang:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.05/Q15

Topic: E.02. Cerebellum

Title: Enriched environment shortens the duration of action potentials in cerebellar granule cells

Authors: *A. ESHRA[1], P. HIRRLINGER[2], S. HALLERMANN[1];
[1]Univ. Leipzig, Leipzig, Germany; [2]Medizinisch-Experimentelles Zentrum, Med. Faculty, Univ. of Leipzig, Leipzig, Germany

Abstract: Environmental enrichment for rodents is known to enhance motor performance. Structural and molecular changes have been reported to be coupled with enriched environment, but functional alterations of single neurons remain elusive. Here, we compared mice grown up under control conditions and enriched environment. We tested the motor performance on a rotarod and subsequently preformed whole cell patch clamp recordings in cerebellar slices focusing on granule cells of lobule IX, which is known to receive extensive vestibular input. Mice grown up in an enriched environment stayed longer on an accelerating rotarod. Electrophysiological analyses revealed normal passive properties of granule cells and a functional adaptation to the enriched environment, manifested in faster action potentials with more depolarized voltage threshold and larger amplitude. Furthermore, the maximal firing frequency of action potentials was higher in mice grown up in an enriched environment. These data show that enriched environment causes specific alterations in the biophysical properties of neurons. Furthermore, we speculate that the ability of cerebellar granule cells to generate higher firing frequencies improves motor performance.

Disclosures: **A. Eshra:** None. **P. Hirrlinger:** None. **S. Hallermann:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.06/Q16

Topic: E.02. Cerebellum

Support: Advanced Research Activities in Biomedical and Agroalimentary Technologies (MIS 5002469)

Title: Functional imaging of the monkey cerebellar cortex during action execution and observation

Authors: *H. SAVAKI[1], **V. RAOS**[1,2];
[1]IACM/FORTH, Heraklion, Greece; [2]Univ. of Crete Med. Sch., Heraklion, Greece

Abstract: We employed the ^{14}C-deoxyglucose quantitative autoradiographic method to obtain high-resolution functional maps of activity in the cerebellar cortex of six rhesus monkeys who

reached and grasped an object either in the light (EL) or in the dark (ED), of three monkeys who observed the same reaching-to-grasp movements executed by another subject (O), and of two monkeys who observed non-object-directed (purposeless) forelimb movements (BM). The extent and intensity of activations in the cerebellar cortical areas of the monkeys grasping in the light and of the monkeys observing either object-directed or purposeless forelimb movements were compared to those of the two fixation control monkeys (Cf). The activity pattern in two dark control monkeys (Cd) was used as reference for the measurement of effects in the animals grasping in the dark. Execution of reaching-to-grasp movements, both in the light and in the dark, activated the forelimb representation of the cerebellar hemispheric extensions of vermian lobules IV, V, VI and VIII, ipsilaterally to the moving forelimb. Moreover, crus II posterior in the ansiform lobule was activated bilaterally only by the execution of visually-guided movements. The activations induced by the observation of reaching-to-grasp movements were similar in extent and intensity with those induced by the observation of purposeless forelimb movements and therefore the O and BM monkeys were pooled together. Action observation activated the forelimb representation of the cerebellar hemispheric extensions of vermian lobules IV, V, VI, as well as the crus II posterior in the ansiform lobule, bilaterally. These results demonstrate that the cerebellar cortex, in addition to its involvement in the production of movement, plays an active role in the perception of observed movements.

Disclosures: H. Savaki: None. **V. Raos:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.07/Q17

Topic: E.02. Cerebellum

Support: NIH NS088567
Roy J. Carver Charitable Trust Bipolar Disorder Research Program of Excellence

Title: Dynamic interactions among the anterior cingulate, amygdala and cerebellum during acquisition of trace eyeblink conditioning in rats

Authors: *H. E. HALVERSON[1], J. KIM[2], J. H. FREEMAN[2];
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Abstract: Eyeblink conditioning is a simple form of associative learning where a conditioned stimulus (CS), e.g., a tone) is repeatedly paired with an unconditioned stimulus (US, e.g., a periorbital shock) to yield a cerebellum-dependent conditioned response. Cerebellar learning during trace eyeblink conditioning, when there is a temporal gap between the CS and US, requires mossy input from the CS and persistent mossy fiber input from the forebrain. The

amygdala has also been shown to modulate cerebellar learning during eyeblink conditioning, through modulating mossy fiber inputs necessary for learning. The necessity of forebrain and cerebellum for acquisition and expression of trace eyeblink conditioned responses has been well-established, however, the dynamic interactions among these structures during learning has not been investigated. The current experiment used simultaneous tetrode recordings in anterior cingulate (right), amygdala (right) and anterior interpositus nucleus (left) during acquisition of trace eyeblink conditioning (250 ms tone/500 ms trace interval) to investigate the evolution of dynamic interactions among these necessary structures during forebrain dependent cerebellar learning. Single unit analysis revealed greater activity during the CS and trace interval in each area on trials when the rat showed a conditioned response relative to non-response trials, and alternatively more activity was found after the US on trials when the rat failed to show a conditioned response. Spectral power analyses showed that theta emerged in all three regions across learning. However, the theta emerged earlier in cerebellum than in the anterior cingulate or amygdala. Coherence was observed between each iteration of pairs and was greatest between cerebellum and anterior cingulate. The synchronous activity between areas became more organized in time during the sessions when the rats transitioned from an unlearned to a learned state. These results indicate that the cerebellum has dynamic interactions with the forebrain during acquisition of trace eyeblink conditioning and these interactions may be playing a larger role in the learning process than previously thought.

Disclosures: H.E. Halverson: None. **J. Kim:** None. **J.H. Freeman:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.08/Q18

Topic: E.02. Cerebellum

Support: NIH DC2390

Title: Predictive processing by Purkinje cells in the vestibular cerebellum during active versus passive rotational and translational self-motion

Authors: *O. ZOBEIRI[1], K. E. CULLEN[2];
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Abstract: The ability to distinguish between self-generated (reafference) vs. externally-applied (exafference) sensory signals is fundamental for ensuring accurate motor control as well as perceptual stability. This is particularly evident in the context of the vestibular system, in which the same central neurons that receive direct afferent input also project to motor neurons that

control vestibulo-spinal reflexes (VSR). Notably, while VSRs are essential for providing a postural response to unexpected perturbation, they are impeding during active head motion. Previous studies by our group have shown that central VSR neurons selectively code passive head movements. Although the cerebellum is hypothesized to play a crucial role in the cancellation of their responses to active motion, the exact underlying neural mechanism is unknown. Accordingly, here we recorded from Purkinje cells in the vestibular cerebellum (anterior vermis, lobules IV-V) in rhesus monkeys during comparable active & passive rotational and translational head movements. We first found that simple spike activity encodes passive head motion in a direction-dependent manner. Accordingly, for each Purkinje cell, we first developed a linear dynamic model of its neuronal response based on passive head movements kinematics in each direction. Then to compare each neuron's responses to active versus passive movements, we fit comparable models to neuronal responses during preferred and non-preferred active head movements. We found that neuronal sensitivities were markedly attenuated in the active condition (~70%, $p<0.01$). Next, we compared the patterning of climbing fiber inputs to the Purkinje cells for passive vs. active head movements and found that the probability of a complex spike firing increased (~60%, $p<0.01$) relative to baseline immediately following (20ms) the onset of head movement in the passive condition. In contrast, we found no increase in the probability of complex spike firing in the active condition, suggesting that complex spikes are preferentially elicited in response to externally-applied versus self-generated vestibular inputs. We speculate that the higher probability of complex spikes following the onset of a passive movement facilitates an increase in the sensitivity of the simple spike response to the vestibular stimulation. Taken together, these results provide new insights into the computations performed by Purkinje cells in anterior vermis that underlie the suppression of vestibular reafference.

Disclosures: O. Zobeiri: None. **K.E. Cullen:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.09/R1

Topic: E.02. Cerebellum

Support: Conacyt CB-154645

Title: Differential axonal projections from substantia nigra and ventral tegmental area into cerebellum

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[2]Inst. of Physics, Univ. Autonoma De San Luis Potosi, San Luis Potosi, Mexico

Abstract: Both the mesencephalic dopaminergic nucleus substantia nigra (A9) and the cerebellum play important roles in the control and integration of motor skills. The presence of the Dopaminergic markers tyrosine hydroxylase (TH) and Dopamine type 2 receptors (D2r) in the cerebellum, and the fact that there are no dopaminergic neurons in the cerebellum, suggests a possible direct interconnection between substantia nigra and the cerebellum.
In order to determine whether exists axonal projections from the substantia nigra *pars* compacta into cerebellum. We performed pressure injections of the anterograde tracer Dextran Alexa-Fluor 546 into the SNc as well as into VTA of adult p90 mice. 10 days later, the fluorescent signal was detected in different areas of the cerebellum. Conversely, injection of the retrograde tracer FluoroGold in the cerebellum produced the recovery of fluorescent signal within the substantia nigra and the VTA as well. We found that both the lateral part of substantia nigra and substantia nigra pars compacta project axons into lobules 4/5 and 3 of the cerebellum, whereas substantia nigra *pars* reticulata does project axon terminals mainly into lobule 6 and scarcely within lobules 4/5 and 3. We also found that VTA sends axonal projection into lobule 3. Moreover, when FluoroGold stained slices were counter-immunostained against TH, we found that the projection from VTA was predominately Dopaminergic whereas the nigral projections were mainly non-Dopaminergic. Our results reveals the presence of differential axonal projections from the ventral mesencephalon to the cerebellum. We speculate that this interconnection might be involved in fine motor integration.

Disclosures: A. Juárez-Tello: None. **J.A. Mendez:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.10/R2

Topic: E.02. Cerebellum

Title: Sphingolipid levels in cerebellar compartments and its implications in Purkinje cell degeneration

Authors: *F. G. C. BLOT[1], M. KRIJNEN[1], S. M. DEN HOEDT[2], C. R. OSORIO[1], M. T. MULDER[2], M. SCHONEWILLE[1];
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Abstract: Lipid metabolism is known to be essential for Purkinje cell survival and degeneration. Knock-out mouse models for various enzymes in the sphingolipid metabolism pathway present a patterned degeneration throughout the cerebellar cortex, which is in line with the Zebrin II defined cerebellar modules. This non-homogeneous degeneration has also been observed in wide range of cerebellar pathologies, however, no mechanism has been so far identified to explain this Zebrin II-like patterned degeneration. To elucidate whether sphingolipid metabolism relates to

this phenotype we investigated the compartmentalization of sphingolipids and their relative enzymes. Here, we report differential sphingolipid levels in the cerebellar compartments, together with differential expression of the enzymes involved in this pathway. To assess whether sphingolipid metabolism is implicated in Purkinje cell degeneration, we crossbred mice that lack Sphk1 with mice carrying a mutated Atxn1 [82Q], a model for SCA1 that is featured by Purkinje cell degeneration. The Sphk1 deficiency in the Atxn1[82Q] results in an aggravated PC degeneration and appearance of a Zebrin II-like pattern of degeneration.

Disclosures: F.G.C. Blot: None. **M. Krijnen:** None. **S.M. den Hoedt:** None. **C.R. Osorio:** None. **M.T. Mulder:** None. **M. Schonewille:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.11/R3

Topic: E.02. Cerebellum

Support: Fulbright Scholarship E0576862
NIH R37-NS39395

Title: Whisking related sensorimotor integration in the cerebellar corticonuclear circuit

Authors: *M. P. MEDINA, S. T. BROWN, I. M. RAMAN;
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Abstract: With extracellular recordings of crus I/II Purkinje cells (Pkj) and cerebellar nuclear cells (CbN) of the mouse lateral nucleus, we previously found that sensory input associated with air-puff evoked whisking drives well-timed suppressions of Pkj cell firing that correspond to elevations of CbN cell firing, suggesting that millisecond time-scale disinhibition increases CbN responses to mossy fiber input, augmenting motor output. Here, we investigated Pkj and CbN responses to contrasting, naturalistic sensory stimuli, namely, (1) passive contacts, in which a bar displaces previously stationary whiskers and (2) active contacts, in which mice actively protract their whiskers into the bar. With passive contacts, firing rates of Pkj cells first showed a well-timed transient decrease, and then increased for hundreds of ms, as they did in response to puffs. CbN cells showed transient increases in firing rate that peaked and decayed to baseline before the maximal Purkinje cell response. Passive contacts also reliably elicited Pkj complex spikes (CS). In contrast, with active contacts, well-timed Pkj cell inhibition and CbN cell disinhibition were greatly reduced or absent. Instead, increases in both CbN and Pkj cell firing rate began before contact, suggesting the activity of both cell types was primarily whisking-related. CS were not modulated with active contacts suggesting that self-initiated, likely predictable, sensory inputs are less effective than externally applied, unpredictable, stimuli at engaging the inferior

olive. We next compared Pkj and CbN responses by triggering the averaged responses to active movement onset rather than the sensory contact. Both Pkj and CbN cells showed activity that scaled with protraction amplitude. Changes in Pkj simple spike firing rate correlated well with changes in whisker position, outlasting the time of contact (which was about 50 ms after movement onset). In contrast, CbN cell responses, which preceded Pkj responses, were transient, peaking 8 ms after movement onset, well before contact. CbN firing rate changes correlated well with whisker velocity. These findings provide evidence that (1) externally applied but not self-initiated sensory inputs inhibit and synchronize Pkj cell simple spikes, briefly disinhibiting CbN cells, and elicit Pkj complex spikes; (2) with self-initiated contact, Pkj and CbN cell activity is more strongly related to whisker movements rather than sensory input; and (3) CbN cell responses during active whisking are well correlated to whisker velocity.

Disclosures: **M.P. Medina:** None. **S.T. Brown:** None. **I.M. Raman:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.12/R4

Topic: E.02. Cerebellum

Support: T32 HL07446

Title: The role of dystrophin on synaptic function in cerebellar nuclei

Authors: *T. KREKO-PIERCE[1], J. R. PUGH[2];
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Abstract: Muscular dystrophy is an X-linked myopathy caused by mutations in dystrophin, the protein product of the DMD gene. In skeletal muscle dystrophin is a key component of the multiprotein dystroglycan complex acting as a linker between the intracellular cytoskeleton and the extracellular matrix, thus mediating the structural stability of the plasma membrane. In addition to high expression in the muscle tissue, dystrophin is also expressed in the central nervous system, particularly in neurons of the hippocampus and cerebellum; however, the role of this protein in the CNS remains largely unknown. Interestingly, many individuals with muscular dystrophy display severe cognitive deficits, suggesting the role of dystrophin in normal neuronal function. We hypothesize that dystrophin mutations disrupt normal cerebellar function which contributes to the loss of motor and cognitive function observed in muscular dystrophy patients. To test this hypothesis we use a combination of genetics (mouse model of muscular dystrophy-mdx), patch-clamp slice electrophysiology and immunohistochemistry and focus our analysis on the cells of the deep cerebellar nuclei, the sole output channel of the cerebellum. Using these

approaches we find significant morphological and functional synaptic impairments in the cells of the deep cerebellar nuclei of our mdx mouse.

Disclosures: T. Kreko-Pierce: None. **J.R. Pugh:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.13/R5

Topic: E.02. Cerebellum

Title: Reduced molecular layer interneuron inhibition to Purkinje cell in a mouse model of muscular dystrophy

Authors: *W.-C. WU, R. D. HOWELL, A. ZANOT, A. GINSBERG, J. R. PUGH;
Cell. and Integrative Physiol., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Duchenne muscular dystrophy (DMD) being the most common form of childhood muscular dystrophy is caused by mutations in the gene encoding the protein dystrophin. In addition to muscle-related symptoms, about one third of patients display a range of cognitive deficits thought to result from loss of dystrophin expressed in the brain. While the function of dystrophin in muscle tissue has been researched in detail, its role in the central nervous system is poorly understood. It has been demonstrated that the highest expression of dystrophin in the brain is in cerebellar Purkinje cells, where it is colocalized with postsynaptic $GABA_A$ receptor clusters. We investigated changes in molecular layer interneuron (MLI)-Purkinje cell inhibitory synaptic transmission and Purkinje cell spontaneous firing using *mdx* mice, a mouse model of DMD lacking full-length dystrophin expression. In acute cerebellar slices we found that the amplitude and frequency of both spontaneous and miniature inhibitory postsynaptic currents of *mdx* Purkinje cells are reduced. This is accompanied by a decrease in the number of vesicles released at the basket cell (a type of MLI)-Purkinje cell synapse, suggesting dysfunctional synaptic transmission with possibly fewer release sites being present at MLI-Purkinje cell synapses. Purkinje cell firing activity has been demonstrated to play an integral role in computing behavioral output signals. We found that Purkinje cells from *mdx* mice fire more regularly and in paired basket-Purkinje cell recording we showed that basket cells from *mdx* mice are less capable of driving pauses in Purkinje cell firing. Current DMD treatments have targeted muscle tissue; however, our data shed light on involvement of dystrophin in the cerebellum which pave the way to future therapies targeting CNS-driven symptoms.

Disclosures: W. Wu: None. **R.D. Howell:** None. **A. Zanot:** None. **A. Ginsberg:** None. **J.R. Pugh:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.14/R6

Topic: E.02. Cerebellum

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Singapore Ministry of Education MOE2017-T3-1-002

Title: Dissecting the organization and function of spinal projecting circuits from the interposed nucleus of the cerebellum

Authors: *A. R. THANAWALLA[1], A. K. K. YIP[2], N. ZAINOLABIDIN[2], J. M. CONNER[1], A. I. CHEN[2], E. AZIM[1];
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Abstract: The cerebellum guides the coordination and precision of dexterous forelimb movements. These behaviors ultimately depend on output circuits that reside in the deep cerebellar nuclei (DCN). While most studies have explored DCN output to thalamic and brainstem structures, direct projections from the DCN to the spinal cord have primarily been described through classical tracing studies. The existence of these descending pathways suggests a means for the cerebellum to adjust the activity of spinal motor circuits directly and rapidly update motor output. However, the contributions of direct cerebello-spinal projections to forelimb movement and the specific classes of spinal motor circuits these projections recruit has not been defined.
Using molecular-genetic tools in mice, we describe subpopulations of neurons in the cerebellar interposed nucleus that directly target the cervical spinal cord. Using viral and genetic anterograde and retrograde mapping strategies to characterize their afferent and efferent connectivity, we find that interposed neurons target specific classes of premotor interneurons in the cervical spinal cord, and receive input from discrete supraspinal sensorimotor areas. Additionally, we observe that a distinct subset of spinal projecting interposed neurons also targets the thalamus, suggesting a role for this subpopulation in conveying both ascending and descending information during movement. To further define the diversity of interposed neurons, we performed single-cell gene expression analysis and uncovered putative molecular distinctions

between DCN neurons that reveal novel neuronal subtypes and guide the development of genetic tools for analysis of cerebellar output circuits. Finally, using molecular-genetic perturbation combined with high-resolution quantitative forelimb behavioral assays, we are exploring the role of spinal projecting interposed neurons in skilled forelimb movements. These findings define the organization of direct cerebellar output pathways to forelimb motor circuits in the spinal cord, revealing a potential neural substrate for rapid online correction of dexterous behaviors.

Disclosures: A.R. Thanawalla: None. **A.K.K. Yip:** None. **N. Zainolabidin:** None. **J.M. Conner:** None. **A.I. Chen:** None. **E. Azim:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.15/R7

Topic: E.02. Cerebellum

Support: I01BX003666

Title: Tottering mutation is not associated with breakdown of vermis Purkinje cell simple spike synchrony

Authors: *T. L. CONNOR[1], A. KETTING-OLIVIER[1], J. S. STAHL[2];
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Abstract: Mutations of Cacna1a, the gene coding for the ionophore component of P/Q (CaV2.1) calcium channels, cause cerebellar motor deficits including ataxia and deficient compensatory eye movements. Several lines of evidence that began with recordings of flocculus Purkinje cells (PCs) in the Cacna1a mouse mutant *tottering* (*tg*) support the hypothesis that disruptions in PC autorhythmicity ultimately negate the influence of cerebellar cortex over the PCs' synaptic targets in the deep cerebellar nuclei (DCN) and vestibular nuclei (VN). The mechanism of this interference remains unclear. Simple spike (SS) activity of adjacent PCs is known to exhibit partial synchrony, and this synchrony has been demonstrated to enhance the response of DCN neurons to their excitatory inputs. A possible explanation for cerebellar cortical dysfunction in Canca1a mutants is that the disruptions in PC autorhythmicity also disrupt the normal PC-PC synchrony. To test this idea, we assessed whether PCs of the anterior vermis of *tg* exhibit reductions in PC-PC synchrony. PC pairs (putative and definitively identified by documentation of SS pauses induced by complex spike (CS) activity) were recorded with tetrode electrodes in awake/restrained *tg* and C57BL/6 controls. PCs were located primarily in the depths of vermis folia 4-6. As in previous studies by us and others, *tg* PCs exhibited prominent irregularity in their SS discharge rates. From the cross-correlograms of the activity of each pair, we defined a

synchrony index (SI) as the area in the central ±1.5 ms of the correlogram that falls above the "baseline" cross-correlation (average value over the ±20-40 ms regions of the cross-correlogram), normalized to that baseline cross-correlation. Since the synchronized non-firing that occurs before and after each synchronized spike may be more relevant than the synchronized spikes themselves in controlling PC targets (because the release from inhibition during the non-firing allows PC targets to spike), we also defined an analogous complementary synchrony index (CSI) based on the area falling below the baseline cross-correlation within the central ±3 ms. In 51 *tg* and 51 C57BL/6 PC pairs, SI was non-significantly greater for *tg* vs. C57BL/6 controls (mean±SD were 0.98±0.51 vs. 0.83±0.51), and CSI were essentially equal (*tg*: 0.73±0.49; C57BL/6: 0.74±0.48). Thus, judging from observations in the anterior vermis, the prominent irregularity of PC firing in *tg* is not likely to be inducing cerebellar dysfunction through a deleterious effect on PC-PC synchrony.

Disclosures: T.L. Connor: None. **A. Ketting-Olivier:** None. **J.S. Stahl:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.16/R8

Topic: E.02. Cerebellum

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International Essential Tremor Foundation (IETF) grant (to EKM)

Title: Purkinje cell specific $GABA_{A\alpha1}$ - knockout mice display a tremor phenotype

Authors: *A. K. NIETZ, C. KROOK-MAGNUSON, H. GUTIERREZ, J. KLEIN, C. SAUVE, S. MITCHELL, E. KROOK-MAGNUSON;
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Abstract: Essential tremor (ET) is the most prevalent movement disorder and can become disabling for patients. Previous research has shown aberrant activity in the cerebellum and other regions related to motor control coincide with tremor. The limited number of animal models of essential tremor have made it difficult to study the pathophysiology of tremor, the brain regions involved, and the mechanisms by which anti-tremor drugs exert their effects. Prior studies characterized a tremor model in which the α1 subunit of $GABA_A$ receptors is knocked out globally [1, 2]. Global knockout of the $GABA_{A\alpha1}$-subunit results in significantly reduced inhibitory transmission in the brain and tremor in homozygous knockout mice. Here we show that Purkinje cell specific deletion of the $GABA_{A\alpha1}$ subunit is sufficient to produce tremor,

supporting the idea that cerebellar dysfunction may be sufficient for tremor generation. We recorded spontaneous inhibitory currents (IPSCs) from Purkinje cells (PCs) and molecular layer interneurons (MLIs) in both global and Purkinje cell specific $GABA_{A\alpha1}$ subunit knockout mice. As expected, PCs from PC-specific homozygous knockout mice lacked IPSCs, whereas MLIs did not. Application of the α1 subunit specific agonist zolpidem prolonged synaptic decay in PC-specific knockout MLIs, suggesting the α1 subunit is still present in these cells and confirming the specificity of the knockout. Tremor measurements were performed by suspending animals by the tail from a force transducer. Data were analyzed using the power spectra of the force data. We found that while PC-specific $GABA_{A\alpha1}$ knockouts exhibited less severe tremor, the tremor exhibited had a similar frequency (20-30Hz) as in global knockouts, suggesting similar underlying mechanisms. We also found that ethanol administration (1.25g/kg) was able to ameliorate tremor in PC-specific knockout mice, consistent with previous findings in global knockout mice. PC-specific knockout mice therefore provide a useful tool for future experiments, including experiments aimed at understanding signaling changes giving rise to tremor in PC-specific $GABA_{A\alpha1}$-subunit knockout mice and how drug treatments work to provide symptomatic relief. Our findings in PC-specific $GABA_{A\alpha1}$ - subunit knockout mice reveal that impaired inhibition to cerebellar PCs can be sufficient to induce a tremor phenotype, and therefore highlight the potential significance of the cerebellum, and Purkinje cell inhibition in particular, in tremor.

1. Kralic J.E., et al. *J Clin Invest.* 115(3): 774-9 (2005).
2. Handforth A., et al. *Neuropharmacology* 59(6): 380-7 (2010).

Disclosures: A.K. Nietz: None. **C. Krook-Magnuson:** None. **H. Gutierrez:** None. **J. Klein:** None. **C. Sauve:** None. **S. Mitchell:** None. **E. Krook-Magnuson:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.17/R9

Topic: E.02. Cerebellum

Title: Cerebellar learning and Purkinje cell physiology abnormalities in the prickle2 knock-out mouse model of autism-like behavior

Authors: *P. ABBOTT, J. HARDIE, S. FARLEY, J. FREEMAN, A. BASSUK, K. PARKER; Univ. of Iowa, Iowa City, IA

Abstract: Autism spectrum disorders (ASD) involve abnormalities across brain systems, resulting in a constellation of symptoms including behavioral inflexibility, cognitive dysfunction, learning impairment, altered social interactions, and perceptive time difficulties. Recently, it was discovered that a common gene variant involved in non-canonical Wnt signaling, *prickle2*, was

present in individuals with ASD. Corroborated findings in *prickle2* knock-out and heterozygous mice suggest patterns of behavior similar to individuals with autism including altered social interaction on the three-chambered social task and behavioral inflexibility on the Barnes maze. Additionally, *prickle2* disruption results in hippocampal neuronal abnormalities including reduced dendritic branching, synapse number, and post-synaptic density size. Over the past several decades, there has become an increased focus on the cerebellum's involvement in ASD. Because *prickle2* is expressed in Purkinje cells, this animal model presents a unique opportunity to investigate cerebellar abnormalities that could exist alongside the previously reported autism-like phenotypes. We studied *prickle2*-disrupted mice on delay eyeblink conditioning, a reliable measure of cerebellar-dependent learning. Preliminary data suggest that *prickle2*-disrupted mice have altered learning of conditioned eyelid responses. Additional motor testing suggests no significant impairments in locomotion. We also explored structural and physiological abnormalities in animals with *prickle2* disruption using immunohistochemistry and whole-cell patch clamp recordings. Purkinje cells displayed an aberrant clustering in their respective layer. Recordings show that *prickle2*-null Purkinje cell action potentials have an attenuated afterhyperpolarization, while also displaying an unchanged firing frequency after stepwise current injection. Overall, these data suggest that *prickle2* knock-out mice have cerebellar abnormalities and could inspire future explorations into cerebellar mediated autism-like behavior.

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Poster

405. Cerebellum: Cortex and Nuclei I

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: E.02. Cerebellum

Support: Prodep México, PTC-LIGH-195
CONACyT (LVC) 575913
CONACyT (GMM) 893764

Title: Cerebellar multiunit recording during exploration and grooming behavior in a model of Parkinsonism in male rats

Authors: *J. MANZO[1], M. G. ROCHA[1], L. VÁSQUEZ[1], G. MARÍN[2], M. MIQUEL[3], P. CARILLO[4], M. E. HERNÁNDEZ[1], G. A. CORIA-AVILA[1], *L. I. GARCÍA[1];
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Abstract: Parkinsonism is the combination of characteristic symptoms: tremor at rest, rigidity, bradykinesia-hypokinesia, flexed posture, loss of postural reflexes and the phenomenon of freezing. However, parkinsonism is diagnosed with at least one of the symptoms, i.e., shaking at rest or bradykinesia. As the cerebellum is highly involved in movement control, the aim of our study was to characterize the multiunit activity (MUA) at the cerebellum of male rats during exploration and grooming behavior in a model of parkinsonism. Resembling human parkinsonism, we induced mandibular tremor in male rats by an electrolytic lesion in the ventrolateral striatum (VLS) of the basal ganglia. Wistar male rats (250 to 350 g/bw) were randomly divided into three groups; an intact group that had only implanted the recording electrode (n=12); the sham group (n=12) where the lesion electrode was descended into the VLS but no current was applied; and the lesioned group (n=12) that had implanted the lesion electrode and a current was applied for a bilateral electrolytic lesion (3.5 mA/30s) of the VLS to induce the mandibular tremor. Recording electrodes were implanted to register the MUA of cerebellar neurons at Sim B (n=4), and Crus II (n=4) lobes in the granular layer, and in the deep dentate nuclei (n=4). MUA recordings were performed 48 h after stereotactic surgery and were computed by the Polyview system. Electrophysiological results were analyzed statistically considering the amplitude of traces during exploration and grooming behavior.Results showed statistical differences in Crus II lobe in sham and lesioned groups compared to the intact group, showing a decrease during grooming (P=0.0001), horizontal exploration (P=0.0014), and vertical exploration (P=0.0034). Our data suggested that Crus II lobe had a lower response because a direct interconnection with the VLS, indicating a possible role in parkinsonism of these cerebellar area.

Disclosures: J. Manzo: None. **M.G. Rocha:** None. **L. Vásquez:** None. **G. Marín:** None. **M. Miquel:** None. **P. Carillo:** None. **M.E. Hernández:** None. **G.A. Coria-Avila:** None. **L.I. García:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.19/R11

Topic: E.02. Cerebellum

Title: Purkinje cell encoding of limb position during skilled reach-to-grasp behavior in mice

Authors: *D. J. CALAME, A. L. PERSON;
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Abstract: A leading hypothesis of cerebellar function is that the cerebellum generates an internal model to predict upcoming body kinematics allowing it to facilitate feedforward motor control, making movements smooth and accurate. Previous work in our lab has demonstrated that during

naturalistic reaching movements, the activity in the output of the cerebellum - the deep cerebellar nuclei - is graded in response to the initial velocity of outreach, acting to decelerate the limb to the endpoint on a reach-by-reach basis. Whether upstream elements of the cerebellar circuit learn to control this signaling to structure reach endpoint is unclear. In this study we recorded from Purkinje cells (PCs) while head-fixed mice performed a skilled reaching task in order to understand how simple spikes (SS) and complex spikes (CS) encode limb movements. By tracking paw position using both motion tracking and machine learning throughout the experimental session, we show that SSs are broadly modulated during reach, producing both bursting and pausing patterns. Using a variety of techniques, including multilinear regression of limb end effector kinematics and markerless tracking of limb postures, we show movement kinematics can explain up to 40% of the variance in individual PC firing rates. By adjusting the target position, we also demonstrate that PC SSs modulate their rates differentially during reaches to different distances, consistent with kinematic tuning, providing a possible mechanism by which the deep cerebellar nuclei are modulated during the decelerative phase of movement. We find that CS rate is consistently modulated during reach, and shows endpoint-related activity. Overall, CSs tend to be depressed during the outward phase of reach, consistent with observations in other species.

Disclosures: D.J. Calame: None. **A.L. Person:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.20/R12

Topic: E.02. Cerebellum

Support: NIH NRSA NS103328
McKnight Foundation
Klingenstein Foundation
NSF 1749568

Title: A cerebellar neural mechanism of reach precision and its role in motor learning

Authors: *M. I. BECKER[1], A. L. PERSON[2];
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Abstract: Cerebellar dysfunction results in a unique constellation of behavioral deficits. During reaching movements, a characteristic oscillation of the limb occurs, especially noticeable in the over- and under-shooting of target endpoint, termed dysmetria. Nevertheless, the cerebellar neural mechanisms that impart endpoint precision to reaching movements remain unidentified. Here, we describe the relationship between activity in the anterior Interposed Nucleus (IntA) and

reach kinematics in order to understand how cerebellar output is utilized to actuate precise motor control. Using closed-loop optogenetic manipulations and single-unit recordings in mice, we found that IntA adaptively decelerates the limb to support reach endpoint precision and accuracy. More specifically, we observed that IntA activity is reciprocally scaled to match preceding reach velocity, such that both relatively slow and fast reaches decelerate with differing magnitudes to accurately obtain the target. We next asked whether this mechanism of enhancing reach precision via variable engagement of IntA activity is a product of motor learning. First, we tested whether reaches exhibit motor adaptation in response to a neural activity manipulation that produces hypometric reach endpoints. In preliminary experiments, analysis of trial-over-trial changes in reach kinematics in response to randomly-delivered optogenetic IntA excitation (which causes hypometria) revealed motor adaptation toward eumetric endpoints. In addition, we have established a motor adaptation paradigm in which optogenetic stimulation is applied in a block structure, enabling us to ask whether animals exhibit cumulative kinematic adaptation over trials, and whether this adaptation is accompanied by concomitant changes in IntA activity patterns. Future work will seek to identify how the causal relationship between IntA activity and reach kinematics is utilized and adjusted over the course of motor learning.

Disclosures: M.I. Becker: None. **A.L. Person:** None.

Poster

405. Cerebellum: Cortex and Nuclei I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 405.21/R13

Topic: A.08. Development of Motor/ Sensory/ and Limbic Systems

Support: NIGMS 8P20GM103436

Title: Cerebellar expression and function of Cabin1

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Abstract: Unchecked overproduction of cerebellar cells can lead to medulloblastoma, the most common pediatric brain tumor. Cerebellar granule cells are normally overproduced and pruned into adulthood under the regulation of the transcription factors MADS/myocyte-enhancer-factor 2 (MEF2) and p53, both of which are regulated by Calcineurin-binding-protein 1 (Cabin1). MEF2 and p53 have opposing effects on healthy granule cell populations: MEF2 promotes cell survival and p53 promotes apoptosis. In medulloblastoma, the reverse appears: p53 is associated with tumor proliferation and MEF2 promotes apoptosis of tumor cells, while most other genetic determinants are unknown. A major obstacle in understanding the roles of Cabin1 in normal cerebellar development and tumorigenesis lies in reconciling the opposing roles that Cabin1

targets MEF2 and p53 play in normal versus diseased cells. Determining where and when Cabin1 is expressed during normal development and understanding its roles therein will provide clarification. Our central hypothesis is that Cabin1 functions in the cerebellum as a negative regulator of MEF2 and p53 to ensure appropriate numbers of granule cells are generated, maintained, and remodeled. To address these problems, we investigated Cabin1 expression in cerebellar development and function using double *in situ* hybridization in zebrafish. We also used Cabin1 loss-of-function to assess the effects on organismal behavior and target cell populations. Establishing roles for Cabin1 in the cerebellum will further our understanding of normal cerebellar development and of the molecular pathways that contribute to brain cancers.

Disclosures: K.M. Dunn: None. **D.R. Hammond-Weinberger:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.01/R14

Topic: E.05. Brain-Machine Interface

Support: NIH K12HD073945
NSF EEC-1028725

Title: A computational model of neural connectivity dynamics in response to optogenetic stimulation of non-human primate sensorimotor cortex

Authors: *J. BLOCH[1], E. T. SHEA-BROWN[2,3,5], A. YAZDAN-SHAHMORAD[1,4]; [1]Bioengineering, [2]Applied Mathematics, [3]Physiol. and Biophysics, [4]Electrical and Computer Engin., Univ. of Washington, Seattle, WA; [5]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Neural stimulation has been shown to result in the reorganization of neural connectivity. The details of this reorganization, however, are not well understood. According to the spike-timing dependent plasticity framework, paired stimulation of two cortical regions should increase the connectivity between the two stimulation sites. In-vivo experiments have been inconsistent in producing this targeted connectivity change, likely due to the complexity of network interactions mediating connectivity between a pair of sites. This underlying complexity of the network, in turn, necessitates the advent of more complex frameworks for targeted connectivity change.
In this study we analyze previously published data from Yazdan-Shahmorad et al. to develop a computational model of optogenetically induced network connectivity change. We then solve a controller which could allow us to change connectivity between two sites in a closed-loop, targeted manner. The dataset consists of neural signals recorded through micro-electrocorticography (uECoG) from the primary sensorimotor cortex of non-human primates,

and contains resting-state activity, activity in response to paired optogenetic stimulation protocols, and activity immediately after stimulation sessions.
To this end, we use a Kalman filter based approach to fit a linear state-space dynamical model composed of interactions between all electrodes, optogenetic stimulation, and gaussian noise. We then use this identified model to fit a controller of connectivity between two target electrodes. The state of the model is characterized by frequency band powers and the connectivity is characterized by frequency band coherence. Such a controller could optimize optogenetic stimulation in a closed-loop fashion to maximize the coherence between two neural sites. Future efforts can deploy this model to real-time for neurorehabilitation of aberrant brain connectivity.
A. Yazdan-Shahmorad, "Targeted cortical reorganization using optogenetics in non-human primates," ELife, 2018.

Disclosures: **J. Bloch:** None. **E.T. Shea-Brown:** None. **A. Yazdan-Shahmorad:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.02/R15

Topic: E.05. Brain-Machine Interface

Support: NRF grant 2017R1C1B5017593
IITP grant 2017-0-00451

Title: Calibration-less p300 speller system using massive data and convolutional neural network

Authors: ***J. LEE**[1], M. AHN[1], M. KWON[2], K. WON[2], S. JUN[2];
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Abstract: Most BCI systems require calibration process where data is extracted from the user to construct a subject-specific classifier. This time-consuming process can be tedious for any user since there is no meaningful interaction. In this study, we introduce a novel way of achieving calibration-less BCI system in case of conventional 6 × 6 P300 speller paradigm. P300 speller uses P300 response for classification, and calibration process is essential because short inter-stimulus-interval is set to achieve high information transfer rate. Our idea is to feed large amount of other subjects' data into convolutional neural network (CNN). We first conducted offline analysis, using P300 speller data acquired from 55 subjects. For each subject, there were 2 training sessions of 5 letters each and 4 testing sessions of 7 letters each. As a whole, there were 99,000 trials acquired from all subjects' training session, of which target-nontarget ratio is 1:5. We used EEGNET as our CNN architecture and tested the network on the testing sessions [1]. Number of kernels per layer were increased compared to conventional architectures used for

P300 classification. This is so that CNN can store various forms of P300 signals. We compared our result to stepwise linear discriminant analysis (SWLDA). SWLDA was trained using conventional method and our method as well; to train with large dataset acquired from other subjects'. Our results show that calibration-less BCI system is achievable through simple usage of CNN and massive data acquired from other subjects. CNN, when trained using only the subject's data (conventional approach), achieved average letter accuracy of 93.63%, while CNN, when trained using other subjects' data achieved letter accuracy of 89.22%. As for SWLDA, it achieved 94.28% when using conventional method while achieving only 69.80% using our proposed method. Unlike conventional methods, CNN when combined with large dataset, achieves calibration-less BCI without any complicated methods. Our work goes further to improve this calibration-less, or zero-trained BCI system. Instead of using the output from the network as the system's output, we integrated a linear classifier (SWLDA). This linear classifier receives its label information from pre-trained CNN, and trains itself whenever new data comes in. Using this semi-supervised approach combined with zero-training approach, we were able to further increase the average letter accuracy up to 92.98% that is not statistically different from the performance of conventional method by SWLDA ($p > 0.05$).

Disclosures: **J. Lee:** None. **M. Ahn:** None. **M. Kwon:** None. **K. Won:** None. **S. Jun:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.03/R16

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1F31HD098804-01
A. Alfred Taubman Medical Research Institute

Title: Optimizing decoding performance and power consumption for closed-loop implantable brain-machine interfaces

Authors: ***S. R. NASON**[1], A. K. VASKOV[2], P. G. PATIL[1,3,4,5], C. A. CHESTEK[1,2,5,6];
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Abstract: Clinical translation of current brain-machine interfaces is limited by the need for patients to remain tethered to large processing computers to transmit the typical 96 channels of neural data from a Utah microelectrode array. Fully implantable brain-machine interfaces would mitigate that issue, but they have severe computational limitations compared to laboratory machines. Many groups have investigated algorithms in their laboratories ranging in complexity from linear regressions to deep neural networks, but there has been no systematic evaluation for

relevance to implantable devices. Thus, using the embedded device we previously presented (Bullard 2019), we compare linear regressions (LR), Kalman filters (KF), and shallow feedforward neural networks (NN) in terms of their suitability for implantable neural interfaces, focusing on computational complexity, execution time, and power consumption.
For this analysis, we assume a one-dimensional task and a 1 by 96 word (two byte) input feature vector containing the brain state estimate for a given 50ms time step. Since LR requires substantial neural history for adequate decoding performance, our implementation models a 10 bin Wiener filter (Chestek 2011). Our KF implementation follows the position-velocity steady-state KF approach (Malik 2011). The simple, preliminary NN is a dense, fully connected network of rectified linear units with two 24 unit hidden layers and a linear output unit. To test these algorithms on hardware, we implemented them on the 32-bit Atmel AT32UC3C2256C microcontroller we previously used and programmed it using embedded C. Each algorithm received randomized weight values between 0.1 and 1 and randomized inputs between 1 and 200. We estimated execution time by toggling voltage when the iteration began and ended, where the processor entered a standby sleep state during the remainder of each 50ms bin.
KF, LR, and NN had complexities of 300, 960, and 2,952 floating-point operations per iteration, respectively. The KF had the shortest execution time at 1.86ms, followed by LR at 3.84ms and the NN at 23.8ms. The KF had the lowest power consumption at 4.3mW, followed by LR at 6.6mW (due to the addition of history) and NN at 14mW for the microcontroller.
These results suggest that KFs have the quickest execution time and lowest power requirements to achieve the performance levels demonstrated in the literature. This implies KFs would work well in a closed-loop implantable brain-machine interface, but alternative NNs should be tested to best optimize performance and complexity. In our next steps, we aim to use our embedded device to validate these algorithms in closed-loop with nonhuman primates.

Disclosures: **S.R. Nason:** None. **A.K. Vaskov:** None. **P.G. Patil:** None. **C.A. Chestek:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.04/R17

Topic: E.05. Brain-Machine Interface

Support: Chen Institute of Neuroscience

Title: Deep multi-state dynamic recurrent neural networks for robust brain machine interfaces

Authors: *B. HAGHI[1], S. KELLIS[2], M. ASHOK[1], S. SHAH[1], L. BASHFORD[2], D. KRAMER[3], B. LEE[3], C. LIU[3], R. ANDERSEN[2], A. EMAMI[1];
[1]Electrical Engin., [2]Biol. and Biol. Engin., Caltech, Pasadena, CA; [3]Neurorestoration Ctr. and Neurosurg., USC, Los Angeles, CA

Abstract: Brain-machine interfaces (BMIs) can help spinal cord injury (SCI) patients by decoding neural activity into useful control signals for guiding robotic limbs, computer cursors, or other assistive devices. BMI in its most basic form maps neural signals into kinematics, then closes the loop to enable direct neural control of kinematics. Such systems have shown promise in helping SCI patients; however, improving performance and robustness of these systems remains challenging. Even for simple movements, such as moving a computer cursor to a target on a computer screen, decoding performance can be highly variable over time.
Furthermore, most BMI systems currently run on high-power computer systems. Clinical translation of these systems will require decoders that can adapt to changing neural conditions, and which operate efficiently enough to run on mobile—even implantable—platforms. Recently, machine learning algorithms have shown promise in attaining high performance and robustness in BMIs. Therefore, to address a number of these challenges, we propose a deep multi-state Dynamic Recurrent Neural Network (DRNN) architecture. The DRNN is used for predicting Cartesian representation of kinematics from the open-loop neural data recorded from the posterior parietal cortex (PPC) of a human subject over 39 days in a BMI system. We design the algorithm to achieve a reasonable trade-off between performance, robustness, and to reduce the memory that is required to store the weights for hardware implementation. To achieve a better prediction performance and robustness, we generalize our model by feeding the predictions of the network back to the input. To solve the statistical distribution mismatch between the ground-truth and predictions, we apply a scheduled sampling approach to the model. By configuring the DRNN to operate without history, we reduce the number of memory accesses which has shown to consume the most significant power in neural network accelerators (Watmough et al., 2018). We compare our algorithm with the state-of-the-art methods in the literature to show that it performs favorably: DRNN achieves average correlation coefficients of (0.75, 0.88) for position (X, Y) and (0.74, 0.87) for velocity in X and Y directions. To the best of our knowledge, this is the first demonstration of applying deep learning-based decoders to human PPC data. The results show that multi-state DRNN has the potential to model the non-linear relationships between the neural data and the kinematics for robust BMIs.

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Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.05/R18

Topic: E.05. Brain-Machine Interface

Support: NIH/NINDS GRANT 1R01NS105646-01A1

Title: Source localization of Mu ERD in response to BCI intervention

Authors: K. GJINI, **A. REMSIK**, M. MAZROOYISEBDANI, L. WILLIAMS, *V. A. NAIR, V. PRABHAKARAN;
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Abstract: Objective: This research seeks to obtain source localization of neurophysiological changes associated with BCI intervention sessions for upper extremity motor recovery. We investigated whether Mu rhythm desynchronization of motor brain areas (ERD), compared to rest, changes between the beginning to the end (pre and post) of therapy in all sessions across all subjects (i.e. grandaveraged). **Methods**: N= 16 right hemisphere stroke survivors participated in 9-15 sessions with the BCI. Participants executed hand movements in response to visual cues displayed on a computer screen concordantly with the corresponding audio instructions (e.g., Left, Right, Rest). The 'screening' sessions (i.e. pre and post BCI intervention) contained two runs, each consisting of 15 trials for rest, left hand, and right hand movements (i.e., 5 trials for each of the three conditions, the order of trials in a run was random). All data from the pre and post conditions were then grandaveraged, respectively. eLORETA source estimates were calculated as follows: 1) Clean EEG data were segmented (LEFT hand attempted movements and REST) separately for PRE and POST conditions. 2) Cross-spectra for Mu[8-12 Hz] band were computed and then averaged (i.e. 1 average per each subject separately for Movement (left, right) and REST trials.3) eLORETA of Mu band power estimates at 6239 cortical locations/voxels were obtained, then normalized across all subjects. In the eLORETA implementation, computations are made in a realistic head model using the MNI152 template, with the three-dimensional solution space restricted to cortical gray matter, as determined by the probabilistic Talairach atlas. The specific frequency band cross-spectra (frequency-domain) obtained from the average-reference potential data, were the inputs for source localization. 4) eLORETA estimates between the two conditions [Movement - Rest] were then calculated in cortical space. 5) Statistical group comparison of POST vs PRE estimates were done using a one tailed t-test (ROI-based). **Results**: ERD increases were observed from pre-post ipsilesionally at primary motor cortex (Brodmann Area (BA) 4) and supplementary motor areas (BA 6), and contralesionally at BA 6. Additionally, ERD decrease was observed at contralesional BA 4. **Conclusion**: BCI intervention may help facilitate increased ERD in association with movement of the impaired upper extremity for the stroke-lesioned brain.

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Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.06/R19

Topic: E.05. Brain-Machine Interface

Support: NIH 1R01NS104923-01
ARO MURI contract W911NF-16-1-0368

Title: Accurate prediction of large-scale LFP network dynamics in response to electrical stimulation

Authors: *Y. YANG[1], S. QIAO[3], O. G. SANI[1], B. PESARAN[3], M. M. SHANECHI[1,2];
[1]Ming Hsieh Dept. of Electrical and Computer Engin., [2]Neurosci. Grad. Program, USC, Los Angeles, CA; [3]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Modeling how neural activity recorded from large-scale distributed brain networks responds to electrical stimulation is critical for developing neurotechnology that aims to precisely modulate brain network activity. Here, we develop dynamic input-output (IO) models to describe the real-time LFP network response to continuously-changing stimulation patterns and test them in non-human primates (NHPs). To obtain informative data for learning the IO models, we apply a new stimulation pulse train to fully excite the LFP network. As the input in our models, we use the pulse train frequency and amplitude, which change in real time. As the output in our models, we use LFP power features. We fit and evaluate the IO models using cross-validation, where we use the trained IO models to feed-forward predict the evolution of LFP power features in response to stimulation in the test set. We compare the predicted evolution to the observed evolution. Further, we test if at-rest network connectivity can predict the variable strength of the stimulation effect across the network nodes. We calculate at-rest network connectivity using LFP power features that are recorded without stimulation. We find that our IO models accurately predict the dynamic LFP network response in cross-validation. Also, the dynamic structure of the IO models and modeling the changes in both stimulation amplitude and frequency are essential for this prediction. Moreover, the strength of the dynamic stimulation effect at different brain regions can be predicted from at-rest connectivity within the network. Our results have important implications for future design of closed-loop brain stimulation systems for treatment of neurological and neuropsychiatric disorders and for more precise modulation of brain functions.

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Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

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Program #/Poster #: 406.07/R20

Topic: E.05. Brain-Machine Interface

Support: MURI ARO W911NF-16-1-0368
NYU NIH 1R01NS104923-01
ONR YIP N00014-19-1-2128

Title: Dynamical characteristics of simultaneously-recorded spike and LFP activities underlying movement

Authors: *H. ABBASPOURAZAD[1], Y. WONG[2], B. PESARAN[3], M. M. SHANECHI[1]; [1]Electrical Engin., USC, Los Angeles, CA; [2]Monash Univ., Melbourne, Australia; [3]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Investigating low-dimensional neural dynamics underlying movements has so far focused on a single scale of brain activity, in particular neuronal spikes. However, movements are encoded across multiple spatial and temporal scales of neural activity, from spikes to local field potentials. Thus, examining the dynamical characteristics of spike-field network activity is important in understanding the neural mechanisms underlying movements. Here we developed multiscale dynamical models and a modal analysis to investigate the low-dimensional dynamical characteristics of simultaneously-recorded spike and LFP activities from non-human primates performing a 3D reach-to-grasp task. Our modal analysis allowed us to dissociate the different components of neural dynamics each with a distinct dynamical characteristic. We refer to these components as dynamical modes. We found that while spike and LFP networks had some distinct dynamical modes, they had a strongly present shared mode. This mode was the dominant mode in predicting the movement. In addition, these shared dynamical characteristics were preserved across different experimental sessions and monkeys. Further, these shared modes were not a replicate of the modes in the behavior. Finally, we used a novel multiscale algorithm to identify latent states and dynamics from mixed spike-LFP activity and found that the same shared unified mode was identified again from hybrid spike-LFP activities.

Disclosures: H. Abbaspourazad: None. **Y. Wong:** None. **B. Pesaran:** None. **M.M. Shanechi:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

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Program #/Poster #: 406.08/S1

Topic: E.05. Brain-Machine Interface

Support: ARO W911NF-16-1-0368
ARO W911NF1810434

Title: Decoder for switching state space models with spike-field observations

Authors: *C. Y. SONG[1], H.-L. HSIEH[2], M. M. SHANECHI[1];
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Abstract: The dynamics of brain activity are inherently nonstationary and can change depending on context, for example based on the tasks performed, the stimuli received, or the attention focus maintained. Thus, to decode brain states in naturalistic scenarios, it is necessary to model and track such changes. Switching state space models can provide a basis for modeling a hidden neural state whose dynamics switch based on a discrete context. Previous works have used such a model for decoding behavioral states from a single neural recording modality. However, it is becoming increasingly common to measure the brain at multiple spatiotemporal scales by recording both spike and field activities simultaneously. Thus, tracking non-stationarity necessitates efficient decoders that can detect changes in spike-field neural dynamics and accurately estimate the underlying neural and behavioral states. Here, we develop a framework to address these challenges. We build a multiscale switching dynamical model that assumes an underlying hidden neural state can evolve with dynamics chosen arbitrarily from a finite set based on the value of a switch state. This switch state would also dictate how the neural state is represented in the recorded binary spike events and continuous field signals. Based on this model, we derive an efficient multiscale decoder that simultaneously estimates the underlying neural and switch states from an identified system using spike-field observations. As validation of these methods, we show with closed-loop numerical simulations that our new decoder is able to accurately estimate the switch state while decoding the neural and behavioral states.

Disclosures: C.Y. Song: None. **H. Hsieh:** None. **M.M. Shanechi:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

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Program #/Poster #: 406.09/S2

Topic: E.05. Brain-Machine Interface

Support: BARI: US army #W911NF1810434

Title: Decoding human confidence from neural signals

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Abstract: Confidence is the perceived notion of correctness in our decisions. Understanding how confidence is encoded in neural signals can enhance our understanding of the underlying brain processes and enable decoding of confidence from neural signals. Neural decoders of confidence may enable brain machine interfaces (BMI) that can intelligently assist humans in decision making when they are not confident about their decisions and can pave the way for cooperation between humans and artificial intelligence. Here, we extend an experimental task designed to evoke confident and non-confident decision making in human subjects and use it to explore neural decoding of confidence. In each trial of this task, a character wearing either a hat or a helmet briefly appears on the screen and subjects attempt to dissociate between the two and report their level of confidence in their decision. We study neural signals recorded during the task to find neural correlates of confidence and explore their spatial and temporal characteristics. To rule out potential confounds, we design multiple control tasks to dissociate the neural correlates of confidence from those of visual stimuli processing and movement. Moreover, we build neural decoders that can classify whether the subject was confident in their decision or not. These decoders aggregate the spectral magnitude and phase information across recording channels to classify an individual subject's confidence at a single-trial level. Finally, we compare the confidence related information contained in different brain regions. These results have important implications for understanding brain processes involved in decision making and confidence, and may enable future human-machine collaboration systems.

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Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.10/S3

Topic: E.05. Brain-Machine Interface

Support: ARO # W911NF-16-1-0368
NSF # CCF-1453868
ONR # N00014-19-1-2128

Title: The topology and geometry of motor cortical dynamics underlying 3D movements

Authors: *H.-L. HSIEH[1], B. PESARAN[2], M. M. SHANECHI[1];
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Abstract: Due to their high dimensionality and complexity, precise modeling of neural population activity is challenging. To date, dynamical modeling and dimensionality reduction approaches have been largely linear. These approaches project the high-dimensional neural activity on a low-dimensional linear hyperplane for example using PCA. Thus, these approaches do not account for nonlinearity in neural dynamics. Here, we tackle the problem of dimensionality reduction for neural dynamics from another direction: identifying the underlying topology and geometry of the neural population activity. We develop tools using algebraic topology and differential geometry to explore the structure embedded in neuronal spiking activity recorded from a non-human primate (NHP) during a 3D movement. We also learn a dynamical model on this structure. We show that utilizing topology and geometry in modeling neural dynamics uncovers their nonlinear structure and sheds light on neural mechanisms of motor control.

Disclosures: H. Hsieh: None. **B. Pesaran:** None. **M.M. Shanechi:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

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Program #/Poster #: 406.11/S4

Topic: E.05. Brain-Machine Interface

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ONR YIP Award N00014-19-1-2128

Title: Multiscale spike-field network causality identification during a motor task

Authors: *C. WANG[1], B. PESARAN[2], M. M. SHANECHI[1];
[1]Electrical Engin., USC, Los Angeles, CA; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Neural representations span various spatiotemporal scales from the spiking activity of single neurons to field activity measuring large-scale networks. To help understand neural mechanisms, simultaneous analyses of causal interactions within networks of spike and field activities are required. However, identifying these causal network interactions is challenging as spikes are binary-valued with a fast timescale while fields are continuous-valued with slower timescales. Traditional causality measures are not designed for these multiscale assessments. We build a novel multiscale causality measure in spike-field networks by constructing a combined likelihood function comprised of point process models for spikes and linear Gaussian models for

fields. We model the firing rates of neurons as a function of the history of both field signals and binary spike events. We model the fields as a function of the history of field signals and the history of the latent log-firing rates of neurons as predictors. We also develop a sequential maximum-likelihood parameter estimation procedure and statistical tests for evaluating significance. We evaluate this novel method by estimating multiscale causality graphs within spike-field network activity recorded from the motor cortex of a non-human primate performing a motor task. Once a causality graph is identified for the network, we measure its accuracy by constructing encoding models with the identified graph and assessing their prediction power for spikes and fields. We find that including the identified multiscale spike-to-field and field-to-spike connections improves prediction accuracy compared to when only single-scale spike-to-spike and field-to-field causal connections are modeled. Also, our method outperforms Granger causality in identifying multiscale causality graphs for both spikes and fields. These results validate the multiscale causality identification method, which can help build more accurate encoding models and study multiscale network interactions.

Disclosures: **C. Wang:** None. **M.M. Shanechi:** None. **B. Pesaran:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

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Topic: E.05. Brain-Machine Interface

Support: NIH # 1R01NS104923-01
ARO # W911NF-16-1-0368
ONR # N00014-19-1-2128

Title: A new preferential subspace identification (PSID) algorithm for learning dynamic neural encoding models with behavior-related latent states

Authors: *O. G. SANI[1], B. PESARAN[2], M. M. SHANECHI[1];
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Abstract: Learning a neural encoding model that describes the representation of a desired behavioral state (e.g., movement kinematics) in neural signals is critical in studying the neural mechanisms underlying behavior and in decoding it for brain-machine interfaces (BMIs). Two main approaches are traditionally used to build an encoding model for behavior: representational modeling and neural dynamics modeling. These approaches learn the dynamics of the encoding model either purely based on behavior (representational modeling) or purely based on neural signals (neural dynamics modeling) and as a result, may learn behavior dynamics that are not encoded in neural signals or behavior-unrelated neural dynamics, respectively. Thus new

approaches are needed that can specifically focus on learning behavior-related neural dynamics to help investigate the encoding of behavior. Here, we introduce a novel learning algorithm, termed preferential subspace identification (PSID), that takes the dynamics of both neural signals and behavior into account and learns a neural encoding model with a low-dimensional behavior-related latent state. Using both numerical simulations and local field potentials recorded from non-human primates during a naturalistic motor task, we demonstrate the advantage of the proposed algorithm in learning behavior-related neural dynamics. We also demonstrate how traditional representational modeling and neural dynamics modeling can be viewed as special cases of PSID. This new learning algorithm can help uncover low-dimensional neural dynamics that underlie behavior and can serve as a unifying framework that extends and relates traditional approaches for learning neural encoding models.

Disclosures: O.G. Sani: None. **B. Pesaran:** None. **M.M. Shanechi:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

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Program #/Poster #: 406.13/S6

Topic: E.05. Brain-Machine Interface

Support: ARO MURI contract W911NF-16-1-0368
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Title: Adaptive modeling of neural network dynamics with optimized learning rate

Authors: *P. AHMADIPOUR[1], Y. YANG[1], M. M. SHANECHI[1,2];
[1]Electrical Engin., [2]Neurosci. Grad. Program, USC, Los Angeles, CA

Abstract: Dynamic latent-state modeling has the ability to model large-scale neural network dynamics and perform dimensionality reduction, leading to its wide use in understanding neural mechanisms and decoding brain states. So far, these models have largely assumed that neural network activity is stationary in time. However, neural network dynamics could be non-stationary for example due to learning and plasticity or due to recording instability, especially over long periods of days or weeks. Therefore, developing adaptive latent-state modeling approaches is important to model non-stationary neural network dynamics but has not been achieved. Current adaptive approaches only focus on tracking representational neural encoding models, in which the state is not latent but rather is the behavior itself. Moreover, a key design parameter in any adaptive model is the learning rate, which controls the weighting of past observations in fitting the current model parameters and can significantly affect the accuracy of the fitted models. Thus a method is needed to optimize the learning in real time. To enable accurate tracking of neural network dynamics, we develop an adaptive latent-state modeling

approach and devise a method to automatically optimize its learning rate in real time. We use an adaptive linear state-space model structure. We develop a recursive adaptive learning algorithm to fit the model parameters in an efficient manner in real time. Finally, we devise an online learning rate optimization algorithm that optimizes the real-time tracking performance. We use comprehensive numerical simulations to validate our adaptive approach. We find that our adaptive algorithm can fit more accurate latent-state models than non-adaptive algorithms. We also find that the learning rate significantly affects the accuracy of the adaptively fitted models, and the optimal learning rate strongly varies as a function neural network properties. Finally, we find that our algorithm enables fast convergence to the optimal learning rate, leading to accurate tracking of non-stationary network dynamics. Together these results have significant implications for investigating non-stationary neural network dynamics, and for developing adaptive neuroethologies to decode and modulate brain states.

Disclosures: P. Ahmadipour: None. **Y. Yang:** None. **M.M. Shanechi:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

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Program #/Poster #: 406.14/S7

Topic: E.05. Brain-Machine Interface

Support: ARO MURI W911NF-16-1-0368
NIH 1R01NS104923-01

Title: Estimating event times from spike trains with a point process matched filter

Authors: *N. SADRAS[1], B. PESARAN[2], M. M. SHANECHI[1];
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Abstract: The spatial response field (SRF) of a neuron describes the region of sensory or behavioral space that it is sensitive to. Further, some neurons exhibit temporal response fields (TRFs), which describe a transient temporal response to stimulus or behavioral event onset. The stimulus sensitivity of such neurons can be jointly characterized by a spatiotemporal response field (STRF). For neurons with purely spatial response fields, algorithms such as the point process filter (PPF) can be used to decode stimulus or behavioral states from spike trains in real time. When neurons exhibit TRFs, however, event times must be known ahead of time in order to perform decoding using existing methods. To solve this problem, an event detection method is necessary. To allow for decoding of neural states from spike trains when neurons exhibit STRFs, we develop the point-process matched filter (PPMF), an algorithm that can detect event times from spike trains. The PPMF can be used along with existing decoders to decode neural states

from neurons that exhibit STRFs when event times are unknown. We validate this method on spiking activity recorded from the prefrontal cortex (PFC) of a rhesus macaque monkey performing a delayed-saccade task. The task required the monkey to make saccades from a central fixation point to one of eight peripheral targets. We use the PPMF to detect task-related saccades, and use a maximum-likelihood point process classifier in order to classify detected saccades into one of eight directions. We evaluate our method via cross-validation across sessions for each day. Our results show that the PPMF can indeed detect events from neural activity, and can be used with classification algorithms to allow decoding from STRF-exhibiting neurons when event times are unknown.

Disclosures: **N. Sadras:** None. **M.M. Shanechi:** None. **B. Pesaran:** None.

Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.15/S8

Topic: E.05. Brain-Machine Interface

Support: Wallace H. Coulter Foundation

Title: Neural signal emulation for closed-loop intracortical brain computer interface decoder design

Authors: ***J. BAE**[1], M. G. PERICH[2], L. E. MILLER[3], Z. C. DANZIGER[1];
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Abstract: Intracortical brain computer interfaces (iBCIs) are a promising avenue to restore the motor function of patients with neuromuscular disabilities. However, even with encouraging results from iBCIs for participants with tetraplegia, the extremely limited number of human participants available for development work remains as a crucial restriction limiting the widespread adaptation of iBCIs. A single participant is typical of most human iBCI studies. Even monkey studies generally have only 2 to 3 subjects. To overcome this limitation, we introduce a novel neural signal emulator which can simulate in real time, the intracortical neural activity of motor cortex associated with reaching tasks derived from human hand kinematics.
Easily accessible, high-dimensional kinematic signals from healthy human subjects (19D hand joint angles) are used as time-varying input to a recurrent artificial neural network (RNN) that emulates intracortical neural dynamics. The RNN (with 2 hidden layers) was trained on multiple sessions of intracortical recordings from arm area primary motor cortex (M1) of 2 monkeys who performed an 8-target center out reaching task. In the RNN, a common 1st hidden layer intended

to capture common underlying dynamics was shared across all sessions. A 2nd hidden layer and the output layer were individually trained in each session, allowing for variations in neural dynamics and in the number of outputs between sessions.
We first validated statistically if the output of the RNN is able to reproduce the intracortical neural dynamics associated with the reaching task. The emulated neural signals captured the actual neural signals' spatial and temporal characteristics, including the average firing rates of each unit. In addition, we had subjects drive the neural model in closed-loop experiments where a decoder translated the emulated neural firing into cursor position. Subjects performed the same target task and used the same decoder as a prior iBCI study in a paralyzed patient (Kim et al. 2008). Subjects using our model system showed comparable ability to control the cursor and acquire targets as the patient using the actual iBCI.
The neural spike emulator enables testing in closed-loop iBCI experiments without any invasive procedures, thereby allowing inclusion of a much larger pool of participants. In addition, the emulator provides a standard platform for decoder development and evaluation. The natural neural dynamics exhibited by the emulated signals and the comparable performance of actual and emulated signals in closed-loop experiments support the emulator's great potential to accelerate iBCI studies.

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Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.16/S9

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

Support: Loyola Undergraduate Research Opportunities Program, Interdisciplinary Research Fellowship
Taiwan Ministry of Science and Technology, Grant 104-2314-B-010-024

Title: System modeling of brain-neuromuscular functions for developing brain-computer interface

Authors: M. MENDOZA[1], S. CACERES[1], L.-L. PAN[2], W.-W. YANG[3], L.-W. CHOU[2], *V. C.-F. CHEN[1];
[1]Loyola Univ. Chicago, Chicago, IL; [2]Natl. Yang-Ming Univ., Taipei, Taiwan; [3]Univ. of Illinois at Urbana-Champaign, Champaign, IL

Abstract: Brain-computer interface (BCI) has been developed for the purpose of bypassing the spinal cord and peripheral nervous system to communicate or control a remote device. Prospective designs have claimed to be able to assist people with severe disabilities or apply to

the practice of neurorehabilitation. Nonetheless, due to the complicated and unpredictable nature of such systems, the feasibility and reliability of available BCIs remain to be fully explored. In this study, we applied system identification techniques for black-box modeling, attempting to find the most appropriate BCI system model. We utilized actual encephalography (EEG) and electromyography (EMG) signals acquired noninvasively from twelve human subjects. The EEG and EMG signals were collected simultaneously while the subjects performed thumb isometric flexion at 50% maximal voluntary contraction for 20 seconds. Based on 16-channel EEG signals and a 1-channel EMG signal collected from the surface of the thenar eminence, we generated model criteria for a state space model, an autoregressive with external input (ARX) model, an autoregressive moving average with external input (ARMAX) model, a nonlinear ARX model, and a nonlinear Hammerstein-Wiener estimation model. The models were calculated by using a 1-second epoch (2000 samples and bandpass filtered to focus on mu wave, 7.6 - 12.4 Hz) of the 16-EEG/1-EMG and validated by a successive 1-second epoch. The one-way ANOVA indicates that the Hammerstein-Wiener estimation model performed significantly better (best fits > 90%, $n = 19$, $p < 0.001$) than the other models, suggesting that the more common polynomial models (ARX and ARMAX) are unfit for BCI development. Despite that the Hammerstein-Wiener estimation model performed the best among all the models included in this study, the massive computation power and time required to properly function may affect the development of BCIs based on this model. The current results may be the basis for future designs of BCIs and can be integrated into brain stimulation models for routine neurorehabilitation sessions.

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Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 406.17/S10

Topic: I.07. Data Analysis and Statistics

Support: Internal Funding from Battelle and Ohio State

Title: Building neural decoders that meet user performance expectations using deep neural networks

Authors: ***D. FRIEDENBERG**[1], A. RICH[1], J. VASKO[1], M. A. SCHWEMMER[1], J. TING[2], N. SKOMROCK[1], M. A. BOCKBRADER[3], G. SHARMA[1];
[1]Battelle, Columbus, OH; [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Physical Med. & Rehabil., Ohio State Univ. Col. of Med., Columbus, OH

Abstract: Intracortical brain-computer interfaces (BCIs) have the potential to reduce disability associated with paralysis by translating neural activity into control of assistive devices. Surveys of potential end-users have identified key BCI system features, including high accuracy, rapid response times, multi-functionality, and minimal interruptions for daily set-up. We have developed novel BCI decoding algorithms that are specifically designed for quick, accurate and robust long-term performance in anticipation of BCI technologies transitioning from the lab to the home environment. Our approach is to build upon the deep neural network (DNN) frameworks developed for computer vision and natural language processing applications and adapt them to BCI decoding. We demonstrate that different components of these models can be adapted to solve complex problems that are specific to BCI applications. For instance, by changing the loss function of the model we can build decoders that sustain high performance for over a year without requiring the user to retrain. This is critical for users who have consistently expressed a desire for a system that requires minimal recalibration. Next, we examined architecture and pre-processing decisions and show that some of the pre-processing methods, like the boxcar filter, which are critical for other machine learning methods are not required for DNN models. By removing these filters, our DNN models improved their response time by about 200ms, another key BCI criterion identified by potential users. Additionally, we examine commonly used BCI features and show that they can be optimized simultaneously with the decoder, leading to more informative features and improved decoding performance. By doing so, we are able to extract more useful information from the array as well as to suggest a path forward

for building BCI decoding algorithms for new intracortical interfaces which may exhibit different and unknown signal characteristics that need to be learned.
Results are presented using a chronically-implanted intra-cortical microelectrode array in the motor cortex of a human study participant with C5/C6 quadriplegia from cervical spinal cord injury during an FDA and IRB-approved study.

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Poster

406. Brain-Computer Interface: Algorithms and Analyses

Location: Hall A

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Program #/Poster #: 406.18/S11

Topic: E.05. Brain-Machine Interface

Support: BRAIN Initiative NIMH F32MH118709 ; NINDS T32NS100663
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* The contents do not represent the views of the Department of Veterans Affairs or the US Government. CAUTION: Investigational Device. Limited by Federal Law to Investigational Use.

Title: Development of a novel auditory-reliant intracortical brain computer interface for effector control and communication in patients with tetraplegia

Authors: *D. J. THENGONE[1,2,3,4], T. HOSMAN[1,2,3], J. D. SIMERAL[3,1,2,4], L. R. HOCHBERG[3,1,2,4,5];
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Abstract: Severe neuromuscular impairments due to ALS, brainstem stroke and traumatic brain injuries may result in the loss of volitional muscle control, and in the inability of individuals to communicate. Brain computer interfaces (BCI) offer a unique channel for communication by circumventing the impaired motor pathways and controlling an external effector such as a computer cursor or robotic arm. The successful usage of such BCIs relies on the users' ability to

reliably utilize the feedback from the interface, which is predominantly visual in current BCIs. However patients with the most severe paralysis can have profound visual impairments making the reliable use of a visual-based BCI difficult or even unfeasible. Here we present a novel paradigm that enables iBCI control and communication in an auditory-reliant manner. Building on previous studies, we utilize spatial auditory signals to develop a multiclass auditory BCI for patients with severe motor impairments. Specifically, we propose to utilize head-response transfer functions (HRTF) to represent spatial auditory cues via headphones to the BCI user. To test the feasibility and reliability of HRTF-based feedback signals, we performed a target acquisition task in healthy subjects using manual cursor control. Subjects were instructed to rely on the auditory cues to navigate the cursor to the cued targets. Preliminary psychophysics testing suggests that manual cursor navigation is possible in an auditory-reliant manner using the HRTF filters updated in realtime. In addition to experimental development, detailed benchmark studies were performed to develop custom low latency architecture capable of presenting audio cues in real-time. Our results reveal that factors that influence audio latencies include: 1) the programming language, 2) the audio driver, and 3) the operating system (OS). Specifically, latencies of 320 ± 0.029ms (mean ± std) were recorded from a Windows OS with default audio drivers. This latency was dramatically reduced to 46 ±46ms with a specialized MATLAB realtime audio library and updating to use the ASIO audio driver. The lowest latencies were consistently observed in the Linux OS with the ASIO driver (11 ± 0.001ms). We present an optimized software and hardware architecture necessary to create a reliable low latency auditory interface that is capable of presenting auditory-reliant BCI communication. In sum, the results of this study demonstrate the development and implementation of a novel auditory-reliant intracortical BCI that provides real-time auditory feedback for communication in individuals with severe motor paralysis and sustained visual impairments.

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Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.01/S12

Topic: E.09. Motor Neurons and Muscle

Title: A new protein to improve motor performance with a limited hypertrophic effect in young and old mice

Authors: *M. BOIDO[1], R. SCHELLINO[1], O. BUTENKO[1], J. W. VRIJBLOED[2], R. G. FARIELLO[2], A. VERCELLI[1];

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Abstract: Several diseases (e.g. neuromuscular diseases and sarcopenia) determine skeletal muscle atrophy and weakness, representing an important clinical problem. Current efforts to improve muscle performance are focused on muscle trophism via inhibition of the myostatin pathway. The activin receptor (ActR-Fc), a myostatin inhibitor, can induce a remarkable gain in body weight: however such muscular hypertrophy per se is not sufficient to ensure a prolonged muscle performance. We speculate that increasing muscle mass without providing adequate incremental innervation could be counterproductive for sustaining long term efforts. We synthetized a novel protein (ActR-Fc-nLG3) by combining the C-terminal agrin nLG3 fragment (a key molecule in development/maintenance of NMJs) to the soluble activin receptor, to support both the myogenic and neurogenic component of muscle performance. Nine-week-old (young) and 22-month-old (aged) C57BL/6j male mice received subcutaneously 10 mg/kg ActR-Fc-nLG3; other age-matched animals received ActR-Fc or vehicle (control). Body weight and motor performances were monitored by rotarod or treadmill. After sacrifice, gastrocnemius, quadriceps and triceps muscles were collected and histologically analyzed. ActR-Fc-nLG3 administration determined a moderate but significant body and muscle weight increase compared to control (although inferior to ActR-Fc). In the young animals, ActR-Fc-nLG3 ensured a remarkable increased performance in the rotarod test. Moreover, concerning the old mice undergone treadmill exercise, during weeks, control and ActR-Fc groups started decreasing the distance run which at study end was significantly shorter (18.2% and 14.4% less, respectively) than at the beginning, whereas ActR-Fc-nLG3 group showed an increase of 2.8% from baseline. Finally, histology demonstrated that, in both treated groups, ActR-Fc-nLG3 administration increased the enfolding of junctional folds of the motor endplates, amplifying the surface area for acetylcholine receptors. Our results support the hypothesis that improving nerve-muscle interaction is an important factor for sustaining long term muscle activity: our work raises the hope that a possible therapy may be developed not only for sarcopenia, but also for many other neuromuscular disorders.

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Poster

407. Neuro-Muscle Interactions

Location: Hall A

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Program #/Poster #: 407.02/S13

Topic: E.09. Motor Neurons and Muscle

Support: CONACYT 256990 CQDL

Title: Lumbosacral ventral root avulsion: Activation pattern of perineal muscles during micturition and motorneuron survival in a rabbit model

Authors: Y. JUÁREZ-ALCOCER[1], A. BELLO-ZAMORA[2], C. ACOSTA-ORTEGA[3], R. ZEMPOALTECA[2], J. MORALES-MEDINA[4], F. CASTELÁN[5], M. MARTÍNEZ-GÓMEZ[5], ***D. L. CORONA QUINTANILLA**[6];
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Abstract: At the lumbosacral level (L6-S2) somato-visceral reflexes are integrated to regulate functions of the lower urinary tract and pelvic-perineal floor muscles. Together, they are a continuous target of plasticity processes due to different factors, such as reproductive experience or mechanical damage, mainly, spinal cord injuries (SC). One of the processes that generates its remodeling is the rupture of nerves from the pelvic-perineal floor to the SC, such as the rupture or ventral nerve roots avulsion (VRA), mainly those of the lumbosacral plexus. Previous studies propose that the VRA of the lumbosacral plexus affects functions that depend on structural interactions between the SC, sympathetic / parasympathetic ganglia and nerves, altering the communication between these pathways. Also, it could affect the activation of the perineal muscles, such as the bulbspongiosus and the survival of their motor neurons (MN). The objective of this study was to determine the effect of the ventral roots avulsion at L6-S2 level on the activation of the bulbospongiosus muscle and the survival of their MN in the female rabbit. For this purpose, we used female rabbits (8 ± 2 months) divided into: 1) Sham (n = 5) and 2) L6-S2VRA (n = 5), which were anesthetized with 2 ml of sodium pentobarbital, were dissected. in the perineal region and bilaterally injected Fluroro ruby tracer (5µl bilateral) into the bulbospongiosus. After 15 days, all animals are anesthetized with 2 ml of sodium pentobarbital; Sham simulates the avulsion and L6-S2 VRA broke the ventral roots of L6-S2. After 15 days, the animals were anesthetized with 20% urethane (i.p.). A cystometrograms, urethral pressure and electromyograms of the bulbospongiosus muscle was recorded. After obtaining 4 micturition reflexes, intracardiac perfusion was performed with paraformaldehyde and 9% saline and spinal segments L6-S2 were obtained to place them 48 hrs in paraformaldehyde and then cryoprotected with 10, 20 and 30% sucrose solutions. Subsequently, transversal slices of 30 µ in cryostat were made. L6-S2 VRA affected bladder and urethral function; produced urinary retention; as well as decrease in the duration of activation frequency and MN number of the bulbospongiosus muscle. This would cause alterations in the activation of the bulbospongiosus during micturition, as well as changes in the frequency of activation and some alterations in the voiding.

Disclosures: Y. Juárez-Alcocer: None. **A. Bello-Zamora:** None. **C. Acosta-Ortega:** None. **R. Zempoalteca:** None. **J. Morales-Medina:** None. **F. Castelán:** None. **M. Martínez-Gómez:** None. **D.L. Corona Quintanilla:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.03/S14

Topic: E.09. Motor Neurons and Muscle

Support: CIHR Grant 148793
NSERC PGSD
Killam Trust

Title: Muscle derived BMP4 regulates morphology and function of the neuromuscular junction in mice

Authors: J. HARRISON, B. PODOR, ***V. F. RAFUSE**;
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Abstract: Growth factors are well known to regulate morphological and electrophysiological properties of synapses including the neuromuscular junction (NMJ). For example, in *Drosophila*, mutants lacking proteins in the Bone Morphogenic Protein 4 (BMP4) signaling pathway have abnormally small muscle fibers, smaller NMJs with ultrastructure synaptic defects, smaller evoked excitatory junctional potentials, reduced frequency of spontaneous neurotransmitter release and an ~ 4-fold decrease in quantal content. Whether BMP4 signaling has a similar role at vertebrate NMJs is currently unknown. To examine this question, we generated doxycycline inducible, muscle-specific BMP4 null mice by crossing HSA-rtTA/TRE-Cre mice with mutant mice where exon 4 of the BMP4 gene is flanked by *loxP* sites (referred to here as BMP4 flox mice). Doxycycline induced knock-down of BMP4 expression in muscle was initiated at three months of age. Motor behavior tasks were examined pre- and post-doxycycline administration while electrophysiological and morphological characteristics of the NMJ were recorded 1-3 months after BMP4 knock-down. We found that BMP4 flox mice had a greater latency to fall on the rotarod turning at 40 rpm compared to wildtype littermates 14 days after doxycycline administration. Morphologically, Type I, but not Type IIa muscle fiber cross-sectional areas in soleus muscles were significantly smaller in BMP4 flox mice 3 months after doxycycline administration. Interestingly, NMJs were larger in BMP4 mice compared to the controls due to a significant increase in acetylcholine receptor cluster number and distribution. Electrophysiological studies showed that the amplitude and frequency of miniature endplate potentials (mEPPs) were significantly smaller in BMP4 flox mice compared to their wild-type littermates. Similarly, evoked EPP amplitude and quantal content were also significantly smaller in BMP4 floxed mice while the number of neurotransmission failures at high stimulus frequencies was significantly higher in muscles lacking BMP4. Finally, ex vivo contractile force recordings indicated that soleus muscles in BMP4 flox mice were significantly weaker that their

wild-type counterparts. Consequently, as observed in *Drosophila*, these results indicate that muscle derived BMP4 regulates morphological and electrophysiological attributes of the NMJ in mice. We are currently exploring the possibility that abnormalities caused by the loss of muscle derived BMP4 are associated with an accelerated, premature ageing phenotype.

Disclosures: J. Harrison: None. **B. Podor:** None. **V.F. Rafuse:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.04/S15

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant 1 R15 NS101608-01A1

Title: The chromatin remodeling enzyme, kismet, negatively regulates BMP signaling at the *Drosophila* neuromuscular junction

Authors: *J. A. PRESTON, B. HARSIN, F. L. W. LIEBL;
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Abstract: Retrograde bone morphogenic protein (BMP) signaling at the *Drosophila* neuromuscular junction (NMJ) is critical for developing and mature motor neurons. It is involved in proper growth and localization of developing neurons and continued functionality in mature neurons. We have found that BMP signaling is increased in motor neurons of larvae with mutations in the gene coding for a chromodomain helicase DNA binding protein, Kismet (Kis). We also showed that BMP signaling is significantly reduced in *Enhancer of zeste [E(z)]* mutants. E(z) is a methyltransferase in the Polycomb Repressive Complex 2 (PRC2). Thus, Kis and PRC2 act in opposition to regulate BMP signaling. Both *kis* and *E(z)* mutants, however, exhibit similar deficits in neurotransmission characterized by significant reductions in evoked endplate junctional currents and quantal content. To determine how Kis may regulate BMP signaling, we examined BMP signaling after tissue specific knockdown of Kis. Knockdown of Kis in postsynaptic muscle but not presynaptic motor neurons or glial cells mimics the *kis* mutant increase in intersynaptic BMP signaling. Next, we will examine transcript levels of Glass Bottom Boat (Gbb), Mad, and BMP receptors in *kis* mutants and determine if E(z) may oppose Kis at these loci. Mutations in the human orthologs to *kis*, *CHD7* and *CHD8*, are correlated with neurodevelopmental diseases CHARGE and a subtype of autism spectrum disorder (ASD), respectively. A better characterization of the interaction between Kismet and BMP signaling at the mature *Drosophila* NMJ could lead to a better understanding of the pathology of CHARGE and ASD.

Disclosures: **J.A. Preston:** None. **B. Harsin:** None. **F.L.W. Liebl:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.05/S16

Topic: E.09. Motor Neurons and Muscle

Support: NIA grant AG051510

Title: GRP78 is essential for NMJ formation and maintenance

Authors: *H. JING[1], L. LI[2], G. XING[1], H. ZHANG[1], Z. DONG[1], W. CUI[1], Z. YU[1], N. GAO[1], W. XIONG[1], L. MEI[1];
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Abstract: The neuromuscular junction (NMJ) is a peripheral synapse between motor nerves and skeletal muscle fibers. It is critical to muscle contraction. NMJ formation is controlled by agrin, a factor released from motor neurons that binds to LRP4 (a LDLR family member) to activate the receptor tyrosine kinase MuSK in muscle cells and thus promotes the aggregation of acetylcholine receptors (AChRs). Recent data suggest that the agrin-LRP4-MuSK signaling is also important for NMJ maintenance. However, mechanisms controlling the signaling pathway are not well understood. To this end, we attempted to identified proteins that interact with LRP4 with an idea that they may regulates its stability or function. We purified the LRP4 complex from HSA-Flag-LRP4 transgenic mice where Flag-LRP4 is specifically expressed in skeletal muscles and identified GRP78 by mass spectrometry. GRP78 is a molecular chaperone critical for protein transport. We show that GRP78 was concentrated at the NMJ, and at postsynaptic sarcoplasmic reticulum. Muscle-specific knockout of GRP78 reduced LRP4 in muscles and leads NMJ deficits including excessive axon arborization and reduced AChR clusters. To characterize the roles of GRP78 in adult, we generated HSA-Cre^{ER};GRP78$^{F/F}$ mice, in which Cre could be induced by tamoxifen. We found that NMJs became fragmented and denervated in adult mice where GRP78 was inducibly knocked out. These results demonstrate a critical role of GRP78 in NMJ formation and maintenance, identify a novel play in regulating the agrin signaling pathway.

Disclosures: **H. Jing:** None. **L. Li:** None. **G. Xing:** None. **H. Zhang:** None. **Z. Dong:** None. **W. Cui:** None. **Z. Yu:** None. **N. Gao:** None. **W. Xiong:** None. **L. Mei:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.06/S17

Topic: E.09. Motor Neurons and Muscle

Support: DFF-5051-00195
CF17-0949
NNF17OC0028928

Title: Nonlinear properties of song bird vocal muscles change over postnatal development

Authors: *I. ADAM, C. P. H. ELEMANS;
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Abstract: Motor skill learning typically occurs in a period when the brain is navigating a body that is still growing and developing. How the changing body, neural circuit formation and motor coding influence each other remains largely unknown. Over the course of song learning in songbirds the behavioral output, namely song, dramatically decreases in variability, which has mostly been attributed to changes in neural circuitry. However, the contraction speed of vocal muscles also changes and nearly doubles over the course of sensory guided motor learning. Because muscle force sets acoustic parameters such as pitch, amplitude and entropy, we investigate how nonlinear properties of force production change depending on muscle contraction speed. First, we focused on the effect of firing frequency on nonlinear force summation in syringeal muscles over song learning in male zebra finches. By comparing recorded data to twitch summation models, we find that identical stimulation patterns yield strikingly dissimilar force profiles depending on the isometric twitch speed of the muscle. The magnitude of nonlinear force summation depends on the stimulation frequency as well as muscle speed and sex of the animal.
Furthermore, we investigated to what extent spike timing at sub millisecond precision can penetrate into force profiles depending on isometric muscle speed. We find that sub millisecond spike timing has effects on maximal tension, timing of maximal tension as well as the shape of force profiles at physiologically relevant stimulation frequencies. In conclusion, we find that force profiles elicited by stimulation patterns within the physiological range of the songbird vocal system change dramatically depending on muscle speed. To achieve a stable acoustic target thus requires adaptation of the motor code to the changing muscle properties over development.

Disclosures: I. Adam: None. **C.P.H. Elemans:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.07/S18

Topic: E.09. Motor Neurons and Muscle

Title: Fine-resolution motor control of birdsong

Authors: *A. MAXWELL, I. ADAM, H. RÖSSLER, E. BØGH HANSEN, C. P. ELEMANS; Univ. of Southern Denmark, Odense M, Denmark

Abstract: The degree of fine control in any complex behaviour is limited by the minimal force step available, i.e. the resolution, when activating muscles. Control resolution is set by the number and size distribution of motor units (MUs). While locomotor muscles have MUs of thousands of muscle fibers, extraocular muscles contain MUs as small as 5-10 muscle fibers allowing fine eye motion control. Songbirds have fine control over acoustic parameters of their song, but very little is known how the motor pool is organized. Here we combine estimates of MU size, *in vitro* muscle force measurements and *in vitro* sound production to predict the control resolution of a key acoustic parameter, fundamental frequency (f_o), in zebra finches.
Superfast syringeal muscles are innervated by motor neurons in the tracheosyringeal part of the hypoglossal motor nucleus (nXIIts). To measure the number of syringeal MUs in male zebra finches, we quantified the number of motor neurons in left and right nXIIts root and major dorsal/ventral branches with laminin/neurofilament stained cross sections of the trachea and the syrinx, respectively. Because nXIIts also contains a small portion of efferent fibers, our data slightly overestimates the number of MUs. As a proxy for MU size, we measured the innervation ratio of syringeal musculature, and quantified the number of muscle fibers in a syringeal cross section. We found that each side of the syrinx consist of 3,000-4,500 muscle fibers innervated by 750-800 myelinated motor neurons. The mean innervation ratios are among the lowest known at only 3-5 muscle fibers/motor neuron.
To estimate the detailed level of control over acoustic parameters such small MUs provide we focused on the effect of recruitment on f_o. We first measured force kinetics and maximal isometric tension generated by *musculus syringealis ventralis* (VS) fibers *in vitro*. To measure how VS force generation increases f_o, we induced sound production using an *in vitro* syrinx preparation and dynamically actuated the VS insertion site while measuring VS shortening as a function of force. Our results suggest that full activation of the VS provides a f_o range of about 500-800 Hz, which is consistent with *in vivo* recordings. Combined, our data suggests that zebra finches have extremely precise control over f_o in the order of 1-2 Hz/MU.

Disclosures: A. Maxwell: None. **I. Adam:** None. **H. Rössler:** None. **E. Bøgh Hansen:** None. **C.P. Elemans:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.08/T1

Topic: E.09. Motor Neurons and Muscle

Support: Broad Institute Grant

Title: Inter individual variability and reliability of cortico spinal excitability modulation across intermittent, continuous and sham theta burst stimulation in humans

Authors: *P. O. B. BOUCHER, R. A. OZDEMIR, S. TADAYON, E. SANTARNECCHI, A. PASCUAL-LEONE, M. SHAFI;
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Abstract: Highly variable inter and intra individual results across transcranial theta burst stimulation (TBS) studies challenge the contention that TBS is more effective and reliable in modulating cortico-spinal excitability as other longer, less tolerated repetitive transcranial magnetic stimulation (TMS) protocols as initially proposed in preliminary studies. We compared two classically defined TBS protocols, continuous TBS (cTBS) and intermittent TBS (iTBS), to their sham equivalents with 24 participants in repeat neuronavigated visits (at least 48h between visits; test-retest performed 1 month apart). Inter and intra individual variability in modulation of corticospinal excitability was measured by motor evoked potentials (MEPs). Averaged MEPs collected before and after each TBS protocol at timed intervals were compared within a visit, to other TBS-modulated MEPs in separate visits, and across repeat visits of the same protocol to assess the relative suppression/facilitation of each TBS protocol and its reliability. cTBS suppresses MEPs as compared to sham stimulation and iTBS facilitates MEPs as compared to baseline MEPs in participants' initial visits. However, none of the protocols modulate cortico-spinal excitability in repeat visits. As well, sham stimulation demonstrated a faciliatory effect above baseline MEPs and even above iTBS at some time points in the initial visit but had no modulatory effect in the repeat visit. In the first within-subject study to test both iTBS and cTBS against sham stimulation across repeat visits, the effectiveness and most tellingly the reliability of both TBS protocols is challenged. Covariates such as genetics, geodesic distance between repeat visit hotspots, and cortical thickness are modelled with MEPs to determine the effect, if any, these variables play in the modulation of cortico-spinal excitability by TBS protocols. Finally, the neurophysiological substrate of sham TBS effects, as well as the need to account for placebo-like effects when assessing TMS efficacy, are also discussed.

Disclosures: P.O.B. Boucher: None. **R.A. Ozdemir:** None. **S. Tadayon:** None. **E. Santarnecchi:** None. **A. Pascual-Leone:** None. **M. Shafi:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.09/T2

Topic: E.09. Motor Neurons and Muscle

Support: Swedish Research Council Grant 2016-02184
Swedish Research Council Grant 2014-02048
Swedish Research Council Grant 2014-07603
Göran Gustafsson foundation

Title: Interfacing motor neurons and skeletal muscle cells with thousands of bi-directional microelectrodes

Authors: *M. RADIVOJEVIC, M. LEWANDOWSKA, E. BOGATIKOV, A. R. PUNGA; Neurosci., Uppsala Univ., Uppsala, Sweden

Abstract: Impaired signal transmission between motor neurons and muscle fibers causes neuromuscular disorders such as amyotrophic lateral sclerosis, myasthenia gravis and muscular dystrophies. Despite efforts dedicated for developing new drugs for these diseases, there are still no curative treatments, and new therapeutic strategies are needed. Formulation of new therapies can be aided through advancing the knowledge about the functional interplay between subcellular elements of the neuromuscular system. Such insights, however, require experimental ability to noninvasively access and control electrical signals arising across different compartments of motor neurons and myotubes. To provide such access, we cultured primary rodent motor neurons and skeletal muscle cells directly on high-density microelectrode arrays. These arrays comprise 26,400 planar microelectrodes packed within an area of ~8.1 mm^2, providing a density of 3,264 electrodes per mm^2. Each of the electrodes can serve as recording and stimulation source, enabling bi-directional communication with any cell interfacing the array. Here we firstly present a method for electrical imaging of individual motor neurons and myotubes across hundreds of microelectrodes. Secondly, we present a method for extracellular electrical stimulation of targeted motor neurons. Finally, we present experimental approach to investigate excitability of myotubes in response to extracellular stimulation. We used spike-sorting algorithms to electrically identify individual cells in the cultures and to track action-potential (AP) propagation across subcellular compartments. We were able to monitor the AP initiation, axonal propagation and dendritic backpropagation in motor neurons. Likewise, the method enabled us to detect the AP initiation and to track bi-directional AP propagation across the sarcolemma of individual myotubes. We used extracellular voltage stimulation to activate individual cells in neuronal and myotube cultures. Stimulation directed at the axon initial

segment provided activation of targeted motor neurons. The presented methods could aid the development of a platform for preclinical studies of neuromuscular disorders.

Disclosures: M. Radivojevic: None. **M. Lewandowska:** None. **E. Bogatikov:** None. **A.R. Punga:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.10/T3

Topic: E.09. Motor Neurons and Muscle

Support: Myaware Charity scholarship

Title: Exploring novel treatments for disorders that feature defects of the neuromuscular junction structure

Authors: *M. PANEA, J. COSSINS, R. WEBSTER, D. BEESON;
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Abstract: Congenital myasthenic syndromes (CMS) are a group of inherited disorders that affect the signal transmission at the neuromuscular junction (NMJ) and share the clinical feature of fatigable muscle weakness. In *DOK7*-CMS compromised neurotransmission is due to small and destabilised NMJs. DOK7 is a cytoplasmic-adaptor protein which amplifies the signalling from muscle-specific receptor tyrosine kinase (MuSK) that is responsible for the formation and stabilisation of the NMJ. It has recently been shown that large amounts of DOK7 protein in muscles generate enlarged NMJs in mice which are very efficient in signal transmission, have no reported detrimental effects and can ultimately lead to a complete recovery for disease model mice.
In this study we aim to identify small molecules that can specifically upregulate the amount of DOK7 in muscles as a potential therapy for CMS. By using an AAV system we generated enlarged NMJs in wild-type mice to further investigate the morphology of these synapses and show that their electrophysiology is similar to the normal-sized NMJs. Use of this system to infect wild-type C2C12 cells combined with titration of the levels of DOK7 showed that an approximate 2-3 fold increase in DOK7 protein levels is sufficient to generate AChR clusters. 5431 small molecules from a library of muscle-specific compounds were used in screening experiments. Initial screening was performed in triplicates on a C2C12 reporter cell line designed to contain a 1-Kb sequence of the DOK7 promoter fused to firefly Luciferase. Confirmatory results were performed for potential candidate upregulating compounds using a similar EGFP reporter cell line. Five potential hits that show significant upregulation after being tested on both reporter cell lines are being further investigated. They will provide the basis for a more detailed

analysis of the effects of DOK7-upregulation in cell culture biological assays and ultimately for *in vivo* testing.

Disclosures: **M. Panea:** None. **J. Cossins:** None. **R. Webster:** None. **D. Beeson:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.11/T4

Topic: E.09. Motor Neurons and Muscle

Support: RQRV Grant #RQS00220
NSERC RN000207
CIHR Grant RNI00252
CIHR: CGS-M

Title: Characterisation of human NMJs in aging using electrostimulation for enhanced biopsy-NMJ sampling

Authors: *S. MARCHAND[1,2], J. VALLÉE[1,2], C. PION[3,4], J. LAI[3], J. A. MORAIS[5], M. BELANGER[3], M. AUBERTIN-LEHEUDRE[3,4], R. ROBITAILLE[1,2];
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Abstract: Several changes occur during normal aging contributing to a loss of skeletal muscle mass and function leading to higher rates of morbidity and mortality, hospitalizations and poor quality of life. Among important factors contributing to muscle changes are alterations of the neuromuscular junction (NMJ). Works using rodents revealed that the NMJ is a tripartite synapse composed of the presynaptic motor nerve terminal, the postsynaptic fiber and perisynaptic Schwann cells (PSCs), glial cells at the NMJ, each component being involved in the functional and structural properties of the synapse. However, the human NMJ remains widely understudied in part due to poor recovery of NMJ material from muscle biopsies. We therefore developed an approach using electrostimulation to identify the best location to perform a muscle biopsy of the vastus lateralis to enhance NMJ sampling (BeeNMJ). We obtained neuromuscular material from a cohort of 9 young (18-30 years) and 16 older adults (over 55 years) to study the alterations of the NMJs throughout aging process. An array of physiological characteristics of the participants (physical activity level, muscle strength, EMG, etc.) was assessed in a separate visit prior to the biopsies. Muscle samples were stained by immunohistochemistry for the nerve terminal (NF-

M/SV2), postsynaptic nAChRs (α-btx), PSCs (s100β) and muscle fiber type (MHC I) and imaged using a FV-1000 or a LSM880 confocal microscope. NMJs were characterised for each of their components and compared between age groups. The data showed that human NMJs were significantly different from their rodent counterparts, especially regarding postsynaptic and PSCs morphology. Comparative analyses also revealed that NMJ structure remained relatively stable throughout aging. However, a higher proportion of denervated NMJ and lower glial coverage of the NMJ were observed in older individuals. These data will be correlated with participants' physiological data to identify the critical NMJ parameters that best reflect the physiological state of each individual. Our results should enable a better understanding of the underlying mechanisms leading to neuromuscular aging and develop better therapeutic approaches to limit muscle weakening in aging.

Disclosures: S. Marchand: None. **J. Vallée:** None. **C. Pion:** None. **J. Lai:** None. **J.A. Morais:** None. **M. Belanger:** None. **M. Aubertin-Leheudre:** None. **R. Robitaille:** None.

Poster

407. Neuro-Muscle Interactions

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.12/T5

Topic: E.09. Motor Neurons and Muscle

Support: AG051510

Title: A role of lamin A/C in preventing neuromuscular junction decline in aged mice

Authors: *N. GAO[1], K. ZHAO[2], G. XING[1], H. JING[1], L. XIONG[1], H. GUO[1], W.-C. XIONG[1], L. MEI[1];
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Abstract: During aging, skeletal muscles become atrophic and lose contractile force. This affects a large population of elderly regardless of ethnicity, gender, and wealth and is the most common cause of age-related loss of independence, frailty, and mortality. As the elderly proportion in the population continues to increase, the potential social and economic burden of muscle aging is becoming enormous. The neuromuscular junction (NMJ) is a synapse between motor neuron terminals and skeletal muscle fibers that transmit signals from motor neurons to muscle fibers. The neuromuscular transmission is critical for the control of muscle contraction and is thus essential for our physical mobility and daily life. Extensive research has revealed insight into the pathophysiological mechanisms of muscle aging. However, although NMJ structures and functions are disrupted in aged animals, little is known about underlying mechanisms. In contrast to NMJ formation, which has been studied extensively, much less is understood about mechanisms of NMJ maintenance, in particular in aged animals. To this end,

we screened for proteins that are reduced in aged muscles and identified lamin A/C, intermediate filament proteins that determine the interphase nuclear architecture. The lmna gene is mutated in Hutchinson-Gilford progeria syndrome (HGPS), a disease of accelerated aging and premature osteoporosis. We found muscle-specific lamin A/C mutant mice had no problem in forming NMJs, but displayed age-related NMJ deficits including reduced AChR cluster size, increased cluster fragments, diminished innervation, and impairment neuromuscular transmission. These deficits were not observed in mice lacking lamin A/C in motor neurons. These results suggest a role of lamin A/C in maintaining proper NMJ structure and function. Experiments are under way to investigate underlying mechanisms.
Key words: aging, neuromuscular junction, Lamin A/C

Disclosures: **N. Gao:** None. **K. Zhao:** None. **G. Xing:** None. **H. Jing:** None. **L. Xiong:** None. **H. Guo:** None. **W. Xiong:** None. **L. Mei:** None.

Poster

407. Neuro-Muscle Interactions

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Program #/Poster #: 407.13/T6

Topic: E.09. Motor Neurons and Muscle

Support: PAPIIT-UNAM IN212916 (MMG)
CONACyT 628536 (CHB)
CONACyT 732830 (DAZL)
NIH 1 R01 DK120307-01

Title: Optimus parameters of electrical stimulation for ischiocavernosus and bulbospongiosus nerves in nulliparous female rabbit

Authors: *D. A. ZACAPA[1], C. HERNÁNDEZ-BONILLA[2], D. L. CORONA-QUINTANILLA[2], R. ZEMPOALTECA[2], F. CASTELÁN[3], M. ROMERO-ORTEGA[4], M. MARTÍNEZ-GÓMEZ[3];
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Abstract: Neuromodulation by electrical stimulation of sacral spinal roots and peripheral nerves have been a therapeutic option to treat pelvic floor disorders. Clinical studies have been demonstrated that electrical stimulation of the pudendal nerve is a viable alternative for neuromodulation, since it innervates the urethral sphincter and some pelvic floor muscles (ischiocavernosus (Iscm) and bulbospongiosus (Bsm) muscles). However, it has not been evaluated the electrical stimulation benefits on specific perineal nerves to develop an effective

treatment. In this study, optimal electrical stimulation parameters were determined for ischiocavernosus (Iscn) and bulbospongiosus (Bsn) nerves. Electromyograms (EMG) (Iscn and Bsn) during electrical nerve stimulation were record in nulliparous anesthetized female rabbits (urethane 1.5 g/Kg body weight, intraperitoneal)11.7 ± 0.3 months age and 4.39 ± 0.13 Kg weight. In a first stage, we applied a single square pulse (1 ms), until find the current to evoke the minimum electromyographic activity in perineal muscles. That was defined as electrical current threshold value (CThV), specifically to Iscm or Bsm.
Results obtained show that the CThV for Isc was 1.42 ± 0.24 μA and for Bs 0.41 ± 0.19 μA. To 6 CThV were observed three components in the EMG records of Iscm, amplitude was 5.26 V ± 0.59, 6.46 V ± 1.5 and 5.9 V ± 0.9 respectively. For Bsm (4 CThV) the amplitude was 8.49 V ± 4.1, 7.98 V ± 2.99 and 2.36 V ± 1.8. The intensity of current applied at 6 CThV on Iscn was 6.31 ± 1.15 μA and at 4 CThV on Bsn was 1.15 ± 0.27 μA.
Our results suggest that the Iscn need more CThV to activate the Iscm. Meanwhile, the Bsn required minor CThV. These results showed that to treatment effective is necessary applying electrical stimulation differential in specific nerves.

Disclosures: D.A. Zacapa: None. **C. Hernández-Bonilla:** None. **D.L. Corona-Quintanilla:** None. **R. Zempoalteca:** None. **F. Castelán:** None. **M. Romero-Ortega:** None. **M. Martínez-Gómez:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.14/T7

Topic: E.09. Motor Neurons and Muscle

Support: PAPIIT-UNAM IN212916 (MMG)
CONACyT 628536 (CHB)

Title: Effect of multiparity on the force generated by the obturator internus and bulboglandularis muscles in the vagina and urethra of the female rabbit

Authors: *C. HERNÁNDEZ BONILLA[1], R. ZEMPOALTECA[2], D. L. CORONA QUINTANILLA[2], F. CASTELÁN[3], M. MARTÍNEZ GÓMEZ[3];
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Abstract: The parturition is a mechanical and hormonal event, which has been linked to alterations in the pelvic floor muscles and its innervation. Studies in female rabbits (*Oryctolagus cuniculus*) have showed that the multiparity modifies fiber type composition and the contractile

properties of pelvic floor muscles, affecting the genesis of vaginal pressure. However, there are other striated muscles anatomically related to the pelvic vagina and urethra as the obturator internus (OI) and bulboglandularis (Bg) muscles that could be damaged by the effect of multiparity. The aim of this study was to evaluate in young nulliparous and young multiparous rabbits, the contractile force in isometric conditions and the pressure generated on the pelvic vagina and urethra by OI and Bg muscles. Twenty-four Chinchilla-breed female rabbits (11-12 months old) were used in this study. Animals were divided in two groups: young virgin nulliparous (n=12) and young multiparous (n=12). Each condition (nulliparous and multiparous) was randomly distributed into two experimental groups. In the first group, was evaluated the pelvic intravaginal pressure (IVP) and intraurethral pressure (IUP) generated by the contraction of the OI and Bg muscles (n=6 per group). The second group was evaluated contractile force developed by OI muscle (n=6 per group). We observed contraction of the OI muscle at 100 Hz, this contraction generated a high IVP in nulliparous females and low in multiparous. The IUP generated by the contraction of OI muscle did not show significant differences between groups. During the contraction of Bg muscle at 100 Hz, the IVP generated was greater in female nulliparous respect to multiparous. The IUP generated by the contraction of the Bg muscle in nulliparous females was greater in comparison to multiparous. The contractile force generated by OI muscle did not show significant differences between groups and the Bg was not possible registered. The results showed that the OI and Bg muscles contribute in a differential way to the generation of IVP and IUP. Likewise, the multiparity affects muscles differently because the contractile properties of the OI muscle were unchanged. This in turn, could modify reproductive and non-reproductive processes.

Disclosures: C. Hernández Bonilla: None. **R. Zempoalteca:** None. **D.L. Corona Quintanilla:** None. **F. Castelán:** None. **M. Martínez Gómez:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.15/T8

Topic: E.09. Motor Neurons and Muscle

Title: Identification and expression of glutamate receptors genes in innervated and uninnervated regions of the ventral diaphragm muscle of the moth Manduca sexta

Authors: A. MORALES BENITEZ, K. M. YATSKO, R. J. BAYLINE;
Washington & Jefferson Col., Washington, PA

Abstract: Modulation of rhythmic patterns of myogenic muscles by motor neurons occurs in many organisms. The ventral diaphragm muscle (VDM) of the tobacco hornworm *Manduca sexta* is myogenic and controls the movement of hemolymph. The VDM shares many similarities

with the dorsal heart muscle. The VDM receives innervation in the most posterior (abdominal segment 6—A6) and anterior (A2-A3) segments. Segments A4 and A5 are uninnervated. While the heart exhibits both anterograde and retrograde cardiac activity, only the fast anterograde contractions are initiated by neural control of the anterograde pacemakers via glutamatergic innervation. Similarly, the VDM experiences an increase in contraction rate following bath application of glutamate. However when the uninnervated A4-A5 segments of the muscle are isolated, glutamate induces a decreased contraction rate. We hypothesized that a difference in expression of glutamate receptor genes accounted for these contrasting responses in the muscle. Specifically, abdominal segments A4 and A5 were expected to present inhibitory chloride channels while segments A6 and A3 were expected to show excitatory GluRIIA receptors. BLAST searches of the *M. sexta* genome revealed potential peptide sequence matches for ionotropic and metabotropic glutamate receptors. We extracted and purified RNA from the adult VDM to generate regional cDNA. We used PCR to amplify sequences of interest using primers for the putative *M. sexta* GluRIIA and GluRCl receptors. Purified PCR products were ligated into the TOPO plasmid to transform bacteria. After successfully growing our colonies and running PCR, we gathered enough evidence to suggest the VDM might present both excitatory GluRIIA and inhibitory GluRCl receptor channels. Initial results suggest that GluRIIA channels are absent from the uninnervated regions of the muscle, while GluRCl channels are present throughout the muscle. This suggests a mechanism by which glutamate could decrease contraction rate in the uninnervated sections via specific activation of the GluRCl channels.

Disclosures: **A. Morales Benitez:** None. **K.M. Yatsko:** None. **R.J. Bayline:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.16/T9

Topic: E.09. Motor Neurons and Muscle

Support: NIH GRANT R01AG055545
NIH GRANT R56AG051501
NIH GRANT R21NS106313

Title: The synaptic associated microRNA, miR-133b, plays an important role in the progression of Duchenne muscular dystrophy

Authors: T. TAETZSCH[1], ***D. SHAPIRO**[1,2], G. VALDEZ[3,1];
[1]Fralin Biomed. Res. Inst., Roanoke, VA; [2]The Grad. Program in Mol. Biology, Cell Biol. and Biochem., [3]Dept. of Mol. Biology, Cell Biology, and Biochem., Brown Univ., Providence, RI

Abstract: The muscle and synaptically enriched microRNA, miR-133b, has been implicated in the biogenesis and maturation of muscle fibers. While mir-133b is increased in Duchenne muscular dystrophy (DMD), it remains unknown whether the induction of miR-133b is required to mitigate muscle fiberdegeneration in this disease. To assess the role of miR-133b in DMD-affected skeletal muscles, we genetically ablated miR-133b from the mdx mouse model for DMD. We have found that loss of miR-133b exacerbates muscle degeneration in the tibialis anterior (TA) muscle. In the absence of miR-133b, the TA becomes populated with muscle fibers exhibiting a rather small cross-sectional area (CSA) and containing centralized myonuclei. Additionally, loss of miR-133b increases both the size of the interstitial space around muscle fibers and the number of mononucleated cells contained within it. To determine if miR-133b directly impacts muscle fiber regeneration following acute injury, we performed cardiotoxin (CTX) injury of the TA muscle of healthy miR-133b null mice and their littermate controls. We did not observe differences in the size of regenerating muscle fibers following CTX-injury in miR-133b null TA, suggesting that the impact of miR-133b on muscle fibersize in mdx muscle is related to a chronic aspect of the disease. Along these lines, RNA seq analysis revealed that while the impact on the transcriptome of healthy miR-133b null muscle is limited, a wide range of genes are impacted by miR-133b deletion in mdx muscle, including a number of previously identified miR-133b targets as well as several members of the TGF-β pathway including LTBP4, a gene associated with increased severity of DMD. Combined, our data suggest that miR-133b functions to slow muscle degeneration in DMD.

Disclosures: **T. Taetzsch:** None. **D. Shapiro:** None. **G. Valdez:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

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Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant R01AG055545
NIH Grant R56AG051501
NIH Grant R21NS106313

Title: Argonaute 2 plays a local role in maintaining and repairing injured and ALS-afflicted NMJs

Authors: *T. TAETZSCH[1], D. SHAPIRO[2], G. VALDEZ[2];
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Abstract: The ability of microRNAs to mediate stress-responses in skeletal muscles, including neuromuscular junctions (NMJs), depends on the function of a variety of proteins. Here, we

examined levels and distribution of genes involved in microRNA biogenesis, transport and translational repression in skeletal muscles and their synapses. We found that expression of genes for miRNA biogenesis (DGCR8, Exportin), components of the RNA induced silencing complex (Argonaute2, Dicer, TARBP2, TNRC6a) and RISC-interactors (HSP90a, PCBP1) decreases as muscles mature. Corroborating these findings, we found reduced protein levels of Argonaute 2 (Ago2), the catalytic core of the RNA induced silencing complex (RISC), in muscles from juvenile and young adult compared to newborn mice. Interestingly, Ago2 concentrates at NMJs as animals mature, suggesting that Ago2 levels and distribution are linked to the innervation status of the NMJ. To examine this possibility, we assessed Ago2 in skeletal muscles following severing of innervating motor axons. Immunostaining revealed that Ago2 vacates denervated NMJs, and then gradually returns as motor axons re-innervate postsynaptic sites. We next asked whether Ago2 expression and distribution are affected in Amyotrophic Lateral Sclerosis (ALS), a disease that causes progressive degeneration of NMJs. Surprisingly, Ago2 is largely missing from intact NMJs in presymptomatic and remains absent during the symptomatic stage of the disease in $SOD1^{G93A}$ mice, a mouse model for ALS. Together, these findings suggest that Ago2 plays a local role in repairing NMJs, and its absence may contribute to NMJ degeneration in ALS.

Disclosures: T. Taetzsch: None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.18/T11

Topic: E.09. Motor Neurons and Muscle

Support: FONDECYT 1170614
GASTOS OPERACIONALES BECA DOCTORADO NACIONAL. 21161461

Title: Dynamics of nicotinic acetylcholine receptor subpopulations at the normal and injured neuromuscular synapse *in vivo*

Authors: *D. ZELADA, F. BERMEDO-GARCÍA, J. P. HENRÍQUEZ;
Cell Biol., Univ. De Concepción, Concepcion, Chile

Abstract: Introduction: The vertebrate neuromuscular junction (NMJ) is a cholinergic peripheral synapse where the post-synaptic muscle membrane specialization interacts with a pre-synaptic motor neuron terminal. NMJ injury leads to the denervation of post-synaptic apparatuses with morphological and functional impairment of the nicotinic acetylcholine receptor (nAChR) aggregates. Our aim is to explore the dynamics of nAChRs subpopulations at the injured NMJ.

Materials and Methods: *Levator auris longus* (LAL) muscles were denervated by facial nerve crush or resection. To analyze nAChR dynamics, we initially labelled surface nAChRs by *in vivo* injection of Alexa-488-Bungarotoxin (BTX). After different times, newly incorporated AChRs at the muscle surface were labelled with Alexa-555-BTX and then, intracellular AChRs were labelled with Alexa-647-BTX. Injured and control non-denervated NMJs were analyzed by confocal microscopy.
Results: Despite an initial preservation of post-synaptic structures, long-term facial nerve resection leads to a remarkable loss in their pretzel-like morphology. Pre-existing surface (S) nAChR stability decreases as NMJ degeneration proceeds, evidenced by a remarkable increase in the 555/488-BTX staining ratio within each post-synapse. Interestingly, new (N) nAChRs were peripherally incorporated into the remaining postsynaptic domains. Also, denervation-induced ectopic (E) nAChR clusters exhibited reduced stability, which displayed both plaque and O-shape morphologies. Remarkably, we found that altered nAChRs turnover precedes the morphological dismantlement of the post-synaptic domain.
Discussion: Our results indicate that NMJ injury leads to an increase of nAChR turnover, suggesting that early morphological preservation of the post-synapse after injury does not correlate with its stability.

Disclosures: D. Zelada: None. **F. Bermedo-García:** None. **J.P. Henríquez:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

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Program #/Poster #: 407.19/T12

Topic: E.09. Motor Neurons and Muscle

Support: T-32 Training Grant HL105355
NIH R01 HL146114

Title: Effect of posture on transdiaphragmatic pressure

Authors: C. WHITNEY[1], M. PAREJA-CAJIAO[2], H. M. GRANSEE[2], G. C. SIECK[3], C. B. MANTILLA[4];
[1]Biomed. Engin., Mayo Clin. Grad. Sch. of Biomed. Sci., Rochester, MN; [3]Physiol. & Biomed. Engin., [4]Anesthesiol., [2]Mayo Clin., Rochester, MN

Abstract: Transdiaphragmatic pressure (Pdi) is commonly used as a surrogate for diaphragm muscle force generation during ventilatory (eupnea, sigh, hypercapnia/hypoxia, airway occlusion) and expulsive (cough, sneeze) behaviors. Most studies have measured Pdi while animals are in a supine position, and have thus far failed to address how position and postural adjustments can affect Pdi. Accordingly, in the present study we focused on the effect of position

on Pdi measurements in rats. Adult Sprague Dawley rats (4 female, 4 male) were anesthetized with ketamine/xylazine. On the first day, animals were placed in supine, prone, right curled and left curled positions within a BUXCO FinePointe whole body plethysmography system to record respiratory rate and tidal volumes during eupnea and hypoxia-hypercapnia. The following day, Millar mikro tip pressure transducers were placed in the stomach (gastric pressure - Pgas) and esophagus (esophageal pressure - Peso), and Pdi was measured as the difference between Pgas and Peso. In each animal, Pdi generated during eupnea and hypoxia-hypercapnia was measured while the animals were placed in the supine, prone, right curled and left curled positions. Tukey-Kramer post-hoc test was used to compare the effect of posture and behavior (eupnea/hypercapnia-hypoxia) on Pdi amplitude, breathing frequency, and duty cycle. From these results, we concluded no differences were found across postures in the bound (isometric) or unbound state of the diaphragm muscle. The postural invariance of Pdi measurements across ventilatory behaviors supports the use of Pdi for diaphragm muscle force.

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Poster

407. Neuro-Muscle Interactions

Location: Hall A

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Program #/Poster #: 407.20/T13

Topic: E.09. Motor Neurons and Muscle

Support: NIH grant R01AG055545
NIH grant R56AG051501
NIH grant R21NS106313

Title: Deletion of Lynx1 promotes compensatory reinnervation of NMJs and delays ALS onset

Authors: S. K. VAUGHAN[1,2,3], ***K. T. REGA**[1], T. D. MYERS[1], G. VALDEZ[1,3];
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Abstract: Dysregulated cholinergic transmission at the neuromuscular junction (NMJ) has been shown to directly contribute to neuromuscular degeneration. Cholinergic dysregulation has also been implicated in amyotrophic lateral sclerosis (ALS) pathology, and thus may contribute to disease progression. Specifically, ALS-afflicted animals have increased spontaneous activity due to elevated ACh at the synaptic cleft, and heightened expression of immature nicotinic acetylcholine receptors (nAChRs) in muscle fibers. Thus, modifying the sensitivity of nAChRs to ACh may reduce deleterious effects of cholinergic dysfunction on NMJs and muscle fibers. In

unpublished findings, we have gathered significant data showing that Lynx1 desensitizes nAChRs at the NMJ to ACh, which contributes to NMJ maintenance and stability. In this study, we asked if Lynx1 also functions to preserve and repair NMJs during the progression of ALS. We found that Lynx1 levels decrease in skeletal muscles before NMJs degenerate in $SOD1^{G93A}$ mice. To determine if this decrease contributes to ALS-pathogenesis, we examined $SOD1^{G93A}$ mice lacking Lynx1 ($SOD1^{G93A}$;$Lynx1^{-/-}$). Interestingly, deletion of Lynx1 significantly delays the onset of ALS symptoms without altering the lifespan of male $SOD1^{G93A}$ mice. Since NMJs undergo bouts of degeneration and regeneration, a process called compensatory reinnervation, during the initial onset of symptoms, these findings suggested that loss of Lynx1 promotes compensatory reinnervation. To test this possibility, we used a fibular nerve crush model to examine NMJ reinnervation in $Lynx1^{-/-}$ and control mice. In $Lynx1^{-/-}$ mice, NMJs are reinnervated at a faster rate compared to control mice, supporting the hypothesis that skeletal muscles in $SOD1^{G93A}$ mice reduce endogenous Lynx1 levels to promote compensatory reinnervation. However, we also found that complete loss of Lynx1 delays cellular changes necessary for NMJs to fully repair following denervation. These include the elimination of redundant axons innervating the same muscle fiber, and the shedding of extensions that go beyond the postsynaptic region. Deletion of Lynx1 also exacerbates denervation-induced fragmentation of postsynaptic sites, and the appearance of extra-synaptic clusters of nAChRs. Not surprisingly, these morphological changes at NMJs have downstream effects on muscle fibers, and the expression of genes involved in NMJs stability, myogenesis, and muscle atrophy. Altogether, these data shed light on the therapeutic potential of Lynx1 for promoting repair of NMJs, and delaying ALS disease progression.

Disclosures: **S.K. Vaughan:** None. **K.T. Rega:** None. **T.D. Myers:** None. **G. Valdez:** None.

Poster

407. Neuro-Muscle Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 407.21/T14

Topic: E.09. Motor Neurons and Muscle

Support: NIH grant R01AG055545
NIH grant R56AG051501
NIH grant R21NS106313

Title: Lynx1 modulates nAChRs in adult skeletal muscles and contributes to NMJ maintenance

Authors: *S. K. VAUGHAN[1,2,3], S. BARBAT[4], T. D. MYERS[1], B. S. PRADHAN[5], T. J. PROSZYNSKI[5], R. ROBITAILLE[4], G. VALDEZ[1,3];
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Providence, RI; [4]Neurosci., Univ. De Montréal, Montreal, QC, Canada; [5]Cell Biol., Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Cholinergic transmission is essential for voluntary movement and plays a central role in sculpting and maintaining neuromuscular junctions (NMJs) and muscle fibers. Unfortunately, cholinergic transmission becomes dysregulated with advancing age and recent discoveries show that such changes contribute to age-related neuromuscular degeneration. Thus, finding ways to mitigate the effects of dysregulated cholinergic activity may prevent NMJ degeneration. Here, we asked if Lynx1, a GPI-anchored protein shown to modulate cholinergic transmission in the brain, plays a similar role at NMJs. We first show that Lynx1 mRNA is expressed by skeletal muscles and C2C12 myotubes. Furthermore, the expression of Lynx1 in muscle fibers is developmentally regulated and has a direct correlation with cholinergic activity. We show that Lynx1 localizes to the NMJ in young adult mice and interacts with NMJ-associated nAChRs. Importantly, electrophysiological analysis revealed that Lynx1 functions to desensitize nAChRs to ACh at the NMJ. We then examined the role of Lynx1 in the development and maintenance of NMJs and muscle fibers. Using $Lynx1^{-/-}$ animals, we determined that Lynx1 has no significant impact on NMJ development. However, deletion of Lynx1 significantly increases the incidence of NMJs with age-related morphological features in young adult and middle-aged mice. Deletion of Lynx1 also accelerates nAChR turnover and alters expression of genes that function to stabilize the postsynaptic region of the NMJ. Together, these findings demonstrate that Lynx1 is a promising candidate molecule for mitigating the deleterious effects of aberrant cholinergic transmission on aging NMJs and skeletal muscles.

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Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.01/T15

Topic: F.03. Neuroendocrine Processes

Support: NIH HD090606

Title: Development of the murine vasopressin system

Authors: E. J. SOMMER, E. A. AULINO, ***H. K. CALDWELL**;
Biol. Sciences, Biomed. Sciences, Brain Hlth. Res. Inst., Kent State Univ., Kent, OH

Abstract: While there has been extensive study of the arginine vasopressin (Avp) system in adults, much less is known, or understood, about the early development of this system. In most

adult mammals, Avp is primarily produced in parvocellular neurons of the supraoptic and paraventricular nucleus of the hypothalamus and to a lesser extent in a handful of other brain nuclei. Avp has also been identified in cells other than neurons, including astrocytes. Given the mounting evidence that Avp's sister neuropeptide, oxytocin, is important for brain development in the perinatal period, it seems appropriate to examine the developing Avp system as well, since both are important modulators of social behavior. Previous work from our lab has found Avp mRNA in the mouse brain as early as embryonic day (E) 12.5. However, as mRNA expression is not necessarily indicative of ligand expression, we decided use immunohistochemistry to identify Avp-immunoreactive cells. Mouse brains of both sexes were collected at E14.5, E16.5, E18.5, and postnatal day (P) 2, and stained for Avp. Preliminary data suggest that there are Avp-immunoreactive cells throughout the brain in both males and females as early as E16.5. However, further work is needed to determine the specific cell types in which Avp is expressed, as well as the precise neuroanatomical localization of Avp-immunoreactivity.

Disclosures: H.K. Caldwell: None. **E.A. Aulino:** None. **E.J. Sommer:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.02/T16

Topic: F.03. Neuroendocrine Processes

Support: NIH R01 MH114994
The Good Nature Institute

Title: Peripheral vasopressin 1A receptors in neonatal mice

Authors: *K. R. DAY, M. A. GREENWOOD, E. A. D. HAMMOCK;
Psychology, Florida State Univ., Tallahassee, FL

Abstract: The process of parturition leads to an infant experiencing a dramatic spike in circulating vasopressin (AVP) that is unique to neonates exposed to vaginal delivery. Massive neuroendocrine activation of the HPA axis is believed to provide protection from the stresses of hypoxia and hypercapnia that are naturally associated with labor. Prior research suggests AVP may provide analgesic effects via vasopressin receptor 1a (AVPR1A), providing additional need for investigation as to which tissues the massive amounts of AVP may be acting on at birth. Utilizing 0.050nM I^{125} radioligand with high binding specificity for AVPR1A (Vasopressin (Linear), V-1A Antagonist (Phenylacetyl1, 0-Me-D-Tyr2, [^{125}I-Arg6]-)) we performed autoradiography to investigate which organs and tissues may be sensitive to the surge of AVP associated with vaginal delivery. Whole post-natal day 0 (P0) male (n=3) and female (n=3) C57/BL6j wildtype mice were cryosectioned in the sagittal plane in 20 micron sections. We

determined specificity of binding by comparing receptor binding observed in wildtype mice to *Avpr1a* KO male (n=3) and female (n=3) mice. To identify potential nonspecific binding, adjacent tissue sections for each specimen were competed with 1000nM of unlabeled vasopressin. For unbiased region of interest measurements of AVPR1A, post processing Cresyl Violet staining was completed. Image stacking, analysis and quantification were performed with ImageJ software and the TurboReg plugin. We confirmed that AVPR1A is present in the periphery of neonatal male and female C57/BL6j wildtype mice. Our results identify the robust presence of AVPR1A in the oronasal cavity, anogenital region, bone, spinal cord, and bladder of P0 male and female wildtype mice. Additional nonspecific binding can be seen in brown adipose tissue, liver, and fecal matter. Sex-specific variations in the presence of AVPR1A in anogenital regions can be observed, with greater binding in the female anogenital area. Identification of perinatal tissues and organs with AVPR1A will lead to a better understanding if the role of AVP at birth in the transition to postnatal life.
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Disclosures: K.R. Day: None. **M.A. Greenwood:** None. **E.A.D. Hammock:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.03/T17

Topic: F.02. Behavioral Neuroendocrinology

Support: National Institute for Environmental Studies [1416AT001]

Title: Behavioral traits associated with neurodevelopmental disorders in the mice lacking both vasopressin 1a and 1b receptors

Authors: *F. MAEKAWA[1,2], E. KIMURA[1,3], K. SANO[1,4], T. ENDO[5], K. NAKAMURA[6,2]; [1]Natl. Inst. for Envrn. Studies, Tsukuba, Japan; [2]Saitama Univ., Saitama, Japan; [3]Japan Society for the Promotion of Sci., Tokyo, Japan; [4]Univ. of Tsukuba, Tsukuba, Japan; [5]Phenovance Research & Technol., Kashiwa, Japan; [6]Natl. Res. Inst. Child Hlth. & Develop., Setagaya, Japan

Abstract: Background: Vasopressin is a hormone synthesized in hypothalamic nuclei and is secreted not only as a hormone from posterior pituitary but also as a neuropeptide within brain. Vasopressin regulates the execution of communicative and adaptive behaviors, and the effect of vasopressin on these behaviors is thought to be mediated by vasopressin 1a and 1b receptors (V1aR and V1bR, respectively), predominant receptors in mouse brain. Although it has been reported that genetic deletion of either receptors causes different types of behavioral impairments in mice, it has remained unclear whether genetic deletion of both receptors causes either additive,

synergistic, or different actions. **Aim**: In this study, we examined the effects of gene deletion of both V1aR and V1bR on neonatal ultrasonic vocalization (USV), an index of communicative behavior, and adaptive behavior under new environment in group-housed condition.**Methods:**The USV of postnatal pups was measured for 1 min in sound-attenuate chamber, and the USV frequencies of 60-100 kHz were analyzed. After maturation, the various aspects of mouse behavior were recorded using the apparatus named IntelliCage. The visit number to corners, small rooms for drinking water, during 24 hours after the first entry into IntelliCage was used as an index of adaptive behavior.**Results:** We could detect significant alternation of frequency and duration of USV in the mice lacking both V1aR and V1bR. The adaptation to IntelliCage was also significantly decreased in the mice lacking both V1aR and V1bR. Such changes were not detected in the mice deficient in either receptor.**Discussion**: These results suggest the possibility that, as far as the USV and the adaptation to new environment are concerned, V1aR and V1bR compensate each other and severe impairments occur only if both receptors are deficient.

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Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.04/T18

Topic: F.03. Neuroendocrine Processes

Support: NIMH-MH002386
CONACYT CB-283279
DGAPA-UNAM-PAPIIT IN216214

Title: Molecular signatures for co-transmission within vasopressinergic neurons of mouse and rat central nervous system

Authors: L. ZHANG[1], V. S. HERNANDEZ[1], *L. E. EIDEN[2];
[1]Physiology/Medicine, Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; [2]Section on Mol. Neurosci., NIH, NIMH-IRP, Bethesda, MD

Abstract: Using high-resolution ISH methods, radio-ribonucleotide and RNAscope® 2.5 HD duplex assay, with VGLUT1, VGLUT2 and VGAT probes as well as AVP probes, we investigated the co-transmission signatures of vasopressinergic neurons throughout the central nervous system of the rat and mouse. The mainly glutamatergic AVP cell groups include: hypothalamic paraventricular (PVN, both media and lateral division and all antero-posterior span) and supraoptic (SON) nuclei, accessory nuclei (which include nucleus circularis and the

posterior fornical nucleus, as well as the tufted cells of the olfactory bulb). We remark here on a newly-identified vasopressinergic cell group, comprising layer V glutamatergic pyramidal neurons of the lateral entorhinal cortex, which also are glutamatergic. The mainly GABAergic AVP cell groups include those of the suprachiasmatic nucleus (SCN), the bed nucleus of the stria terminalis, medial posterior internal division (BSTmpi), the intra-amygdala division of BST (BSTIA), and the central amygdala. These data suggest that the traditional parsing of vasopressinergic cell groups into magno- and parvocellular should include also an additional neurochemical classification based on co-transmitter phenotype.

Disclosures: L.E. Eiden: None. **L. Zhang:** None. **V.S. Hernandez:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.05/T19

Topic: F.02. Behavioral Neuroendocrinology

Support: The University at Buffalo Research Foundation Award #64755
National Science Foundation, IOS-1754878

Title: Behavioral and neural investigations in the Brattleboro rat reveal a role for central vasopressin projections in behavioral arousal during adolescence

Authors: *K. SCHATZ[1], L. M. BROWN[1], A. R. BARRETT[1], L. C. ROTH[2], V. GRINEVICH[3], M. J. PAUL[4];
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Abstract: The neuropeptide, arginine vasopressin (AVP), has been implicated in a number of neurodevelopmental disorders that impact social and emotional development (e.g., autism and ADHD). Nevertheless, we do not understand how AVP modulates behavioral development or how its altered function contributes to neurodevelopmental disorders. The Brattleboro rat, which lacks AVP due to a mutation in the *Avp* gene, is an ideal model to study the impact of life-long AVP disruption. Brattleboro rats suffer from diabetes insipidus, excessive drinking and urination, due to the loss of AVP action on the kidney. Recently, we found that male and female adolescent homozygous Brattleboro rats (Homs) engage in less social play than their Het littermates, and amount of play behavior was correlated with the amount of behavioral arousal in an open field. This suggests that AVP may modulate levels of social play through its regulation of arousal. To further investigate the link between arousal and social behavior, our first experiment tested whether adolescent Hom rats exhibit decreased behavioral arousal in an open field test compared

to wild type (WT) littermates. Consistent with previous findings, Homs traveled less distance, spent more time inactive, and entered the center of the arena fewer times than WT littermates. We then asked which pathway(s) mediate AVP's actions on arousal. In experiment 2, we infused a recombinant adeno-associated virus containing an AVP promoter driving expression of a functional AVP gene (rAAV-AVP) or a Venus fluorescent marker (rAAV-Venus) into the paraventricular nucleus of the hypothalamus (PVN) of male and female adolescent Hom rats. The rAAV-AVP selectively rescued AVP production in magnocellular cells and fibers of the PVN, including projections to limbic areas such as the lateral habenula, hippocampus, medial amygdala, and lateral septum. These findings support previous reports indicating extraneurohypophysial projections of PVN AVP magnocellular cells. Hom rAAV-AVP infused rats showed a marked decrease in water intake, but behavioral arousal in the open field was unaffected. These data implicate a role for AVP in the regulation of arousal during adolescence. Furthermore, the present findings indicate that the hypoaroused phenotype of adolescent Hom rats is not due to the loss of AVP function in magnocellular cells or a side effect of diabetes insipidus, but favors the hypothesis that central, parvocellular AVP mechanisms underlie the regulation of arousal during adolescence. Ongoing studies are testing the role of hindbrain AVP projections in the regulation of arousal and social behavior during adolescence.

Disclosures: K. Schatz: None. **L.M. Brown:** None. **A.R. Barrett:** None. **L.C. Roth:** None. **V. Grinevich:** None. **M.J. Paul:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.06/T20

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R21MH115680

Title: Effect of sex and autism diagnosis on oxytocin and vasopressin 1a receptors in the substantia nigra, hippocampus, and primary visual cortex of the human brain

Authors: *M. C. PALUMBO[1], T. C. SIMMONS[1], A. L. SMITH[2], M. M. GOODMAN[2], K. L. BALES[1], S. M. FREEMAN[1,3];
[1]California Natl. Primate Res. Ctr., Univ. of California Davis, Davis, CA; [2]Ctr. for Translational Social Neurosci., Emory Univ., Atlanta, GA; [3]Dept. of Biol., Utah State Univ., Logan, UT

Abstract: Oxytocin (OT) and vasopressin (AVP) influence complex social behaviors across mammals. In humans, clinical trials have begun using OT to treat Autism Spectrum Disorder (ASD), a condition characterized in part by deficits in sociality. However, our knowledge of the OT and AVP systems in the human brain is limited. This study used competitive binding

receptor autoradiography to quantify putative oxytocin receptor (OXTR) and vasopressin receptor 1a (AVPR1a) densities in postmortem human brain tissue from individuals with ASD and neurotypical (NT) specimens. We examined three regions previously shown to contain dense OXTR or AVPR1a receptor binding in the human brain: the substantia nigra (SN), hippocampus, and primary visual cortex (V1). The NIH NeuroBioBank provided us with the following specimens: 8-9 ASD males, 6-8 ASD females, 7-8 NT males, and 6-7 NT females. In the SN, OXTR density was lower in ASD females compared to ASD males (p=0.0293), although neither group was significantly different from NT. Previous reports have postulated sex differences in ASD severity, and our results in the SN provide neuroanatomical support for this idea. Additionally, among ASD specimens, we found a significant negative association between OXTR density in the SN and the patient's Autism Diagnostic Interview-Revised (ADI-R) scores for quantitative abnormalities in reciprocal social interaction (p=0.0479; r=-0.6709) and for abnormality of development (p=0.0144; r=-0.7738). The higher the ADI-R score, the more severe the symptoms, so our results show that lower OXTR densities in the SN correlate with more severe social symptoms. We did not find any effect of ASD or sex on AVPR1a density in the hippocampus or V1, nor were there significant associations between age and either OXTR or AVPR1a density in any region of interest. We did find significant positive associations in ASD between AVPR1a density in the dentate gyrus and ADI-R scores for abnormality of development (p=0.0273; r=0.7641), as well as between OXTR density and social interaction ADI-R scores in all subregions of V1. Correlations between ADI-R scores and receptor binding in multiple brain regions associated with sensory processing provide some of the first neuroanatomical evidence underlying ASD symptom severity.

Disclosures: M.C. Palumbo: None. **T.C. Simmons:** None. **A.L. Smith:** None. **M.M. Goodman:** None. **K.L. Bales:** None. **S.M. Freeman:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.07/U1

Topic: F.02. Behavioral Neuroendocrinology

Support: JSPS KAKENHI Grant JP 16K10004
MEXT KAKENHI Grant JP 17H05967
MEXT KAKENHI Grant JP 19H04922

Title: Useful tools for oxytocin studies: A new reporter and a new cre recombinase driver to precisely recapitulate oxytocin receptor expression profiles in mouse brains

Authors: *Y. U. INOUE[1], R. KANEKO[2], Y. MORIMOTO[1], T. INOUE[1];
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Abstract: Oxytocin (Oxt), a natural brain peptide produced within the hypothalamus, plays an important role in regulating social behaviors. It is also expected as a potential therapeutic for social deficits in autism spectrum disorders. Oxytocin receptor (Oxtr) is abundantly expressed in the brain and its relationship with social behaviors have also been extensively studied in mouse brains. However, multiple independent tools have been used to visualize Oxtr expressions and the resulted expression patterns are not fully consistent with each other. First, commercially available antibodies don't have enough specificities to Oxtr protein, and detection of *Oxtr* mRNA by *in situ* hybridization has been reported to be difficult. Secondly, several reporter lines and Cre recombinase driver lines have been generated by BAC transgenesis or ES cell-based gene targeting, yet they have somewhat different expression profiles probably because of the different integrity and context for their reporter/driver expressions in each line. Here, we employ CRISPR/Cas9 genome editing technology to generate novel knock-in mouse lines to precisely recapitulate the endogenous Oxtr expression profiles. We establish *Oxtr-PAtag-T2A-tdTomato* reporter line and *Oxtr-PAtag-T2A-iCre* driver line, in which fluorescent reporter or codon-improved Cre recombinase is bicistronically expressed within the Oxtr-expressing cells depending on T2A peptide cleavage. Furthermore, in order to directly determine the endogenous Oxtr localizations, PA-tag is fused to C-terminus of the receptor protein. By using these knock-in lines, we carefully analyze the co-localization of tdTomato/iCre with PA-tag in the brain tissues to obtain a precise Oxtr expression atlas. As the results, in addition to the previously reported brain regions where Oxtr expressions have been detected, we confirmed the receptor expressions in the other regions such as the nucleus accumbens. It is noteworthy that *Oxtr-PAtag-T2A-tdTomato* reporter line can be maintained in the homozygous state without the laborious genotyping procedure and the tdTomato fluorescence can immediately illuminate brain tissues without immunostaining. Our knock-in mouse lines could thus serve useful tools to investigate roles of Oxt-Oxtr system in the social brain.

Disclosures: **Y.U. Inoue:** None. **R. Kaneko:** None. **Y. Morimoto:** None. **T. Inoue:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.08/U2

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS 1353859
NIH R15HD090606

Brain Health Research Institute Blue Award

Title: Disruption of oxytocin signaling during mouse fetal development results in sex-specific deficits in adult social behaviors

Authors: *E. A. AULINO[1,2], H. K. CALDWELL[1,2];
[1]Dept. of Biol. Sci., [2]Brain Hlth. Res. Inst., Kent State Univ., Kent, OH

Abstract: In adults, the neuropeptide oxytocin (Oxt) is known to be important to the neuromodulation of social behaviors in both males and females. However, there is also evidence to suggest that the Oxt system plays an important role in the neurochemical 'shaping' of the social brain during early development. For instance, mice whose Oxt signaling is impaired during fetal life have heighted inter-male aggression and impaired social-recognition in adulthood; these same deficits are not observed when the Oxt receptor (Oxtr) is genetically disrupted two weeks after birth. Even in a non-traditional model species, such as prairie voles, disruption of the Oxt system in neonates results in sex-specific behavioral changes in adulthood. Taken together, these studies point to Oxt having an important role in early brain development – specifically being involved in the development of neural circuitry integral to sex-specific behavior. Based in part on the aforementioned studies, as well as data from our lab that has found that there are significant sex-differences in the development of the Oxt system in the embryonic mouse brain, we sought to perform functional studies linking the fetal Oxt system to changes in adult social behaviors. We hypothesized that transient disruption of Oxtr signaling during fetal life would result in sex-specific behavioral differences in adulthood. To test this hypothesis, we performed transuterine microinjections of either saline or an Oxtr antagonist into the lateral ventricles of embryonic day (E) 16.5 in C57BL/6J mice and observed their behavior in adulthood. We found that transient disruption of Oxtr signaling at E16.5 resulted in increased aggressive behavior in adult males and impaired social recognition memory in adult females. While additional study is still needed to determine the mechanism by which these behavioral alterations have occurred, these results suggest that the Oxt system is important to sex-specific brain organization.

Disclosures: E.A. Aulino: None. **H.K. Caldwell:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.09/U3

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH R01 MH114994
The Good Nature Institute

NIH T32 5T32DC000044-23

Title: Mice prefer oxytocin-containing social stimuli

Authors: *M. TABBAA, E. A. D. HAMMOCK;
Psychology, Florida State Univ., Tallahassee, FL

Abstract: Oxytocin (OXT) regulates species typical social behaviors and interacts with early life experience to shape adult responses. Recent data from our lab characterizing oxytocin receptor (OXTR) ligand binding in the mouth and nasal cavity as well as the brain and behavioral response of orally-applied OXT in pre-weanling mice suggest that socially-acquired OXT may affect sensory processing and subsequent brain development through peripheral OXTR. Peripheral OXTRs may be activated in social contexts to drive social interactions throughout life. If OXT is exchanged between individuals and this influences social behavior, then mice should be able to distinguish between a conspecific with and without OXT as indicated by behavioral preference in a two-choice assay. To begin to address this hypothesis, we tested the social preference of adult C57 male (N=13) and female (N=12) mice for OXT containing wild-type (WT) versus OXT knock-out (KO) same-sex stimulus mice in a 30-minute three-chambered choice test. Next, we asked if mice would show a preference for same-sex mouse bedding with and without OXT. Bedding was collected from cages containing only OXT KO mice and was then contaminated with either synthetic OXT or saline. One week later, this test was repeated to evaluate the responses to opposite-sex bedding with and without OXT. Behaviors were video recorded and later scored by a researcher blind to stimulus mice genotype and bedding condition. During the social preference test for OXT WT versus KO same sex stimulus mice, both males and females spent more time sniffing the face of OXT WT mice compared to OXT KO mice. In the soiled bedding test, there were no differences in investigation of same sex OXT KO bedding containing OXT compared to saline. However, males spent more time investigating female bedding containing OXT compared to saline. In contrast, females did not show a preference for male OXT KO bedding containing OXT over saline. Combined, these data indicate the possibility for socially-acquired OXT to modulate species-typical social interactions, with potential for sex-specific effects. Next, we will attempt to replicate and extend these findings using mice with a deletion of *Oxtr* from peripheral sensory ganglia.

Disclosures: M. Tabbaa: None. **E.A.D. Hammock:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.10/U4

Topic: F.02. Behavioral Neuroendocrinology

Support: NRF-2016R1D1A1B03934263
NRF-2019R1A2C1002963

Title: Oxytocin modulation in social pair-induced fear behavior

Authors: *M. JANG, T. JUNG, J. NOH;
Dankook Univ., Yongin, Korea, Republic of

Abstract: We previously reported that the pair exposure with conspecific during fear conditioning in adolescent rats helped to cope with both freezing response and fear memory and those effects were correlated with cellular activity in the medial prefrontal cortex (mPFC). Oxytocin can be proposed as a candidate substance for pair exposure effect due to reporting that oxytocin controls social behavior and its receptors are widely expressed in the mPFC but its role for social pair remains unclear. To determine this issue, we performed a passive avoidance test (0.7 mA, 5 s) in pair-exposed adolescent rats treated by oxytocin receptor antagonist atosiban peripherally (via i.p injection) and centrally (injected in mPFC). Peripheral administration of atosiban before learning session significantly increased the freezing behavior and decreased the black room preference in pair exposed rats compared with saline-injected pair rats. Atosiban-injection via stereotaxically implanted guide cannula into the mPFC in pair rats significantly increased the freezing behavior and the step-through latency into the black room compared with ACSF-injected pair rats. Our finding indicates endogenous oxytocin released in paired condition play an important role in the modulation of fear-related behavior and fear memory in adolescent rats.

Disclosures: **M. Jang:** None. **T. Jung:** None. **J. Noh:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.11/U5

Topic: F.02. Behavioral Neuroendocrinology

Support: The Good Nature Institute

Title: Modulation of pupillary behavior by oxytocin in mice

Authors: *M. A. GREENWOOD[1], E. A. HAMMOCK[2];
[1]Psychology,Program in Neurosci., [2]Psychology, Florida State Univ., Tallahassee, FL

Abstract: Oxytocin (OXT) is a peptide with important regulatory roles in both physiological and behavioral contexts. OXT binds to the OXT receptor (OXTR) in the central and peripheral nervous systems, with diverse patterns of expression dependent on many variables including but

not limited to species, sex, and stage of development. OXTR have previously been identified in the eye across rodent species. However, the functionality of these receptors has not been thoroughly explored. The aim of this project was to investigate the role of OXTR on pupillary changes under constant luminance. Adult transgenic male and female *Oxt* and *Oxtr* wild-type and knockout mice were used to assess differences in pupillary responsiveness to the topical application of OXT directly to the eye. These assessments in adult mice demonstrated, relative to wild-type mice (n=20), a significantly dilated pupil in OXTR knockout mice (n=9) and a constricted baseline pupil diameter in *Oxt* knockout mice (n=10), which was rescued by the topical application of OXT (n=8). These data support a potential developmental role for OXTR and an activational role for OXT on autonomic regulation in the visual system.

Disclosures: M.A. Greenwood: None. **E.A. Hammock:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.12/U6

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF DGE-1745038
NSF DGE-1143954
R01 MH107515-01A1

Title: Behavioral effects of an oxytocin receptor antagonist on a mouse modeling the complete deletion of the Williams Syndrome critical region

Authors: *K. R. NYGAARD[1], N. D. KOPP[2], E. MINAKOVA[3], S. E. MALONEY[4], J. D. DOUGHERTY[1];
[1]Genet. and Psychiatry, [2]Genet., [3]Pediatrics, [4]Psychiatry, Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Atypical behaviors, including increased sociability and a propensity for non-social phobias, are hallmarks of Williams Syndrome (WS). Caused by a relatively small genetic deletion, WS thus offers a unique view of the genetic contributions of behavioral phenotypes. While some genes have easily been mapped to the cardiac phenotypes of WS, the neurogenetics underlying the behavioral phenotypes are unknown. Dysregulation of the oxytocin system has been observed in people with WS but the mechanisms behind it and behavioral consequences of it are not well understood. Our objective is to elucidate the mechanisms and consequences of dysregulated oxytocin in WS by examining the effects of an oxytocin receptor antagonist on a mouse model with a hemizygous deletion of the entire syntenic WS critical region. Prior studies in the lab have shown increased oxytocin in the hypothalamus of these Complete Deletion (CD)

mice. As the hypothalamus is critical in fear memory, we chose to examine the behavior of our CD mice in the Conditioned Fear task. In this task, associative learning is examined by measuring freezing behavior in response to pairing both contextual and auditory cues with an adverse stimulus. Blinded to genotypes, I examined the freezing behavior of adult CD and wild-type (WT) mice of both sexes on a C57BL/6J background. The CD mice demonstrated increased freezing to the stimulus during acquisition but showed decreased freezing relative to WT littermates in response to the auditory cue 48 hours later. These data suggest deficits in amygdalar-dependent conditioning in mice hemizygous for the WS critical region. We hypothesized the abnormal freezing response in CD mice was influenced by the dysregulated oxytocin system. To test this hypothesis, CD and WT mice were split into two groups. Both groups were fitted with a cannula to facilitate drug delivery directly to the left lateral ventricle. Half of each group was given an oxytocin receptor antagonist, while the others received a normal saline vehicle solution. We are currently collecting and analyzing the freezing behavior measured in the Conditioned Fear task. In the future, we hope to expand this design to other phenotypes of the CD mice in order to best understand the impact of oxytocin on the various behaviors. These results will help to inform the mechanisms and consequences of oxytocin dysregulation in WS. The knowledge can also help inform other social or anxiety disorders, and mechanisms of oxytocin in general.

Disclosures: K.R. Nygaard: None. **N.D. Kopp:** None. **E. Minakova:** None. **S.E. Maloney:** None. **J.D. Dougherty:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.13/U7

Topic: F.03. Neuroendocrine Processes

Support: NIH MH114994
The Good Nature Institute

Title: Molecular anatomy of oxytocin receptors in peripheral sensory ganglia of neonatal mice

Authors: *R. VAIDYANATHAN, E. A. HAMMOCK;
Psychology, Program in Neurosci., Florida State Univ., Tallahassee, FL

Abstract: Neonatal mice abundantly express oxytocin receptors (OXTRs) in a range of peripheral tissues where circulating oxytocin (OXT) can mediate tissue-specific effects via peripheral OXTR. OXTR-containing regions in the face such as the mandibular and maxillary periodontium, eye, and whisker pads are innervated by sensory afferents of the trigeminal ganglion. Similarly, other OXTR-containing regions in the body such as the anogenital region

and adrenal glands are innervated by the dorsal root ganglia. We have previously identified *Oxtr* mRNA in the trigeminal sensory ganglia (TG) and dorsal root ganglia (DRG) of neonatal mice. In this study we aim to A) refine the anatomy of *Oxtr* in the TG, and B) to determine if peripheral OXTR are synthesized in the peripheral sensory ganglia. Previously, we identified by in-situ hybridization that *Oxtr* mRNA colocalize with different types of sensory neurons in the TG. Mechanosensory neurons (TrkB positive) show the highest co-expression of *Oxtr*, but nociceptive neurons (TrkA positive) and proprioceptive neurons (TrkC positive) show low and moderate levels of co-expression with *Oxtr* in the TG, respectively. In our current study, we use markers for thermoreceptive neurons to identify if *Oxtr* in the TG colocalize with warm or cold-sensitive neural populations. Preliminary data suggest that *Oxtr* co-localizes with thermoreceptive neurons. To address aim B, we selectively eliminate *Oxtr* from sensory ganglia (TG and DRG) using a Cre-lox P approach and quantify peripheral OXTR ligand-binding in the whole body of neonatal C57BL/6J mice. Our preliminary findings suggest that OXTR are not fully eliminated in the periphery due to the deletion of *Oxtr* from sensory ganglia. We predict that some of these peripheral regions will show a decrease in OXTR-ligand binding compared to their WT controls.

Disclosures: R. Vaidyanathan: None. **E.A. Hammock:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.14/U8

Topic: F.03. Neuroendocrine Processes

Support: R01MH116176

Title: Brain wide input output mapping of hypothalamic oxytocin neurons

Authors: *S. SON, Y. KIM;
Dept. of Neural and Behavioral Sci., Col. of Medicine, Penn State Univ., Hershey, PA

Abstract: Oxytocin (OXT) is the evolutionarily conserved neuropeptide in mammals, which is produced by neurons of the paraventricular hypothalamic (PVH) and the supraoptic (SO) nucleus. Many studies have been conducted to show that OXT neurons projected to broad brain regions and the spinal cord, and revealed underlying mechanisms of OXT to the circuit-specific behaviors. However, it is unclear how OXT neurons within the hypothalamic nuclei are topographically organized with regard to input and output throughout the whole-brain. Here, we employed viral vector-based techniques, which combined with adeno-associated virus (AAV) and monosynaptic rabies tracing techniques, to label axonal output and pre-synaptic input of OXT neurons in mice. We imaged the whole mouse brains at cellular resolution using serial two-

photon tomography and used whole-brain data processing pipeline to achieve quantitative input-output mapping of hypothalamic OXT neurons. The OXT neurons of PVH projected dorsally to the lateral zone of hypothalamic and continued into the medulla through the midbrain and the pons. This pathway provides dense input to the substantia nigra, the ventral tegmental area, the periaqueductal gray (PAG), and the parabrachial nucleus. The OXT neurons also projected to the forebrain regions; the nucleus accumbens (ACB), the lateral septal nucleus (LS), and olfactory cortex areas. Both of the OXT neurons in PVH and SO were ventrally projected into the tuberal nucleus (TU), then reached to the median eminence. Rabies-virus-based input mapping indicated that OXT neurons of the PVH received synaptic input from the hypothalamus, the thalamus, and the striatum, while inputs in the SO were mainly from the hypothalamic area. Interestingly, the brain regions previously associated with social behavior, learning, and memory were reciprocally connected, including the ventromedial hypothalamic nucleus, the parasubthalamic nucleus, the lateral hypothalamic area, the central amygdalar nucleus, the bed nuclei of the stria terminalis, the PAG, the LS, and the ACB. In summary, our data established the anatomical organization of OXT neurons in order to understand the neural circuit mechanisms of OXT neurons in regulating different circuit specific behaviors.

Disclosures: S. Son: None. **Y. Kim:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.15/U9

Topic: F.03. Neuroendocrine Processes

Title: Decreased GRIN1 receptor subunit protein expression in the basolateral amygdala of phencyclidine-treated oxytocin knockout mice

Authors: *K. M. RODRIGUEZ[1,3], H. K. CALDWELL[2,3];
[1]Sch. of Biomed. Sci., [2]Dept. of Biol. Sci., Kent State Univ., Kent, OH; [3]Kent State Univ., Brain Hlth. Res. Inst., Kent, OH

Abstract: It is hypothesized that the oxytocin (Oxt) system, known for its neural modulation of social behaviors, may contribute to the social impairments observed in many neuropsychiatric disorders, including schizophrenia. Support for this hypothesis comes from some studies demonstrating that some patients diagnosed with schizophrenia that have higher plasma Oxt levels also have less severe positive symptoms and exhibit fewer social deficits. There is also evidence that genetic polymorphisms of the *Oxt* and *Oxt* receptor (*Oxtr*) genes may also contribute to symptom severity and treatment efficacy in some individuals diagnosed with schizophrenia. Interestingly, Oxt is known to interact with many of the neurotransmitters disrupted in schizophrenia, including dopamine and glutamate; both of which are also important

for the modulation of social behavior. However, how Oxt and these neurotransmitters interact is unclear. The glutamate hypothesis of schizophrenia supposes that the hypo-functioning of N-methyl-D-aspartate (NMDA) receptors are involved in the negative and cognitive impairments of schizophrenia. Work in our lab has found that Oxt knockout (-/-) mice treated acutely or subchronically with phencyclidine (PCP), an NMDA receptor antagonist, have greater impairments in prepulse inhibition of the startle reflex and sociability, respectively, compared to controls. Thus, here we sought to investigate how a PCP model of schizophrenia and genetic disruption of Oxt, i.e. Oxt -/- mice, affected expression of NMDA receptor subunits. Specifically, we focused on GRIN1, GRIN2a and GRIN2b, as genetic polymorphisms in these receptor subunits are correlated with schizophrenia. We found differential expression patterns in brain areas involved in social behavior. Specifically, as compared to controls, PCP-treated Oxt -/- mice had increased *GRIN1* and *GRIN2a* mRNA expression in the basolateral amygdala (BLA). However, protein expression of GRIN1 was decreased in the BLA. Using this mouse model, future work will explore how Oxt and glutamate may interact in the BLA to affect social behavior.

Disclosures: K.M. Rodriguez: None. **H.K. Caldwell:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.16/U10

Topic: F.03. Neuroendocrine Processes

Support: NTU
The Ministry of Science and Technology

Title: Synapsin Ia regulates the oxytocin release in the hypothalamic-neurohypophysial system of adult male rats

Authors: *C.-T. HUANG[1], E.-C. CHEN[1], C.-T. WANG[1,2,3,4];
[1]Inst. of Mol. and Cell. Biol., [2]Dept. of Life Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Genome and Systems Biol. Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan

Abstract: Oxytocin (OT), a hormone that regulates delivery and lactation, is synthesized from the magnocellular neurons (MCNs) of paraventricular nucleus (PVN) and supraoptic nucleus (SON) in the hypothalamic-neurohypophysis system (HNS). With the deepening of research, many studies indicated that OT neurons can project their axon terminals into different brain regions, including amygdala, nucleus accumbens (NAc), ventromedial nucleus (VMN), and dorsal vagal complex (DVC), thus regulating emotion, motivation, glucose homeostasis, gastric

motility, and social behavior. However, the molecular mechanism underlying the regulation of OT release remains unclear. In the HNS, the axon terminals of OT neurons contain two types of vesicles, i.e., OT-laden large dense-core vesicles (LDCVs) and microvesicles (MVs) similar to synaptic vesicles (SVs). Although the release from both types of vesicles can be mediated by Ca^{2+}-dependent exocytosis, a specific protein Syn Ia is exclusively expressed in MVs/SVs and regulates the exocytosis through phosphorylation. In this study, we aimed to determine the role of Syn Ia in regulating the OT release from the HNS. First, we designed a series of the OT neuron-specific constructs expressing the exocytosis reporter and Syn Ia (or the Syn Ia mutant). Next, we transfected the SON-OT neurons with these constructs in adult male SD rats by using *in vivo* electroporation. To further detect the expression of these constructs, we performed immunostaining by labeling the exocytosis reporter. As the results, we found that the immunoreactivity of the LDCV-specific reporter (Neurophysin-pHVenus) was relatively localized to the OT immunoreactivity compared to that of the general vesicle reporter (Synaptobrevin II-pHVenus). Furthermore, by using ELISA, we detected the changes in OT level in the plasma of transfected rats before and after *in vivo* electroporation. We found that overexpressing Syn Ia in the SON-OT neurons can increase the OT release to peripheral plasma compared to the control, but overexpressing the Syn Ia mutant cannot. Thus, our results suggest that Syn Ia may regulate the OT release from the HNS.

Disclosures: C. Huang: None. **E. Chen:** None. **C. Wang:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.17/U11

Topic: F.03. Neuroendocrine Processes

Support: CONACYT: CB-238744
DGAPA-UNAM-PAPIIT-IN216918
CB-238313, 221092
PAPIIT : IA208118

Title: Water deprivation influences predator-triggered fear response: Finding a causal role for the hypothalamic vasopressin system ascending innervations to limbic regions

Authors: *E. C. GUERRA[1], T. G. PADILLA[1], V. S. HERNÁNDEZ[1], M. C. CÁRDENAS-AGUAYO[1], O. R. HERNÁNDEZ-PÉREZ[1], F. JÁUREGUI[2], S. LUQUÍN[2], L. E. EIDEN[3], L. ZHANG[1];
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Abstract: We have previously reported that the hypothalamic vasopressinergic magnocellular neurosecretory neuron (AVPMNN) system possesses ascending projections to the lateral habenula, amygdala, hippocampus and locus coeruleus which may have synapse organizing functions when it is persistently up-regulated. Here, we devised a predator odor + cat exposure test to assess purposeful movement, freezing behavior and Fos expression, comparing C57BL6 mice deprived of water for 24 hours (WD24) with control mice. Inside a hood, mice were housed individually in small wire cages (15cm x 15 cm x 45cm) modified from standard wire cage mice traps. Cat urine material was collected from domestic cat litter boxes and was inserted into 50 mL Falcon tubes. During the experiment, the opened Falcon tubes were introduced individually into each cage while recording mice behavior. After five minutes, a young male cat was introduced to the hood. This procedure lasted 5 min. Perfusion and fixation were performed 90 min later for Fos expression assessment. Predator odor triggered purposeful movements such as approaching and withdrawal from the Falcon tube, but this behavior decreased during the 5 min observation period. The WD group had significantly higher counts of both purposeful movement and immobility during cat presentation. Moreover, Fos expression assessment showed that most of the olfactory pathway, as well as limbic regions such as the dentate gyrus, hippocampal CA3, MPO, BNST, PVN, LS, claustrum, MeA, and CeA, were active with significantly higher level of Fos expression in the WD24 group. Caudate-putamen was unexpectedly active in the WD group. To further elucidate the causal involvement of AVPMNN system, GluR1 and PSD95, two major postsynaptic density proteins expression levels were assayed in the lateral habenula, central amygdala and ventral hippocampus, with semiquantitative immunoblotting in basal, WD24, water deprived for 48 h (WD48) and WD48 + 24 h and 48 h of hydration restoration groups. A statistically significant increase was observed between the basal and WD48 groups in PSD95 expression of the amygdala, lateral habenula, and ventral hippocampus as well as GluR1 expression in the ventral hippocampus. Additionally, using 10 nm AVP, 30 min incubation of ventral hippocampus acute slices, significant pERK expression was observed with Western blot. This data suggests that thirst, an internal water-electrolytes imbalance due to water deprivation, which potently up-regulated the AVPMNN system as one of the main homeostatic mechanisms, could modify neuronal activation patterns in both sensorial and limbic systems to determine a behavioral response to facilitate survival.

Disclosures: E.C. Guerra: None. **T.G. Padilla:** None. **V.S. Hernández:** None. **M.C. Cárdenas-Aguayo:** None. **O.R. Hernández-Pérez:** None. **F. Jáuregui:** None. **S. Luquín:** None. **L.E. Eiden:** None. **L. Zhang:** None.

Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.18/U12

Topic: F.02. Behavioral Neuroendocrinology

Support: NIMH K08MH092697
NICHD P01 HD33113

Title: OXTR and AVPR1A polymorphisms are associated with religious experience in a functional neuroimaging genetic study

Authors: *L. DAI[1], J. KING[2], M. FERGUSON[2], J. NIELSON[2], H. NAZARLOO[5], C. CARTER[6], J. S. ANDERSON[3], J. R. KORENBERG[4];
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Abstract: The origins of religious experience remain controversial. The relationship between self and other, whether beings or dieties, is a concept common to social science and religious philosophy. Therefore, the brain systems mediating these may overlap, suggesting the social neuropeptides oxytocin (OT), arginine vasopressin (AVP) and their receptors, the OXTR and the V1a, respectively, may also be involved in the brain networks of religious experience. In our prior studies, we have shown that endogenous OT, but not AVP, was associated with BOLD imaging in anterior and posterior cingulate cortex, ventromedial prefrontal cortex and anterior insula, the hubs of default mode and paralimbic networks that are involved in self-awareness and self-other, when individuals felt the Spirit. In this report, we explored the hypothesis that the genetic variance of the receptors, OXTR and AVPR1A, might be independently associated with specific brain substrates in response to three religiously evocative tasks, and combined with self-experience reports, in a cohort of individuals trained to report spiritual responses. Our results showed that DNA polymorphisms of OXTR (rs2254298, rs2268498) were associated with left dorsolateral prefrontal cortex, right inferior parietal and left posterior cingulate cortex. The AVPR1A promoter-region microsatellite repeat length of RS3 was associated with right ventrolateral orbitofrontal cortex. These brain substrates were also involved in default mode network, self-other and high cortical processes, suggesting the genetic variance, present for birth, possibly acting throughout development, and the other, reflecting the variable interaction and results of a spectrum of developmental and experiential events. Putting together with our prior studies, these results indicate that both the original state of the receptors, and the endogenous peptide, are involved in religious experience, most strongly in regions involved in early developing brain systems but also with the later appearing brain systems for the more complex aspects of self involving others.

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Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.19/U13

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH T32 NS048004
Whitehall Foundation grant
NARSAD Young Investigator grant
Sloan Research Fellowship
Searle Scholars Award
Klingenstein-Simons Fellowship
Brain Research Foundation grant

Title: Sexually dimorphic control of parenting behavior by the medial amygdala

Authors: *R. K. HU, P. B. CHEN, Y. E. WU, L. PAN, S. HUANG, P. MICEVYCH, W. HONG;
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Abstract: Social behaviors encompass a broad set of behaviors critical for the survival and well-being of an individual. The diverse catalog and contextual display of social behaviors can greatly vary between the sexes, and disruption of social behaviors, which is a prominent feature in psychiatric disorders, often appears in a sexually dimorphic manner. A major challenge in neuroscience lies in understanding how sex differences, from molecules to circuits, synergistically contribute to sex differences in behavioral displays. Parenting and infanticidal behaviors are opposing pup-directed behaviors that are sexually dimorphic. Although studies have implicated the medial preoptic area as a key region for regulating parental behaviors, little is known about the contribution of other brain regions beyond the medial preoptic area in parenting, and even less is known about brain regions regulating infanticidal behavior and its relationship to parenting.
The medial amygdala (MeA) is a sexually dimorphic brain region involved in adult-directed social behaviors. A longstanding view is that the MeA is not part of the main circuitry that promotes parenting behavior towards pups, but may be involved in suppressing parental behaviors. In this study, we establish a sexually dimorphic role for the MeA in regulating pup-directed behaviors. Contrary to traditional views, activation of GABAergic neurons in the posterodorsal region of the MeA (MeApd) actually promotes parenting in females, whereas GABAergic neurons in males regulate both parenting and infanticidal behavior in an activity level-dependent manner. In contrast, glutamatergic neurons in the MeApd exert the same effect on both males and females in promoting self-grooming behavior. Using single-cell RNA

sequencing, we comprehensively characterize cell type heterogeneity and transcription profiles in the MeA between males and females, and find that sex differences are specifically represented in GABAergic neurons. This supports a sex-specific role for GABAergic neurons, but not glutamatergic neurons, in behavioral and circuit functions. Collectively, these findings demonstrate that GABAergic neurons in the MeApd control parental and infanticidal behaviors in an activity level-dependent, sexually dimorphic manner, and provide important insight into the connection between sex differences at the levels of molecules, cells, and circuits in regulating sexually dimorphic behaviors.

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Poster

408. Hormone Modulation of Behavior and Physiology I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 408.20/U14

Topic: F.03. Neuroendocrine Processes

Support: CAPES (Finance code 001)
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CNPq, 132836/2014-9

Title: Effects of corticosterone and estrogen on HPA axis activity and telomeres length in brain areas of female rats

Authors: P. C. G. BARCELLOS[1], L. C. ZANELATTO[2], B. A. A. S. LEMOS[3], R. T. C. SALOMA-RODRIGUES[3], *C. R. FRANCI[1];
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Abstract: The telomeres are structures present at the endings of chromosomes and act to stabilize the DNA during cell division. Throughout the aging telomere shortening undergoes a process, but other factors can enhance this process, such as chronic stress. Chronic stress induces changes in the functioning of an organism, including increased glucocorticoid levels, which may affect physical and psychological state, such as anxiety and depression. Recent work correlates chronic psychosocial stress to the reduction of the telomere length of certain cells and estrogen may also influence telomere repair. However, it is unclear whether increased glucocorticoid concentrations influence telomere length in brain tissue cells. The objective of this study was to verify whether chronic exposure to glucocorticoids promotes changes in telomere length of encephalic areas involved in the control of hypothalamic-hypophysis-adrenal (HPA) axis activity

and whether estrogen modulates these changes. Methods: Wistar female rats were ovariectomized and daily treated with estrogen cypionate, at doses of 50 and 100 ug/kg. The animals were subdivided into treated with corticosterone or its respective vehicle for 28 days. On the day following the end of corticosterone injections, the animals were sacrificed by decapitation to the withdrawal of blood samples, brain, and pituitary. Plasma corticosterone, progesterone, luteinizing hormone, follicle stimulating hormone, and estradiol were determined by radioimmunoassay. Results: Treatment with estrogen increased the secretion of corticosterone (326.03±32.99 / 361.20±34.87 ng/mL) and progesterone (5.53±0.85 / 5.47±1.26 ng/mL), compared to their respective control group treated with oil and saline (134.70±6.12 ng/mL /1.05±0.15 ng/mL) The combined treatment maintained of estrogen and corticosterone maintained plasma corticosterone and progesterone similar to the control treated with oil. mRNA levels for CRH, AVP, and POMC were reduced by estrogen. The telomeres length in the paraventricular nucleus was not altered by corticosterone and estrogen, but in the central amygdala and dorsal hippocampus, estradiol cypionate shortened telomeres of the cells in the brain nuclei. Thus, the treatment with corticosterone did not alter the length of telomeres, and estrogen doesn't have a protective factor in the brain tissue of the central amygdala and the dorsal hippocampus. Financial support: CAPES, CNPq and FAPESP agencies from Brazil.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.01/U15

Topic: F.04. Stress and the Brain

Support: CNR-DISVA-Sardegna Ricerche

Title: Early life stress induces changes on specific brain areas for cognitive and motivational functions: Inside the multimodal effects of maternal separation in C57BL6J mice

Authors: *G. TALANI[1], D. COLOMBO[2], E. SAOLINI[2], G. M. CALANDRA[2], F. BIGGIO[2], G. BIGGIO[2,1], E. SANNA[2,1];
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Abstract: Stress occurring early in life may be predictive for the development of neuropsychiatric disorders as well as increased vulnerability to drug use disorders in adulthood. Repeated maternal separation (RMS) in rodents is a powerful model to investigate the consequences of neonatal stress on brain plasticity and vulnerability to ethanol (EtOH) abuse. Here we extended our recent findings about the potential mechanisms involved in the long-term

effects of RMS in C57BL/6J adult mice by evaluating the changes in neuronal plasticity at both GABAergic and glutamatergic synapses in the hippocampus (hip) and nucleus accumbens (NAcc), areas involved in learning and memory function and drug reward, respectively. Patch-clamp experiments performed in the hip revealed that RMS causes a significant enhancement in the tonic component of the GABAergic inhibition in dentate gyrus granule cells from male, but not females, mice. RMS is also accompanied in males by a marked increase in the frequency of GABAergic IPSCs recorded in the same neurons. Interestingly, all these changes induced by RMS were paralleled by an impairment in LTD formation in the CA1 subregion in male but not in female mice, an effect that may involve an increased function of the endocannabinoid system and which was accompanied by an impaired cognitive performance in the Barnes maze test. RMS is also associated in males with a marked increase of EtOH intake and preference in the two-bottle free choice paradigm, while in females this result is not statistically relevant. We observed a significant RMS-induced changes in synaptic plasticity with a reduction of LTD formation in the NAcc MSNs, an effect that is accompanied with an impairment of the AMPA/NMDA ratio. Interestingly, these functional and behavioral changes were no longer appreciable in RMS male mice when treated with a single injection of beta-ethinylestradiol at PND3, suggesting that alteration in the hormonal asset may strongly influences the neuronal and behavioral impairments induced by RMS. Taken together, these findings demonstrate that RMS is associated with long-lasting effects on synaptic plasticity in both hip and NAcc of C57BL/6J male mice, alteration of learning and memory as well as goal directed behavior. In line with previous findings, our data may support a gender-dependent effect of RMS. Supported by CNR-DISVA-Sardegna Ricerche

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.02/U16

Topic: F.04. Stress and the Brain

Support: NIMH Grant 1R01MH107556-01

Title: Effects of maternal separation paired with juvenile social isolation on adult anxiety-like behavior and perineuronal net-ensheathed parvalbumin interneurons in male and female rats

Authors: *K. R. GILDAWIE, J. A. HONEYCUTT, H. C. BRENHOUSE;
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Abstract: Exposure to early life adversity leads to behavioral and neurological dysfunction, and increased vulnerability to a multitude of neuropsychiatric and inflammatory disorders, such as depression, anxiety, and schizophrenia. Notably, clinical studies demonstrate an additive effect of adversity, where repeated stressful life events throughout childhood results in more severe neuropsychiatric symptoms. It is not understood, however, whether differential timing of later stressors alters the neurobiological consequences of early life adversity in a sex-dependent manner. Research demonstrates that the formation of perineuronal nets (PNNs) - which preferentially enwrap fast-spiking parvalbumin (PV)-expressing interneurons - is essential for proper neurodevelopment. PNNs exhibit delayed and protracted formation throughout the brain, gradually maturing from the postnatal time period through early adulthood. We have previously observed that adversity during developmental periods where PNNs are not yet fully formed could disrupt the formation of PNNs surrounding PV+ cells in the prefrontal cortex (PFC). To further investigate the later-life behavioral and cellular implications of multiple developmental stressors, male and female rat pups underwent maternal separation (MS) for 4 hours per day from postnatal day (P) 2-20 (or control rearing), followed by juvenile social isolation (SI) from P21-35 (or standard pair-housing). SI rats were then pair-housed with cage mates matched for sex, age, and experimental group until early adulthood (P70), when anxiety-like behavior was measured using the open field test and elevated zero maze. At P85, rats were perfused and brains were collected, cryoprotected, and sliced on a freezing microtome to 40 µm slices. Tissue sections containing the prelimbic (PL) and infralimbic (IL) PFC were stained using *Wisteria floribunda* agglutinin, anti-PV antibody, and NeuroTrace (for layer specificity analyses). Z-stacks were obtained (6 stacks per section) using fluorescent microscopy in 3 consecutive sections of the PFC and the FIJI macro plugin PIPSQUEAK was used to quantify the number and intensity of PNNs, PV cells, and PNNs ensheathing PV cells. We present findings elucidating the association between adult PFC PNN dysfunction and enhanced anxiety-like behavior following multiple instances of adversity throughout development. These data provide critical information regarding the differential windows of vulnerability to chronic stress exposure in males and females, which have the potential to aid in the development and application of interventions for children who have experienced early life adversity.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.03/U17

Topic: F.04. Stress and the Brain

Title: Early life stress in mice causes a change in the excitation-inhibition balance in prefrontal cortex neurons during development

Authors: *H. KARST[1], L. I. VAN MOURIK[1], M. JOELS[2];
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Abstract: The excitation-inhibition (EI) balance plays an important role during maturation of the brain. A disturbance of the balance is thought to predispose to the development of psychiatric disorders. Early life stress (ELS) can cause impairments in cognitive and a variety of behavioral functions later in life. It is hypothesized that a disturbance of the EI balance may contribute to these abnormalities. In this study we followed the EI balance during maturation by recording the glutamatergic and GABAergic transmission from neurons of the mouse infralimbic medial prefrontal cortex (imPFC) layer 2/3. Mice were exposed from postnatal day (P)2 until P9 to the limited nesting and bedding model. At P9, P15, P21 (weaning), at 6 weeks (adolescent) and at 10-12 weeks (adult) male mice were used for in vitro electrophysiology. We recorded miniature excitatory (mEPSCs) and inhibitory currents (mIPSCs) in the same neurons. This was done by recording the mEPSCs at -65 mV, the reversal potential for GABA (chloride) currents and by recording the mIPSCs at +10mV, the reversal potential for glutamate currents. Compared to the control mice, the mEPSC frequency in imPFC neurons of ELS mice was remarkably decreased at P9 and P21. The frequency of the mIPSCs, however, was not affected after ELS. When calculating the EI balance by dividing the frequency of the mEPSCs by the frequency of the mIPSCs, the balance was considerably lower in ELS mice. Later in development, from adolescence onwards, the effect of ELS on the EI balance was not detected anymore. An important property of the GABAergic transmission during development is the GABA-switch, the moment when GABA switches from being excitatory to inhibitory, caused by a shift in the reversal potential (Erev) of chloride. We recorded the GABA-switch in slices of ELS and control mice with the perforated patch clamp technique by measuring the Erev for chloride. In control mice, the switch takes place around P14 in the imPFC neurons. In ELS mice the switch takes place earlier, already at P9, but perhaps even sooner, during the ELS period from P2 until P9. Most likely a change in the expression or phosphorylation of the Chloride co-transporters, NKCC1 and KCC2 is responsible for this effect, which we are currently investigating. We conclude that the EI balance in ELS mice is changed early in life, by 1) a decrease in the glutamate transmission and 2) by a shift of the GABA-switch towards an earlier moment in life, both effects probably resulting in a suppression of the excitability.

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Poster

409. Early-Life Stress

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Program #/Poster #: 409.04/U18

Topic: F.04. Stress and the Brain

Support: Swedish Medical Research Council
Swedish Brain Foundation
County Council of Östergötland

Title: Maternal presence/absence during early-life immune challenge: Effects in pups and dams

Authors: *S. CASTANY[1], J. ZAJDEL[1], A. ZAGER[2], A. BLOMQVIST[2], K. SHIONOYA[2], D. ENGBLOM[1];
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Abstract: Maternal-infant interactions profoundly affect the ability of the pups to respond to different kinds of stressors. Stressors such as maternal separation and early-life sickness may have an impact on the hypothalamic-pituitary-adrenal (HPA) axis and brain function of both pups and dams. We examined how maternal separation affected inflammatory gene expression and corticosterone response in mouse pups (8-9 days old) after an acute immune challenge induced by lipopolysaccharide (LPS; 40 μg/kg ip). Maternal separation robustly potentiated the hypothalamic expression of IL1β, IL6, TNFα, COX2, CCL2 and CxCL10, as well as the corticosterone response, seen 3 hours after immune challenge. Such separation-induced potentiation of the inflammatory response and the resulting HPA-axis activation may have detrimental effects if the separation is prolonged or repeated. Little is known about how having sick pups affects the mothers/dams. To address this question, we examined if LPS-induced inflammation in pups elicited hypothalamic inflammatory signaling also in their dams. Surprisingly, systemic inflammation in pups led to an induction of inflammatory gene expression in the hypothalamus of their dams. This was accompanied by HPA axis activation. The induction of inflammatory signaling in dams did not require physical contact with the LPS-injected pups. Collectively, our results highlight how bidirectional mother-infant interaction can modulate the inflammatory response.

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Poster

409. Early-Life Stress

Location: Hall A

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Program #/Poster #: 409.05/U19

Topic: F.04. Stress and the Brain

Support: Hans Fischer Senior Fellowship, IAS-TUM, Munich, Germany
Canadian Institute of Health Research, Canada
The Friends of Canada Fund for Personalized Medicine, Jerusalem, Israel

Prof. Milton Rosenbaum Fund for Research in Psychiatric Sciences, Jerusalem, Israel

Title: Prenatal maternal stress, non-invasive fetal biomarkers and infant neurocognitive development: A prospective cohort study

Authors: *M. C. ANTONELLI[1], C. ZELGERT[2], S. VAKNINE[3], M. MOLINET[2], R. SHARMA[4], P. ZIMMERMANN[2], A. MÜLLER[5], G. SCHMIDT[5], B. HALLER[6], G. BERG[7], B. FABRE[7], H. WU[8], H. SOREQ[3], M. G. FRASCH[9], S. M. LOBMAIER[2];
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Abstract: Maternal stress during pregnancy and during early parenting may program physiological responses and lifetime trajectories of the infant, interact with genetic liabilities and early-life challenges and determine ultimate health status. Seeking a prenatal measure with a preventive clinical significance, we hypothesized that the coordinated roles of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis (HPA) in the integrated stress response may be monitored by measuring several maternal and fetal molecular and biophysical biomarkers. We performed a prospective study in stressed mothers with controls matched 1:1 for parity, maternal age and gestational age at study entry. Pregnant women were administered the Cohen Perceived Stress Scale questionnaire (PSS-10), which categorized them as Stressed Group (SG) for PSS-10 score ≥19. The extent of coupling between maternal and fetal heart rate (mHR, fHR) derived from maternal abdominal ECG was quantified. Assessment by the bivariate phase-rectified signal averaging algorithm (BPRSA) yielded fetal stress index (FSI) values. On the day of parturition, maternal blood and hair strands from the posterior vertex region on the head were collected for cortisol measurements. Cord blood samples and saliva/buccal samples from the newborns were also collected. Maternal and newborn serum was used to measure acetylthiocholine hydrolytic activity of acetyl- and butyrylcholinesterases (AChE, BChE). Infants' cognitive development was assessed by Bayley Scale III of Infant Development (BSID) at 24 months of age when a new saliva sample was taken to be processed for epigenetic biomarkers. Preliminary results show that prenatal maternal stress identified in the third trimester by PSS-10 has an impact on the coordination of fetal and maternal heart rate (captured by FSI) and on fetal oxygenation at birth. Machine learning approaches (with internal validation) indicated that the fetal AChE/BChE ratio is predicted best by joint fetal-maternal stress biomarker FSI, but not by maternal stress levels alone. In conclusion, the maternal and fetal stress status may be shaped by both maternal cortisol and the maternal and fetal AChE/BChE balance, validating our hypothesis that PS-induced programming is reflected in mHR and fHR biomarkers of ANS activity.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.06/U20

Topic: F.04. Stress and the Brain

Support: NIGMS P20GM103643
NICHD R15HD091841

Title: Neonatal pain has lasting effects on fear, anxiety, and somatosensory function through changes in amygdala CRF signaling

Authors: ***S. M. DAVIS**[1], J. ZUKE[1], E. RUSSO[1], D. PADMANABHAN[1], M. A. BURMAN[2]; [2]Psychology, [1]Univ. of New England, Biddeford, ME

Abstract: Human infants in the neonatal intensive care unit (NICU) are exposed to a variety of stressful and painful events and are more likely to suffer from conditions such as chronic pain, anxiety and depression. Our lab has adapted rodent models to investigate the behavioral and neurobiological mechanisms underlying these changes. In particular, rats received either 4 left hindpaw pricks per day for postnatal days (PNDs) 1-7 or 2% lambda-carrageenan injections on PND 1 and 4. Contextual and auditory fear conditioning, elevated plus maze behavior, and somatosensory function were assessed in late childhood (PND 24), adolescence (PND 45), and adulthood (PND 66) in male and female rats. Contrary to our initial hypothesis, we found reductions in auditory fear conditioning across the lifespan in rats exposed to neonatal pain. In addition, we observed reduced anxiety, but enhanced stress-induced tactile hypersensitivity in PND 24 rats exposed to neonatal pain. The stress-induced tactile hypersensitivity was reduced by administration of a CRFR1 antagonist either systemically during neonatal stress on PND 1-7 or intra-amygdala during the activating stressor on PND 24. In addition, a subset of subject's brains were collected on PND 6 and underwent florescent *in situ* hybridization (RNAScope®) for corticotropin releasing factor (CRF) and c-Fos to examine the acute effects of neonatal pain. Expression was examined using automated thresholding and quantification, which revealed that neonatal pain significantly enhanced CRF signaling in the amygdala, but not hypothalamus in male, but not female, rats. All together, these experiments provide evidence that amygdala CRF signalizing is at least one mechanism by which neonatal pain and stress have lasting effects of affective and sensory function.

Disclosures: **S.M. Davis:** None. **J. Zuke:** None. **E. Russo:** None. **D. Padmanabhan:** None. **M.A. Burman:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.07/U21

Topic: F.04. Stress and the Brain

Title: Developmental-stage dependent behavioural and neurochemical deficits produced by embryonic ethanol exposure in zebrafish: A model for FASD

Authors: *A. FACCIOL[1], C. BAILLEUL[2], S. NGUYEN[2], R. T. GERLAI[3];
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Abstract: Fetal Alcohol Spectrum Disorder (FASD) is a disease category that encompasses a large number of deficits resulting from the consumption of alcohol by pregnant women. Symptoms of FASD include morphological, behavioural and cognitive deficits, however, the expression and severity of these deficits is highly variable. One hypothesis for this variability is the varying developmental stage at which alcohol was administered. Although ethanol induced developmental stage-dependent deficits have been investigated in zebrafish, these studies focused on the use of high doses of and/or prolonged exposure to ethanol. While such exposure regimens do produce robust alterations, short exposure to low doses of ethanol will more accurately mimic the mild and more prevalent forms of FASD. To investigate the developmental stage-dependent effects of mild ethanol exposure, we exposed zebrafish embryos to 1% ethanol for 2 hours at one of six key developmental stages: 5 hours post fertilization (hpf), 10hpf, 16hpf, 24hpf, 36hpf and 48hpf. A “No exposure” group was also included to control for the procedure of exposure itself. Following exposure to alcohol or freshwater, zebrafish were raised normally to 1 month of age, at which they were behaviourally tested in an open tank to analyze ethanol-induced alteration in locomotor and anxiety-related behaviours. Following behavioural analysis, brains were dissected and analyzed for neurotransmitter levels, specifically dopamine, serotonin and their metabolites, via HPLC. Although results show no significant effect of ethanol exposure on behaviour in 1 month old fish, neurochemical analysis revealed a developmental stage-dependent decrease in all neurotransmitters analyzed. Specifically, dopamine, serotonin and their metabolites were all lower in ethanol exposed zebrafish compared to control fish but only with exposure at 10, 16 and 24hpf. This study, together with previous studies modeling FASD in zebrafish, suggest that although 1 month of age may be too young for detection of behavioural deficits, the deficits manifest at the neurochemical level. Additionally, the deficits found here in 1 month old fish are concordant with embryonic ethanol-induced neurochemical deficits reported

in adulthood. This study is the first to investigate the developmental stage-dependent effects of short, low dose ethanol on both behavioural and neurochemical phenotypes.

Disclosures: **A. Facciol:** None. **C. Bailleul:** None. **S. Nguyen:** None. **R.T. Gerlai:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.08/U22

Topic: F.04. Stress and the Brain

Title: Maternal separation and monosodium glutamate intake don't affect spatial memory, but increase weight gain and water intake in male Wistar rats

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Abstract: Humans and rodents, like most mammals, share in the early stages of life a bond given by the mother-child relationship, where it has been shown that disruption generates negative effects on the later life of the offspring in the short, medium and long term, altering the neuroendocrine and behavioral response. Monosodium glutamate (GMS) is a food additive widely used to enhance and improve food acceptance, in addition, it has been related to neurotoxicity and long-term consequences in animal models. The objective of this study was to determine if maternal separation during breastfeeding (MSDB) influences the consumption of MSG and whether these two factors produce changes in learning, spatial memory, weight and water intake of separate male rats; compared to their control group. The MSDB protocol was performed from day 1 to 21 of life for a period of 360 minutes daily: 180 in the morning and 180 in the afternoon, during the dark phase. The control group was the animals that did not undergo the maternal separation protocol, nor did them receive GMS. On the postnatal day 30 the animals previously assigned started treatment with MSG. Four work groups were managed: Control group: males without GMS and without maternal separation (n = 7): males with MSDB and GMS (n = 12), males without MSDB and with MSG (n = 11), and finally males with MSDB and without MSG (n = 10). During one month, the GMS groups were awarded two bottles with the same amount of water, one containing the GMS and the other just water, which was changed every 2 days. GMS every 24 hours, body weight every 3 days, and finally day 60 rats were exposed to the widely valid Barnes maze. It was found that animals subjected to the MSDB paradigm, an increase in the consumption of MSG, possibly related to an increase in weight found in the same experimental group. Additionally, in the learning and retrieval tests, significant differences were not found for individuals who were exposed to the MSDB paradigm, but them tending to perform worse in spatial memory tests when compared to their respective controls. The results indicate that early stress may be associated with changes in eating patterns

as well as some cognitive impairments, considering that MSG may possibly be associated with the cognitive performances shown in this study.
Key words: Maternal separation, monosodium glutamate, early stress, spatial memory, Barnes Maze

Disclosures: C. Sierra: None. **Z. Duenas:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.09/U23

Topic: F.04. Stress and the Brain

Support: NIH Grant HD077623
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Title: Effects of social subordination and consumption of an obesogenic diet on total brain size and structural development of cortico-limbic regions: A longitudinal study in infant and juvenile macaques

Authors: *M. H. KYLE[1,2], A. KALDAS[1,2], M. PINCUS[1,2], J. GODFREY[1,2], Z. KOVACS-BALINT[2], E. L. MORIN[1,2], D. DE LEON[2], L. LI[1], B. R. HOWELL[3], M. A. STYNER[4], C. PAYNE[1], K. ETHUN[2], M. E. WILSON[1,2], M. SANCHEZ[1,2];
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Abstract: Although child obesity rates in the United States continue to rise, our understanding of the neurodevelopmental impact of obesogenic diets remains limited. Obesity in children is commonly comorbid with chronic psychosocial stress, which is associated with neurobehavioral alterations and is itself a cumulative risk factor for obesity. It remains poorly-understood, however, how psychosocial stress interacts with consumption of obesogenic diets to affect brain development, and which biological mechanisms underlie these effects. This study longitudinally investigated the potential synergistic impact of postnatal exposure to psychosocial stress and consumption of an obesogenic diet on brain development in female rhesus macaques, while examining underlying biological signals. We used a translational macaque model of social

subordination stress, and followed 41 female rhesus monkeys (n=21 dominant, n=20 subordinate) with access to a low-calorie diet (LCD) only, or to both a LCD and an obesogenic, calorically dense diet (HCD; Choice condition) from birth. Food intake was recorded continuously using automated feeders. Brain structural MRI data was collected during infancy (at 2 weeks, 6 months) and in the juvenile, pre-pubertal period (16 months). Physiological measures of chronic stress activation (hair cortisol accumulation), inflammation (C-reactive protein; CRP), body weight, and kilocalories (Kcals) consumed from each diet were collected in parallel. Subordinate animals had higher CRP levels, and subjects with access to a HCD consumed more Kcals than LCD subjects, but there were no group differences in body weight at any age. Subjects who consumed a HCD had larger overall brain size (intracranial volume; ICV), prefrontal cortex (PFC), insula (INS), and amygdala (AMYG) volumes than those on the LCD. Subordinate animals had larger AMYG, PFC, and hippocampus (HIPP) volumes than dominants, and the overall AMYG growth rate was predicted by cumulative exposure to CRP. Cumulative HCD Kcal consumption predicted PFC and INS growth rates. Our findings suggest that postnatal consumption of an obesogenic diet has robust effects on global brain growth during early development, and that diet and social subordination have region-specific, non-synergistic effects on primate neural development that first appear during infancy and are predicted by inflammation and HCD Kcal consumption.
We are currently analyzing effects of diet and subordination on the development of PFC subregions and the nucleus accumbens, and will also probe maternal Kcal consumption and dosed relative rank as predictors of structural brain alterations.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.10/U24

Topic: F.04. Stress and the Brain

Title: Disrupted development: An investigation of post-natal restricted resources on neurobiological, behavioral and morphological outcomes

Authors: *M. H. KENT[1], K. LAMBERT[1], G. BOWEN[1], S. DESINOR[1], D. VAVRA[1], K. SCIANDRA[1], K. GILLENWATER[1], C. GLORY[1], E. MEISEL[1], R. OLIVARES-NAVARRETE[2], J. PIETZER[2];
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Abstract: The quality of maternal care depends on the availability and quality of necessary resources. Accordingly, limited environmental resources may result in atypical maternal care, disrupting various developmental outcomes of her pups. On postnatal day (PND) 1, maternal Long-Evans rats in the current study were randomly assigned to either a standard resource (SR) group, provided with 4 cups of bedding and 2 paper towels for nesting material, or a limited resource (LR) group, provided with a quarter of the bedding/ nesting materials given to the SR group. Offspring were observed daily and handled weekly to record developmental measurements such as weight, timing of eye-opening, and various morphological measures. After weaning (PND 21), pups were standard housed in same-sex dyads for continued observations. Subsequent behavioral tests revealed a sex by resource interaction in play behavior on PND 28; specifically, SR reduced play attacks in males while it increased play attacks in females. A sex x resource interaction was also observed in anxiety responses in the open field task with SR resulting in more exploratory rear responses in males and, in contrast, fewer rear responses in the females. No effects were observed in a spatial learning task. Focusing on morphological variables, tail and foot length measurements of LR males and females were shorter on PND 7, 14, 21, and 28 but was no longer present after PND 35. Following the behavioral assessments, animals were perfused at 56 days of age so that the brains could be prepared for immunohistochemical assays for the presence of glucocorticoid receptors, microglia, and oxytocin. Interestingly, the same sex x resource interactions were observed as trends in levels of oxytocin and microglia immunoreactivity. Specifically, in the medial forebrain bundle and supraoptic nucleus, LR increased oxytocin-ir in males while decreasing it in females. Further, tail vertebrae were assessed by micro CT. Vertebrae from LR showed an increase in trabecular separation and decrease in bone volume and bone connectivity resulting in lower bone density. Tail tendons showed a decrease in hydroxyproline suggesting a decrease in collagen content in the LR animals. Thus, although the restricted resources only persisted for a brief duration, the effects appear to be far-reaching and pervasive. Considering that 45% of children across the world are raised in impoverished conditions characterized by restricted resources, it is important to explore long-term effects in human experiencing early life stress.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.11/U25

Topic: F.04. Stress and the Brain

Support: Start-Up Funds of Dr. Brummelte

Title: Effects of neonatal procedural pain and reduced maternal care on brain development and cell proliferation in male and female rat pups

Authors: B. M. TIMMERMAN[1], S. M. MOONEY-LEBER[2], *S. BRUMMELTE[1];
[1]Psychology, Wayne State Univ., Detroit, MI; [2]Penn State Univ., University Park, PA

Abstract: Preterm birth accounted for almost 10% of all U.S. live births in 2016. Children born preterm often display impaired cognitive, behavioral, motor, and neural development compared to full-term peers. Prior studies have found a relationship between these alterations and the number of neonatal stressors that preterm infants experience. Utilizing a rodent model, our study investigated how stressors that preterm infants commonly experience in the Neonatal Intensive Care Unit, particularly procedural pain and reduced maternal care, may alter brain development. Male and female rat pups were bred in-house and culled on the day after birth (postnatal day one (PD 1)) so litters had equal numbers of male and female pups. Pups received either repeated needle insertions into their paws (pain group) or stimulation with a paintbrush (touch control). Afterwards, pups were placed back in their home cages either within a tea-ball infuser for 30 minutes to reduce maternal care (isolation) or on the nest with littermates (no isolation). This resulted in the following four groups within each litter (n=8): pain, pain + isolation, touch, touch + isolation. This stress exposure was performed in 4 sessions per day from PD 1 - 4. Animals were sacrificed at PD 8 and brain sections were processed via immunohistochemistry for Ki67, a marker of cell proliferation. Preliminary results suggest that pups that received pain plus isolation had significantly lower cell proliferation in the dentate gyrus compared to pups in the touch plus isolation group. Our findings may indicate that neonatal pain and isolation have a synergistic effect on cell proliferation in the dentate gyrus of 8-day-old rat pups. Future research will investigate additional markers of brain maturation as well as explore the consequences of these early life stressors once animals reach adulthood.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.12/U26

Topic: F.04. Stress and the Brain

Title: Effect of enriched early environments and lipopolysaccharide treatment on retrotransposon expression in stress-sensitive brain regions

Authors: *A. A. AIKEN[1], A. C. KENTNER[2], R. G. HUNTER[1];
[1]Psychology, Univ. of Massachusetts Boston, Boston, MA; [2]Sch. of Arts and Sci., Massachusetts Col. of Pharm. & Hlth. Sci., Boston, MA

Abstract: Environmental enrichment has been observed to both protect the brain and attenuate responses to varied stressors. In the present study, an animal model of maternal immune activation (MIA), which has been associated with a variety of neurodevelopmental defects, was utilized as a stressor. Further, the potential involvement of epigenetic mechanisms was analyzed as they may serve as a means to induce lasting physiological and behavioral changes following stress exposure. Previous work in our lab found that short interspersed elements (SINEs) are repressed epigenetically after acute stress, giving rise to the concept that the mammalian genome may be regulating retrotransposon RNA expression in response to environmental challenges. Thus, SINE RNA expression may be adaptive, an idea supported by observations demonstrating regulated B2 SINE expression in response to restraint stress and heat shock. Once thought of as "junk", transposons have now been linked to multiple nervous system disorders including post-traumatic stress disorder and schizophrenia. As a disorder that is characterized by developmental neuroinflammation and high retrotransposon activity, schizophrenia may, in fact, result from environmental stressors (e.g. MIA) that alter retrotransposon activity in the developing brain. To test for potential effects of housing environment on neural responses to a developmental inflammatory insult, Sprague-Dawley rats were housed and bred in either standard animal care control, social control, or enriched environment housing. On gestational day 11 dams were administered either saline or lipopolysaccharide (LPS) to provide an inflammatory challenge and brains were obtained from male and female offspring on postnatal day 21. Preliminary results revealed dysregulation of B2 in the medial prefrontal cortex, medial preoptic area, and hippocampus indicating B2 involvement in response to the gestational LPS stressor in these regions compared to saline-treated controls. We anticipate that EE housing will ameliorate the observed effects of LPS on B2 expression in order to further regulate the stress response.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.13/U27

Topic: F.04. Stress and the Brain

Support: NIH Clinical and Translational Science Award (UL1TR002366)
UMKC Sarah Morrison Student Research Award
Friends of Psychiatry foundation research grant
UMKC Department of Psychiatry Medical Student research grant

Title: Maternal-fetal reactions to acute emotional stress in prenatal depressed mothers: Correlations with fetal biomagnetometry measures

Authors: *P. CHANDRA[1], A. OYETUNJI[2], K. GUSTAFSON[3];
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Abstract: Introduction: Perinatal maternal stress is now known to have negative programming effects on fetal neurodevelopment, which may be the origins or precursors of neuropsychiatric problems, as postulated by the 'fetal programming hypotheses'. But the underlying mechanisms are not well understood. The autonomic nervous system (ANS) regulates maternal physiologic reactions to unpleasant conditions or perceived threat by altering sympathetic and parasympathetic input but there is limited evidence that the fetus responds to maternal emotional or autonomic state in real-time. The aim of the study is to determine if maternal autonomic reactivity induces a similar response in the fetus. If confirmed, the results would support a model of *in utero* programming; suggest underlying mechanisms by which maternal stress affects fetal outcomes. **Methods:** Fifteen pregnant women between 30-32 weeks gestation, with history of perinatal depression and fifteen control subjects are participating in the study. Simultaneous maternal-fetal magnetocardiograms (MCG) were recorded while women viewed a series of photos with validated emotional valence that ranged from neutral, pleasant, unpleasant and threatening. Simultaneous maternal-fetal biomagnetometry was recorded and subjected to independent component analysis (ICA) to extract and separate maternal and fetal MCGs and other fetal signals. Time-frequency plots were used to assess maternal and fetal autonomic reactivity. **Results:** Our findings noted a typical maternal ANS response with increased vagal input during neutral and pleasant pictures and increased sympathetic input during unpleasant and threatening pictures. Some subjects showed an atypical maternal ANS response with chronic hyper-sympathetic response, low ANS reactivity, high stress index, and reduced sympatho-vagal interaction. We noted that depressed maternal and fetal pairs had a maternal HR and sympathetic drive increased with negative valence of pictures. Fetal HR mirrored maternal HR. Also, fetal breathing movements resulting in increased vagal drive occurred at initiation of unpleasant pictures, and lower HR during threatening pictures. **Conclusions:** These data establish a link between maternal and fetal ANS reactivity that occurs while women view pictures designed to evoke emotional responses. These data support our hypothesis and suggest that maternal emotion and physiologic reactions related to stress may have a direct effect on the autonomic reactivity and neurodevelopment of the fetus. The information gained from the study would help to identify at -risk fetuses and support early intervention during pregnancy.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.14/U28

Topic: F.04. Stress and the Brain

Title: The impact of early developmental stress on anxiety, cognition, and inflammation in adulthood

Authors: *C. HAGEN, J. FIGG, K. BRICE, J. PETERMAN, G. BOEHM, M. CHUMLEY; Texas Christian Univ., Fort Worth, TX

Abstract: It has been widely established that psychological stress has the potential to negatively impact brain health through a variety of mechanisms. Both chronic and acute stress have been shown to alter the inflammatory response which has implications for the progression of neurodegenerative diseases like Alzheimer's disease. This study aims to investigate the effects of stress during early development, specifically maternal stress during pregnancy and its impact on anxiety, learning, and neuroinflammation in the adult offspring. Development while *in utero* is particularly susceptible to stress and can have lasting effects well into adulthood. Here, we measured anxiety behaviors in elevated zero, performance in contextual fear conditioning, and the production of pro-inflammatory cytokines in the hippocampus of adult mice exposed to different early life stress timelines. These timelines were designed to investigate the long-term effects of early postnatal stress as well as how prenatal stress can either attenuate, exacerbate, or supersede these effects. Mice were assigned to one of three conditions: (1) mice undergoing stress during the entire prenatal period in combination with the early postnatal period, (2) mice undergoing stress just during the early postnatal period, (3) and mice undergoing no additional stress at any point. Ultimately, mice in the combination stress paradigm showed cognitive deficits in contextual fear conditioning with no change in anxiety behaviors in elevated zero. Mice in this condition also showed downregulated hippocampal TNF-αand IL-6 while mice that underwent only postnatal stress showed no change in cytokine levels. This suggests an immunosuppressive effect of prenatal stress in combination with postnatal stress.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.15/U29

Topic: F.04. Stress and the Brain

Support: NIMH Grant R01-MH115914
NIMH Grant R01-MH115049

Title: The effects of early life adversity on instrumental reward learning

Authors: *M. GALLO[1], A. A. HAMID[2], D. OFRAY[1], D. SHLEIFER[1], G. MANZANO-NIEVES[2], C. LOPEZ[1], E. HRABARCHUK[1], K. G. BATH[1];
[1]Cognitive, Linguistic and Psychological Sci., [2]Neurosci., Brown Univ., Providence, RI

Abstract: Exposure to early life adversity (ELA) alters the risk for later development of pathology. Here, we use a mouse model of ELA, limited bedding and nesting (LBN) stress, to determine its effects on development of key brain centers associated with reward processing and instrumental reward learning. LBN alters early experience of pups, leading to unpredictable, fragmented care, impacting the reliability and predictability of the early environment. These experiences likely have profound effects on the development of reward systems, possibly contributing to behavioral symptoms associated with risk for major depressive disorder, hedonic responding, addiction, and risk taking behavior. To assess sex and developmental differences between groups, we used adolescent and adult male and female mice for all experiments. Our results showed that LBN rearing was associated with altered development and expression of dopamine receptors in the striatum of mice. Additionally, we note that females exhibiting differences in striatal dopamine receptor expression compared to males. Using variations of a lever press / reward task, we assessed learning rates, willingness to expend effort to receive a reward, and break point for reward responding. Current findings provide a critical starting point to more fully probe the effects of ELA on reward processing, effort allocation, and possible neural substrates underlying disruption in these behaviors following ELA.

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Poster

409. Early-Life Stress

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Program #/Poster #: 409.16/U30

Topic: F.04. Stress and the Brain

Support: NIH-RO1-MH115914 1098 (KGB)
RO1-MH115049 (KGB)

Title: Type of early life adversity confers differential risk for altered timing of development

Authors: *C. DEMAESTRI, M. CRITZ, T. PAN, K. BATH;
Dept. of Cognitive, Linguistic, and Psychological Sci., Brown Univ., Providence, RI

Abstract: Early life adversity (ELA) dramatically increases the risk for developing psychiatric disorders, including anxiety and depression, and is associated with poorer health outcomes. Females have elevated risk for ELA-associated pathology and are twice as likely than males to

develop depression and post-traumatic stress disorder. A significant gap in the literature remains with regard to how the specific type and severity of ELA may influences outcomes and contribute to sex-specific risk. ELA can come in many forms, including extreme poverty, hypervigilant parenting, physical abuse, or parental neglect, to name a few. Importantly, these disparate experiences may provide unique signals to the developing organism about the quality of their environment and drive different effects on brain and behavioral development. Understanding the unique consequences of different forms of ELA on neurobehavioral development will be critical for identifying sex-specific risk factors.
Here, we compare two mouse models of ELA reflecting different forms of adversity with relevance for humans. Specifically, we test the effects of limiting maternal resources or repeated maternal separation on genetic markers of neuronal maturation, somatic and sensory development, and anxiety- like phenotypes during development.
We have identified sex-specific effects of ELA, where mice who are subjected to adversity fail to meet typical developmental milestones. We find that the domain of functioning impacted, and the severity of these effects depend upon both up on the form of stress and sex of the developing animal.
The current work advances our understanding of what developmental mechanisms are impacted in response to ELA, sex-specific vulnerabilities, and the contribution of each to health outcomes. Such findings may reveal targets for earlier interventions or identification of genetic biomarkers of risk/resilience and provide critical groundwork for individualized medicine.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.17/U31

Topic: F.04. Stress and the Brain

Support: NIDA Grant R00 DA035251
NIMH Grant P50 MH096889
NIH Grant T32 GM008620

Title: Early life adversity increases addiction-like behavior for opioid drugs

Authors: *S. C. LEVIS[1,2], J. L. BOLTON[2], B. S. BENTZLEY[3], C. R. PERRONE[1], T. Z. BARAM[2], S. V. MAHLER[1];
[1]Neurobio. and Behavior, [2]Anat. and Neurobio., UC Irvine, Irvine, CA; [3]Psychiatry and Behavioral Sci., Stanford Univ. Med. Ctr., Palo Alto, CA

Abstract: Maternal attachment and quality of maternal care is critical for the neurodevelopmental outcome of offspring across species, including rodents and humans. Fragmented maternal care has been shown to have deleterious effects on the developing brain, and may predispose individuals to develop neuropsychiatric conditions such as anxiety, depression, and addiction. We previously demonstrated that providing limited bedding and nesting (LBN) to early postpartum rodents, a naturalistic rodent model for poverty and early life adversity, causes fragmented maternal care. This leads to anhedonia-like behavior for natural rewards and the abused drug cocaine in adult offspring reared under these conditions. Here we test whether LBN impacts addiction-like behaviors for the opioid drugs of abuse heroin and remifentanil in male and female rats. We examined self-administration, extinction, reinstatement, and economic demand measures of opioid addiction. We also examined heroin-induced neural activity (c-Fos) in LBN and control rats, including in neurons that project to nucleus accumbens (labeled via intra-accumbens injections of the retrograde tracer cholera toxin beta subunit; CTb). Taken together, our data suggest that early-life adversity causes long-lasting neurobiological changes in brain reward and stress circuits that markedly increase risk for addiction to opioid drugs in particular.

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Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.18/U32

Topic: F.04. Stress and the Brain

Title: Exploring mild stress to induce stress inoculation in early life prairie voles

Authors: *O. AKINBO[1], A. J. GRIPPO[2], L. MATUSZEWICH[3];
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Abstract: The experience of brief mild stress during early life may have an inoculating effect to more severe stress in adulthood. Mild stress exposure may increase resiliency by reducing anxiety, emotional distress and cardiovascular dysfunction. Stress inoculation elicits protective effects in humans, other primates, mice and rats. The socially monogamous prairie vole may be an ideal model to investigate whether mild stress has an inoculating effect on later social stress, given their social structure of pair bonding and living in family groups. In an exploratory study, we investigated the feasibility of studying stress inoculation as a function of mild stress applied to prairie voles early in life. Female prairie voles were assigned to a mild stress (n = 32) or

control condition (n = 8), at post-natal day (PND) 21 or PND 29. Mild stress was operationalized by handling the animal with a garden glove or placing in a cup covered with a garden-gloved hand for 30 seconds, either for 1 day or 3 days. These manipulations resulted in the following 10 groups: PND 21 control, PND 21 glove handled for 1 day, PND 21 glove handled for 3 days, PND 21 cup handled for 1 day, PND 21 cup handled for 3 days, PND 29 control, PND 29 glove handled for 1 day, PND 29 glove handled for 3 days, PND 29 cup handled for 1 day, and PND 29 cup handled for 3 days. On the last day of the mild stress protocol, blood was collected in anesthetized animals 10 minutes after the handling and assayed for corticosterone concentrations. Age matched control animals remained undisturbed until anesthetized for blood collection. Corticosterone was slightly higher in the mild stress groups relative to controls. Glove handling yielded higher corticosterone concentrations than cup handling in both age groups. PND 29 control animals exhibited higher basal corticosterone concentrations than PND 21 animals. Finally, when compared to control adult (PND 60-90) female prairie voles from a previous study, both PND 21 and 29 control animals exhibited higher basal corticosterone concentrations. These results indicate that early life prairie voles are a viable model to study stress inoculation. The clinical implications of the results may inform early life intervention strategies to build resiliency in humans.

Disclosures: O. Akinbo: None. **A.J. Grippo:** None. **L. Matuszewich:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.19/U33

Topic: F.04. Stress and the Brain

Support: NIMH Grant 1R01MH107556-01

Title: Modulating behavioral effects of early life adversity by targeting prefrontal cortex NMDA NR2A subunits in adolescent rats: Impact on infant-caretaker communication, cognition, and anxiety

Authors: *L. E. GRANATA, J. A. HONEYCUTT, H. C. BRENHOUSE;
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Abstract: Early adverse experiences are linked to social dysfunction and increased vulnerability to psychiatric disorders. An ethologically relevant model of adversity in rodents is the maternal separation (MS) paradigm. MS during development leads to behavioral deficits in adolescence, with concurrent aberrant development of the prefrontal cortex (PFC). Specifically, the NMDA receptor NR2A subunit and the post-synaptic density protein PSD-95 show increased expression, along with a decrease in parvalbumin (PV)-positive interneurons in rats. PSD-95 is an anchoring

protein, localizing NR2A-containing NMDA receptors to the post-synaptic membrane to facilitate calcium influx/synaptic depolarization. Increased NR2A and PSD-95 following MS likely enhances PFC activity, with possible consequences of PV loss and behavioral deficits. We have shown that after MS, intracranial treatment with TAT2A, a cell-permeable peptide that uncouples NR2A from PSD-95, returned PV expression and measures of anxiety to control levels. We have also reported sex differences in the developmental trajectories of rats exposed to MS, with females showing PV loss and behavioral deficits earlier than males. Here, we aim to elucidate the effects of disrupting NR2A/PSD-95 associations after MS at two developmental timepoints: juvenility or adolescence. To determine the effects of MS and TAT2A treatment, male and female rat pups were separated from dams for 4 hours daily from postnatal day (P)2 - P20. A cannula was surgically implanted in the PFC at P25 (juvenility) or P35 (adolescence) for repeated intracranial injections of TAT2A or vehicle in rats that were then tested in PFC-dependent assessments of anxiety and cognition at P42. Anxiety-like behavior was tested in the open field and elevated zero maze, and cognition was tested via spontaneous alternation in the Y-Maze. To assess the impact of MS on mother-infant communication, juvenile ultrasonic vocalizations (USVs) were collected at weaning and analyzed to determine whether early variations in USV emission can predict later behavior. While USVs are understood as behavioral markers of a rat's affective state, our work shows that juvenile USVs may also reflect a learned response to early experiences, reflected by MS-induced changes in emitted USV number/type. By correlating juvenile USV properties with adolescent behavior, we can determine whether individual differences in response to an aversive experience underlies vulnerability to behavioral dysfunction. Our results demonstrate that specific juvenile USV types predict the degree of anxiety-like behavior in adolescence dependent on rearing condition and TAT2A treatment.

Disclosures: L.E. Granata: None. **J.A. Honeycutt:** None. **H.C. Brenhouse:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.20/U34

Topic: F.04. Stress and the Brain

Support: National Institute on Minority Health and Health Disparities G12 MD007579
RISE R25 GM082406

Title: Microglia activation and depressive-like behavior in adult male and female rats exposed to early-life and adolescence stress

Authors: *K. SANTOS-AVILES[1], L. SAMBOLIN-ESCOBALES[2], M. COLON[3], A. HERNANDEZ[3], J. T. PORTER[4];
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Sci. Univ., Adjuntas, Puerto Rico; [3]Ponce Hlth. Sci. Univ., Ponce, Puerto Rico; [4]Ponce Sch. Med., Ponce, PR

Abstract: High levels of environmental and psychological stressors at key developmental stages such as early life and adolescence have been related to the onset of depression and posttraumatic stress disorder (PTSD) in adults. Depression and PTSD also seem to involve increased peripheral inflammation. Since some children are repeatedly exposed to stressors during both infancy and adolescence, we examined the effects of combining early life stress, modeled by maternal separation (MS), and repeated restraint stress during adolescence on depressive-like and PTSD-related behaviors in an animal model. We also explored whether the combined developmental stressors induced neuroinflammation by measuring the activity of microglia. We hypothesized that the combined developmental stressors would impair fear extinction and increase depressive-like behaviors by enhancing microglia activity in the brain. Male and female rat pups were separated from the dam for 3 hours daily from P1-14, exposed to 3 hours of restraint stress during adolescence (P29-43), and exposed to fear conditioning and extinction and forced swim test as adults. Interestingly, exposure to the combined stressors during key developmental stages did not affect acquisition or extinction of cued fear in either sex. However, the stressed rats showed increased depressive-like behavior. In addition, exposure to the combined stressors increased the microglial activity (Iba-1 immunostaining) in the ventral hippocampus (vHPC) suggesting neuroinflammation. Our follow-up study found that MS alone caused the same depressive-like behavior as the combined stressors in both sexes, suggesting that the early life stress is critical to the development of the depressive-like behavior. Currently, we are evaluating if there is an increase in pro-inflammatory cytokines in the vHPC. Our findings suggest that early life stress may activate hippocampal microglia to produce depressive-like behaviors in adults of both sexes.
Keywords: early life stress, maternal separation, chronic stress, depressive-like behavior, microglia, fear extinction

Disclosures: K. Santos-Aviles: None. **L. Sambolin-Escobales:** None. **M. Colon:** A. Employment/Salary (full or part-time):; Ponce Research Institute. **A. Hernandez:** None. **J.T. Porter:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.21/U35

Topic: F.04. Stress and the Brain

Support: Sigma Xi Grants-in-Aid of Research
OSU Otto S. Cox Genetics Research Fellowship

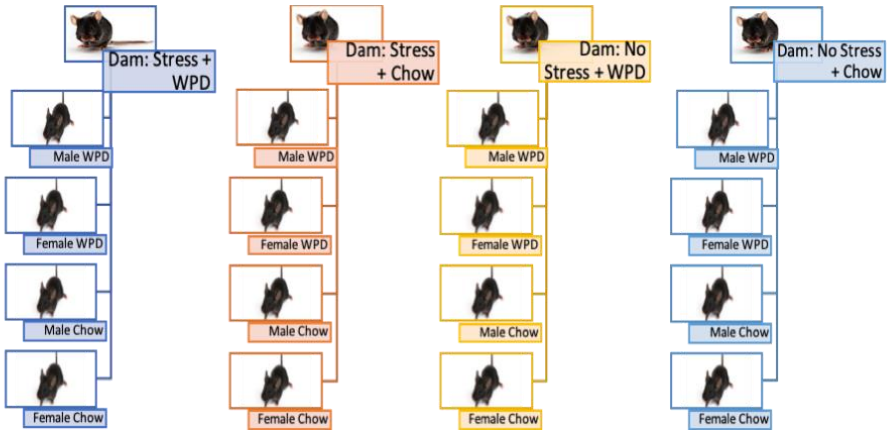
Title: The impact of a Western-pattern diet on the interaction of prenatal stress, maternal behavior, and offspring phenotype

Authors: *N. CLAUSS[1], J. BYRD-CRAVEN[1], P. CAMPBELL[2];
[1]Oklahoma State Univ., Stillwater, OK; [2]Univ. of California- Riverside, Riverside, CA

Abstract: A Western-pattern diet (WPD) during lactation has been found to lead to anxiety reduction in rodent offspring. However, a WPD can also impact maternal behavior during lactation, and previous investigations have yet to disentangle the relative impact of potential nutrition-induced epigenetic changes from diet-induced changes in maternal behavior. To address this gap in the literature, experimentally and sexually naïve female *Mus musculus* (*n* = 32) and their offspring (*n* = 192) were maintained on a chow or WPD and experienced prenatal stress or no stress (8 dams per group). After weaning at PND 21, offspring were housed with same-sex siblings and further divided into groups fed either chow or WPD (12 mice per group; see Fig. 1).

Figure 1. **Maternal experimental groups, lactation diet experimental groups, and weaning diet experimental groups.**

Maternal behavior was assessed at PND 1 and revealed that stressed dams fed a WPD engaged in greater maternal behaviors than stressed dams fed chow. Anxiety-related behavior and exploration in the offspring were tested in the Open Field at PND 23 and PND 70; and reverse transcription followed by quantitative real-time PCR was used to assess mRNA levels of the following genes were examined: ER, DRD1, DRD2, GCR, and OXTR. Results revealed that the relationship between early stress and juvenile anxiety was mediated by gene expression and moderated by lactation diet, indicating that juvenile offspring of non-stressed dams and the stressed dams fed a WPD displayed significantly less anxious behavior than the chow-fed offspring of stressed dams. In adulthood, there was a three-way interaction between early stress, adult diet, and offspring sex to impact adult phenotype, with females driving this interaction. This preliminary data suggests that the lasting effects of developmental stress could be mitigated by a diet that stimulates the same neuroendocrine systems depleted by early stress. If replicated in humans, these results could provide insight into developmental correlates of pathological stress-induced eating and inform intervention development.



Disclosures: **N. Clauss:** None. **J. Byrd-Craven:** None. **P. Campbell:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.22/U36

Topic: F.04. Stress and the Brain

Support: Undergraduate Research Grant from Purdue University Northwest

Title: Investigation of serotonin-related gene expression and socioemotional behaviors as a result of early-life exposure to clomipramine in Sprague-Dawley mother rats

Authors: *B. N. REGULA[1], C. M. RAGAN[2], N. SHAH[3];
[1]Psychology, Purdue Univ. Northwest, Hammond, IN; [2]Psychology, Purdue Univ. Northwest, Westville, IN; [3]Sch. of Med., St. George's Univ., True Blue, Grenada

Abstract: Early exposure to antidepressants can alter brain mechanisms during development. Previous animal models show that administering the tricyclic antidepressant, clomipramine, to rats during a sensitive period in development causes alterations to the serotonergic system and induces obsessive-compulsive disorder (OCD)-like behaviors in adulthood. Similarly, rats that display anxiety-like behaviors show increased gene expression of 5-HT2C in the orbitofrontal cortex (OFC) due to the deficiency of serotonin. However, previous studies have not examined long-term effects of early clomipramine exposure on mothers during the postpartum period. Therefore, our laboratory has used a model of early-life clomipramine exposure to observe its molecular and behavioral effects on Sprague-Dawley dams. During postnatal days 9-16, male and female pups from 8 litters were intraperitoneally-injected with either clomipramine or saline (control) to induce OCD-like behaviors in adulthood. To continue this study, OFC tissue from the mother brains and male brains from the same litter were examined to explore the effects that early clomipramine exposure has on the serotonin receptor, 5-HT2C. RNA from the OFC was isolated and quantitative real-time PCR was used to measure 5-HT2C mRNA expression normalized to the housekeeping gene CYPA. Results showed no significant differences in 5-HT2C gene expression in the OFC in saline vs. clomipramine-treated dams, however the clomipramine-exposed dams made more total hole pokes and engaged in more passive nursing than saline-treated dams. Conversely, clomipramine-exposed males had higher 5-HT2C gene expression in the OFC. The difference in 5-HT2C expression in the OFC between the males and females may be linked to pregnancy/lactation-related stress as the serotonergic system is rather dynamic during the reproduction period. Therefore, to further analyze the differences in gene expression between the males and females, expression of the serotonin transporter, SERT, which is responsible for serotonin reuptake, will be examined in both populations as well as in the offspring.

Disclosures: **B.N. Regula:** None. **C.M. Ragan:** None. **N. Shah:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.23/U37

Topic: F.04. Stress and the Brain

Support: Bezos Foundation
JPB Foundation
CIFAR

Title: Effects of early life stress on social group dynamics in mice

Authors: *G. BATISTA[1], N. W. HODGSON[2], J. SCHNEIDER[3], S. EL-SHAWA[3], J. LEVINE[3], T. K. HENSCH[1,2];
[1]Harvard Univ., Cambridge, MA; [2]Boston Children's Hosp., Boston, MA; [3]Univ. of Toronto Mississauga, Mississauga, ON, Canada

Abstract: Abnormal maturational trajectories following early life stress (ELS) lead to cognitive and social deficits associated with several psychiatric disorders. A comprehensive assessment of the impact of ELS on different social traits in mice is needed to uncover the underlying mechanisms. Here, we employed social network analysis to characterize the interactions of individual young adult (P60-P70) mice housed in more naturalistic groups. We were able to extract network parameters that are modified when animals exposed to control care (CC) or fragmented care (FC) in early life (P2-P9) interact with each other in adulthood. These results were compared to their performance on various standard laboratory tests commonly used to assess social behaviors (dominance, sociability, novelty seeking and empathy). Tracking body and head/tail position information of individuals identified by machine learning algorithm revealed interactions in four categories. This information was then used to compare the organization of social networks comprised of balanced CC and FC cohorts. Several aspects of the networks were notably altered when FC mice were included in the social group. Average clustering, global efficiency and betweenness centrality were increased in CC/FC groups as compared with purely CC/CC interactions. In contrast, average assortativity was reduced in mixed CC/FC networks. These effects were mainly observed in male interactions; however, some changes were also noted in female networks. Sociability over an inanimate object, as measured in a standard three-chamber assay, was not affected in either sex, while social novelty seeking was selectively impaired in males after ELS. Likewise, FC males in the tube test were more likely to be victorious over CC challengers, indicating increased dominance (consistent with their broader territorial urine marking). Elevated observational fear learning, commonly used as a proxy of empathy in mice, was primarily driven by FC females. Taken together, ELS affects distinct aspects of social behavior in mice, not only individually but at an ecological

group level. These findings will guide future research into molecular and circuit mechanisms mediating ELS-induced social impairments.

Disclosures: G. Batista: None. **N.W. Hodgson:** None. **J. Schneider:** None. **S. El-Shawa:** None. **J. Levine:** None. **T.K. Hensch:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.24/U38

Topic: F.04. Stress and the Brain

Support: Faculty Startup Funds

Title: Light at night during development increases susceptibility to an immune challenge in adulthood in female mice

Authors: *R. CHEN, L. K. FONKEN;
Div. of Pharmacol. and Toxicology, Univ. of Texas at Austin, Austin, TX

Abstract: The increase of light at night (LAN) in the past few decades accompanies the increased prevalence rate of mood disorders in young populations. Chronic dim light (~5 lux) at night can alter the circadian system leading to changes in mood as well as neuroimmune changes such as microglia activation. Neuroimmune activation is closely linked to mood disorders: activation of the immune system leads to mood disturbances and elevated expression of both pro- and anti-inflammatory cytokines can accompany mood disorders. However, the effects of dim light at night (LAN) during development remains unclear. Here we aimed to evaluate the effect of dim LAN during critical developmental windows on neuroimmune function and affective behaviors in adulthood. Male and female C57/BL6 mice were exposed to chronic dim LAN [12:12 light (150 lux) /dim (10 lux) cycle] during early life (PND 10 - 24) or adolescence (PND 30 - 44) or a standard light-dark cycle [12: 12 light (150 lux)/dark (0 lux)]. In both sexes, dim LAN exposure during development did not lead to a difference in growth patterns compared to mice housed in the standard light-dark cycle. No spontaneous anxiety- or depressive-like effects were observed in the open field test and the sucrose preference test. Post-LPS (10 ug/kg) pro-inflammatory cytokine levels in the hippocampus (HPC) and medial prefrontal cortex (mPFC) were measured when the animals reached adulthood. In HPC, LPS induced a significant increase in inflammatory markers IL-β and IL-6 in LAN but not control animals. This difference was only shown in female but not male mice. These data may indicate a greater vulnerability to developmental LAN exposure in female mice. Ongoing experiments will examine behavioral outcomes, such as whether anxiety- and depressive-like responses are exacerbated following an immune challenge in mice exposed to LAN during development.

Disclosures: **R. Chen:** None. **L.K. Fonken:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.25/U39

Topic: F.04. Stress and the Brain

Support: NIH R01 HD072968

Title: Mast cells as potential mediators of early life adversity effects on adult physiology and behavior

Authors: ***N. DUQUE-WILCKENS**[1], N. MARADIAGA[2], F. STOELTING[3], E. MACKEY[2], E. SARNO[3], K. THELEN[3], A. MCCAULIFF[3], A. ROBISON[4], A. MOESER[2];
[1]Physiol., [2]Large Animal Clin. Sci., [4]Neurosci., [3]Michigan State Univ., East Lansing, MI

Abstract: Early life adversity (ELA) is linked to an increased susceptibility to adulthood major depressive disorder (MDD), one of the most common and debilitating psychiatric diseases and for which available treatments are largely ineffective. Inflammation in the brain is a key mechanism underlying the pathophysiology of MDD, and studies across species suggest that ELA increases vulnerability to depression by priming the immune system for an amplified and more persistent inflammatory response to environmental stressors. However, the cellular and molecular mechanisms of this long-lasting effect are not fully understood. Mast cells (MCs) are uniquely positioned to drive ELA-mediated inflammatory responses that lead to MDD. First, they are distributed throughout the body, including the brain and meninges, and are highly activated in response to psychological stress. Second, they can release a variety of mediators responsible for initiating, amplifying, and prolonging inflammation; and third, they can disrupt blood-brain barrier integrity, allowing peripheral proinflammatory substances to enter the brain. Surprisingly, to our knowledge, no studies of MC effects on the brain in models of MDD have been performed to date. Using a mouse model of ELA consisting of neonatal maternal separation combined with early weaning (NMSEW), we first found that, compared to normal handling (NH), NMSEW mice exhibit heightened MCs hyperactivity demonstrated by higher basal and restraint stress-induced serum histamine levels. Additionally, analysis of bone marrow-derived MCs (BMMCs) showed that BMMCs derived from adult NMSEW mice had increased proinflammatory cytokine release after allergen stimulation (including TNFalfa and IL-6), together with persistent histone methylation modifications, suggesting that MCs are permanently, epigenetically programmed by NMSEW. Further, preliminary data suggest that meningeal MCs show increased hyperactivity in NMSEW vs NH females. Here we found that exposure to a 2nd hit stress during adulthood reduced social interaction in NMSEW but not NH males, and sucrose preference in NMSEW compared to NH females. Currently, we are using a combination of novel

mouse genetic tools, in vivo imaging technology, and behavioral and immunological studies to fully characterize ELA-driven functional changes in MCs, and establish the extent to which MCs are necessary and/or sufficient for stress-induced phenotypes in ELA exposed mice.

Disclosures: **N. Duque-Wilckens:** None. **N. Maradiaga:** None. **F. Stoelting:** None. **E. Mackey:** None. **E. Sarno:** None. **K. Thelen:** None. **A. McCauliff:** None. **A. Robison:** None. **A. Moeser:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.26/U40

Topic: F.04. Stress and the Brain

Support: NIH R01 HD094809
NIH F32 HD085576

Title: Methylene blue rescues mitochondrial respiration impairments in developing iron-deficient hippocampal neurons

Authors: ***T. W. BASTIAN**, L. M. LANIER, M. K. GEORGIEFF;
Univ. of Minnesota, Minneapolis, MN

Abstract: Iron deficiency (ID) affects an estimated 2 billion people worldwide, including 30-40% of pregnant women and children. ID is particularly deleterious during fetal/neonatal brain development, leading to neurological impairments, including deficits in hippocampal-mediated learning and memory. These deficits often persist into adolescence and adulthood despite early-life iron repletion. Unfortunately, in some iron-deficient populations nutritional iron supplementation is either not realistic or can even be dangerous (e.g., with coexisting malarial infection). Because iron therapy is not always possible or effective, it is necessary to develop alternative treatment strategies targeting the underlying neurodevelopmental deficits of early-life ID.

Iron is necessary for mitochondrial enzymes involved in cellular energy production, supporting metabolically demanding developmental processes such as dendrite growth/branching. Early-life ID impairs mitochondrial respiration, ATP production, and dendritic complexity in the developing brain, with its most pronounced effect on hippocampal neurons. Methylene blue (MB) accumulates in mitochondria, and at low doses, acts as an alternative electron transport shuttle, enhancing mitochondrial energy production. We hypothesized that MB treatment would improve mitochondrial respiration and ATP production in a dose-dependent manner in developing iron-deficient neurons.

Mouse embryonic hippocampal neuronal cultures were treated with deferoxamine (DFO, an iron

chelator) beginning at 3 days in vitro (DIV). At 11 or 14DIV, iron-deficient cultures were treated with varying doses of MB, with and without concomitant iron repletion, or left untreated. Mitochondrial oxygen consumption rate (OCR) and ATP levels were assessed after 7 days (i.e., at 18 or 21DIV).
Consistent with our previous findings, DFO treatment significantly reduced neuronal OCR and ATP levels. When given from 11 to 18DIV, 17nM or 50nM MB partially rescued OCR, while 150nM MB increased cell death in iron-deficient neurons. When given from 14 to 21DIV, iron and 17nM and 50nM MB treatments all partially restored neuronal ATP levels. ATP levels were fully rescued when MB and iron treatments were combined.
Our findings show that MB can improve mitochondrial energy production following early-life ID. Importantly, energy metabolism deficits that persist even after iron treatment can be recovered with combined iron/MB treatment. Thus, stimulating mitochondrial energy production, in addition to or instead of iron repletion, has potential as a therapeutic target for the neurological deficits of early-life ID.

Disclosures: T.W. Bastian: None. **L.M. Lanier:** None. **M.K. Georgieff:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.27/V1

Topic: F.04. Stress and the Brain

Support: CONACYT 238313-2015
CONACYT 221092-2014

Title: Effect of methylphenidate on cell proliferation and memory in adult female rats exposed to chronic stress during the gestation stage

Authors: S. CASTELLANOS DE ALBA, A. FREGOSO-GONZÁLEZ, A. GARCÍA-ZAMUDIO, T. MORALES-SALCEDO, A. AGUILAR-DELGADILLO, J. GARCÍA-ESTRADA, D. FERNANDEZ-QUEZADA, F. JAUREGUI-HUERTA, S. LUQUIN, *Y. RUVALCABA-DELGADILLO;
Univ. de Guadalajara, Guadalajara, Mexico

Abstract: Methylphenidate (MPH) is a drug prescribed for the treatment of some pathologies such as attention deficit hyperactivity disorder (ADHD) in children and adolescents. This disorder is related to the hypo-functionality of the prefrontal cortex (PFC). The PFC and the hippocampus are regions of great relevance in cognitive processes such as memory. They also participate in the regulation of neuroendocrine processes as the stress response. The function of the medial PFC and the hippocampus can be altered due to stressful events. OBJECTIVE:

Evaluate whether chronic treatment with MPH improves cognitive ability (working memory) and modifies cell proliferation in mPFC and hippocampus of adult rats exposed to a model of chronic variable stress (CVS) during pregnancy. METHODS: Female Wistar rats were exposed to CVS during days 13-19 of gestation. After birth, the female offspring of stressed mothers and controls remained in laboratory conditions until four months of age. In this age we evaluate the work memory (tasks alternance / discrimination) with T maze task. After evaluation, we administered chronic treatment with MPH (40 days). In addition, BrdU was administered (days 28, 29 and 30 of the treatment with MPH). After the treatment with MPH, they were exposed again to the working memory test and cell proliferation and astrocyte response were analyzed in the mPFC and hippocampus of the CVS and control groups with and without MPH. RESULTS: We found a decreased number of BrdU + cells in mCPF of the CVS groups with and without MPH treatment compared to the control groups. In this region the control group with MPH treatment showed significantly more BrdU positive cells than the control group without MPH. In the hippocampus, an increase was observed in BrdU + cells in the CVS group treated with MPH compared to the other groups. Regarding the cognitive evaluation, the MPH did not have any beneficial effect in the execution of the work memory task in all the groups.

Disclosures: S. Castellanos de Alba: None. **A. Fregoso-González:** None. **A. García-Zamudio:** None. **T. Morales-Salcedo:** None. **A. Aguilar-Delgadillo:** None. **J. García-Estrada:** None. **D. Fernandez-Quezada:** None. **F. Jauregui-Huerta:** None. **S. Luquin:** None. **Y. Ruvalcaba-Delgadillo:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.28/V2

Topic: F.04. Stress and the Brain

Support: SIR 2014 RBSI14ZV59

Title: Maternal insulin resistance transgenerationally impairs memory via germline histone modifications via germline histone modifications

Authors: *S. FUSCO, M. SPINELLI, S. COCCO, C. RIPOLI, A. MASTRODONATO, G. LIVRIZZI, F. NATALE, M. RINAUDO, C. GRASSI;
Inst. of Human Physiol., Univ. Cattolica Del Sacro Cuore, Rome, Italy

Abstract: Metabolic diseases harm brain health and cognitive functions, but whether maternal metabolic unbalance may affect brain plasticity of next generations is still unclear. To address this question, C57 adult female mice (F0) were fed with either standard or high fat diet (SD or HFD, respectively) from 4 weeks before mating until the 3rd week of suckling. The first

generation of HFD-fed mice, hereinafter referred as F1-HFD, and their descendants (F2-HFD and F3-HFD, respectively) were all fed with SD and were tested by behavioral, electrophysiological and molecular analyses. All HFD-descendant mice showed a lower discrimination index than the SD mice in a standard novel object recognition paradigm (F1-HFD = 56.5 ± 0.8%, F2-HFD = 54.6 ± 0.6%, F3-HFD = 55.8 ± 0.9% vs SD = 68.7 ± 1.0%; n=8-10 per group, $p<0.01$ for each comparison). These effects were associated with a significant impairment of spatial learning and memory in the Morris water maze. Time spent to reach the platform at the 3rd and the 4th training days was increased by +106.2% and +130.1%, respectively in F1-HFD; +78.9% and +91.6% in F2-HFD; +70.3% and +161.1% in F3-HFD, when compared to SD mice (n=8-10 per group, $p<0.05$ for each comparison). Accordingly, electrophysiological analyses on hippocampal brain slices of F1-, F2- and F3-HFD mice revealed severe deficits of long-term potentiation at CA3-CA1 synapses (ranging from -37% in F1-HFD to -52% in F3-HFD; n=11-15 slices from 4-5 mice per group, $p<0.05$ for each comparison). We demonstrated that maternal HFD-dependent insulin resistance induces multigenerational impairment of synaptic plasticity, learning and memory by epigenetically inhibiting Bdnf expression in both germline and hippocampus of progeny. These effects were triggered by downregulation of both TrkB and insulin receptor signaling in maternal ovaries, whose changes affected the recruitment of histone remodelers on BDNF regulatory sequences. Notably, either intraperitoneal administration of BDNF to the mothers or exposure of the offspring to novel enriched environment counteracted the transmission of HFD detrimental effects to the next generations by restoring the BDNF levels via opposite epigenetic changes on the same loci. More importantly, preserved insulin sensitivity in HFD-fed p66Shc KO mice also prevented the cognitive impairment of progeny. Collectively, our data suggest that maternal diet transgenerationally impacts on descendants' brain health via epigenetic modifications susceptible to lifestyle.

Disclosures: **S. Fusco:** None. **M. Spinelli:** None. **S. Cocco:** None. **C. Ripoli:** None. **A. Mastrodonato:** None. **G. Livrizzi:** None. **F. Natale:** None. **M. Rinaudo:** None. **C. Grassi:** None.

Poster

409. Early-Life Stress

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 409.29/V3

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

Support: SNI to EER, BAI, IJ

Title: Chaos analysis of compound action potentials evoked in sural nerves from rats reared without littermates

Authors: *E. E. RODRIGUEZ-TORRES[1], V. MARTÍNEZ-ALVAREZ[2], M. A. LANDA-JIMENEZ[4], P. GONZALEZ-GASPAR[4], J. VIVEROS-ROGEL[1], B. A. ITZA-ORTIZ[1], F. MENENDEZ-CONDE-LARA[1], M. TETLALMATZI-MONTIEL[1], R. JIMENEZ-MUNGUIA[1], I. JIMENEZ-ESTRADA[3];
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Abstract: Behavioral factors (e.g., artificial breeding of the offspring) produce negative effects in the propagation of evoked compound action potentials (eCAP) and in the myelination process of peripheral nerve axons (Segura et al, 2014). In the present study we analyze whether the presence or absence of littermates modify the chaotic fluctuations in area of the eCAPs in sural nerves (SU) from Wistar rats (60 days of age) reared with their mothers. We worked with litters of 9 male rats (littermates were tagged by their weight (±1SD) in light, intermediate, and heavy) and with only one pup. eCAPs were recorded in nerves by means of conventional electrophysiological techniques. Scalar time series were formed by using the area values of the sequentially generated eCAPs (N=1000; 1Hz freq., 2xU) in SU nerves from each group of rats. If each time series corresponds to measurements of one degree of freedom in a dynamical system that evolves near an attractor, it is then possible to reconstruct phase space orbits using methods from chaos theory which we implemented in Python. In the reconstruction of phase space orbits it is necessary to determine the time interval (τ) for observation, and the embedding dimension. Our results show changes in the dynamics of the reconstructed orbits of sural nerves from lightweigth rats and without littermates as compared with those of the control group, the latter being more symmetrical. In the case of heavyweight rats the reconstruction of orbits showed much more background noise, even though eCAP records from all groups are overall quite similar. τ was obtained using the mutual information function which measures the interdependence between two variables, the idea being that $x(t + \tau)$ brings new information, thus a large τ value would mean that there is a good amount of redundancy in the time series of eCAPs. In this sense, control time series has the most redundancy, whereas a loss of redundancy was observed in the other times series, remaining at 8% in the lightweight group, 29% in the heavyweight group, and 14% in the group with no littermates. Therefore, eCAP time series from the lightweight and without littermates groups had the most redundant information loss. Our results indicate that lightweigth and without siblings rats present alterations in the transmission of sensory information from the periphery to the central nervous system.

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

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Program #/Poster #: 410.01/V4

Topic: F.08. Biological Rhythms and Sleep

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PIP CONICET 2014
SECyT UNC 2018
FONCYT PICT 2016 No. 0187

Title: A cross-talk between the molecular clock and the metabolic/redox oscillator operates in glioblastoma cancer cells displaying a differential temporal susceptibility to chemotherapy

Authors: *M. E. GUIDO, P. M. WAGNER;
Ciquibic Conicet Univ. Nacional de Cordoba, Cordoba, Argentina

Abstract: Biological clocks ticking on a near 24 h period (circadian) are constituted by a molecular clock core displaying transcriptional/translational negative feedback loops of clock and clock-controlled genes, and proteins. They are even present in immortalized cell lines driving transcriptional and metabolic rhythms regulating physiological processes. In addition, redox oscillations evolutionally conserved from humans to Archae were found to persist without transcription. Circadian desynchrony as a consequence of modern life (shiftwork, jetlag, etc.) may promote higher cancer risk and metabolic disorders. Nevertheless, little is known about the biological clock on proliferating tumor cells. Here we investigated metabolic/redox rhythms in human glioblastoma T98G cells and whether these cells displayed differential time responses to chemotherapy after treatment with bortezomib (BOR) or SR9009, a Rev- erb agonist. In synchronized cells, rhythms of redox and lipid metabolism were observed while cell viability significantly changed over time after BOR or SR9009 treatment. For BOR, the temporal changes in susceptibility together with the redox cycles were further altered after *Bmal1* knock-down, indicating a significant cross-talk between the molecular clock and the metabolic oscillator. In addition, SR9009 used to affect the molecular clockwork, exhibited significant anti proliferative effects in T98G cells and altered metabolic pathways involving reactive oxygen species and lipid droplets. Our results suggest that an intrinsic metabolic clock works in proliferating cells and the metabolic rhythms generated could be taken into account for a more efficient chronotherapy to determine time of day when glioblastoma cells are more susceptible to treatment.

Disclosures: M.E. Guido: None. **P.M. Wagner:** None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

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Program #/Poster #: 410.02/V5

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R15GM128170

Title: A circadian output center controlling feeding:fasting rhythms in *Drosophila*

Authors: *D. J. CAVANAUGH, A. P. DREYER, M. M. MARTIN, C. V. FULGHAM, D. A. JABR, J. BESHEL;
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Abstract: The circadian system produces ~24-hr rhythms in physiology and behavior and can be divided into three major components: a core molecular clock in the brain, input pathways that synchronize the molecular clock to environmental cycles, and output pathways that couple the clock to overt physiological and behavioral outputs. Most circadian studies in *Drosophila* have been conducted with respect to locomotor behavior, and little is known about circadian control of other outputs such as feeding behavior. We used a recently developed feeding monitor to characterize the contribution to circadian feeding rhythms of two key neuronal populations in the *Drosophila* pars intercerebralis (PI), which is functionally homologous to the mammalian hypothalamus. We demonstrate that thermogenetic manipulations of PI neurons expressing the neuropeptide SIFamide (SIFa) as well as mutations of the *SIFa* gene degrade feeding:fasting rhythms. In contrast, manipulations of a nearby population of PI neurons that express the Drosophila insulin-like peptides (DILPs) affect total food consumption but leave feeding rhythms intact. The distinct contribution of these two PI cell populations to feeding is accompanied by vastly different neuronal connectivity as determined by trans-Tango synaptic mapping. These results for the first time identify a non-clock cell neuronal population in *Drosophila* that regulates feeding: fasting rhythms and furthermore demonstrate dissociable control of circadian and homeostatic aspects of feeding regulation by molecularly-defined neurons in a putative circadian output hub.

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

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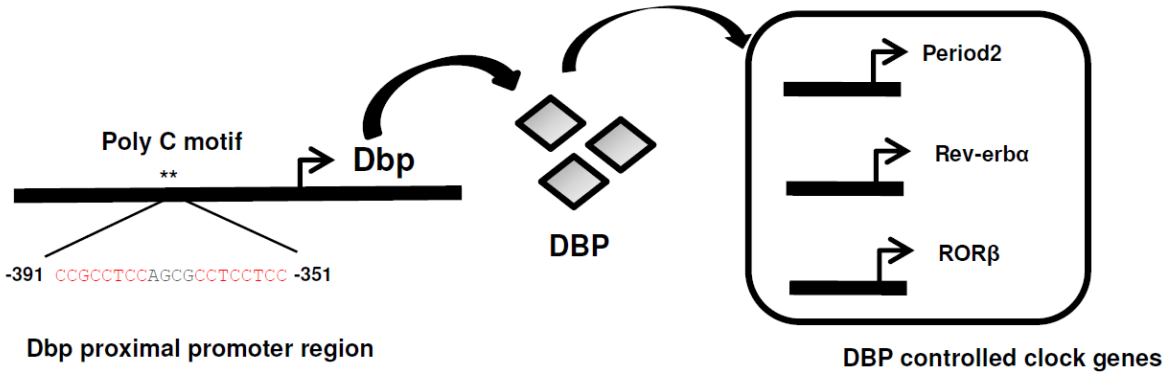
BK21 Plus Ministry of Education, Korea. (10Z20130012243)

Title: The poly(C) motif in the proximal promoter region of the D-box binding protein (DBP) drives its high-amplitude oscillation

Authors: *S. KIM[1], P. KWON[1], K.-T. KIM[1], T.-Y. ROH[1], H.-M. KIM[1], H. YI[2], H.-O. KU[2]; [1]POSTECH, Pohang, Korea, Republic of; [2]Vet Drugs and Biologics Division, Animal and Plant Quarantine Agency, Gimcheon, Korea, Republic of

Abstract: DBP, also known as D-site albumin promoter binding protein, is a transcription activator that binds to the D-box of its target gene. It has been known that there are more than 2000 D-boxes that govern about 10% of the clock-controlled gene. DBP supports the rhythmic transcription of the D-box containing clock-genes, in part by displaying high-amplitude cycling of its own transcripts compared to other circadian clock genes. However, the underlying mechanism of its high-amplitude cycling remains elusive. Here, we demonstrated that the poly(C) motif within the DBP proximal promoter provoked the transcriptional activation, in addition to a well-known E-box element. Furthermore, we generated poly(C) deleted cell line to demonstrate the endogenous effect of the poly(C) motif within the DBP promoter. We investigated that RNA polymerase 2 (Pol2) recruitment on the DBP promoter was decreased in poly(C) deleted cell line. Next, Assay for Transposase Accessible Chromatin (ATAC)-qPCR showed that the poly(C) motif induced higher chromatin accessibility within the region of DBP promoter. Finally, we identified that the oscillation amplitude of endogenous *DBP* mRNA of the poly(C) deleted cell line was decreased, which also affected the oscillation of other clock genes that are controlled by DBP. Taken together, our results provide new insights into the function of the poly(C) motif as a novel enhancer of DBP, along with its significance in the regulation of circadian rhythms.



Disclosures: **S. Kim:** None. **P. Kwon:** None. **K. Kim:** None. **T. Roh:** None. **H. Kim:** None. **H. Yi:** None. **H. Ku:** None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

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Program #/Poster #: 410.04/V7

Topic: F.08. Biological Rhythms and Sleep

Support: CIHR MOP142458

Title: The circadian gene Bmal1 in the striatum regulates alcohol intake and preference in male and female mice

Authors: ***N. DE ZAVALIA**, P. SOLIS, G. PARENT, S. FERRARO, K. SHÖTTNER, S. AMIR;
Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Perturbations in the function of core circadian clock components such as *BMAL1*, *Per1* and *Per2*, or to the circadian environment, can affect alcohol intake and are associated with alcohol use disorder. The neuronal pathways that mediate this disorder are not well understood. The striatum is of special interest because it is involved in ethanol drinking and it is the site of ethanol neuroadaptations. However, the molecular and cellular mechanisms that mediate alcohol-induced striatal neuroplasticity and neuroadaptation remain to be defined. The aim of this work was to study ethanol intake in mice containing a functional mutation in *Bmal1* gene in the striatum. We created a conditional *Bmal1* knock-out mouse using the Cre-loxP system. *Bmal1*$^{lox/lox}$ mice were crossed to *Gpr88-cre* mice to specifically knock out *Bmal1* in Gpr88 expressing striatal medium spiny neurons. 12-18 week old WT, Knock-out (KO) and Heterozygote (HET) male and female mice were tested in: (A) 24 h intermittent ethanol (15% v/v) access protocol; (B) 24 h intermittent ethanol access protocol with ascending concentrations

of ethanol (5-45% v/v); (C) sucrose (0.25 or 2% m/v) access protocol. Blood alcohol concentration (BAC) levels after an alcohol injection (3.6 g/kg, 20% v/v) were measure at different times. Selective depletion of *Bmal1* mRNA and protein was observed in the striatum of KO mice compared with WT mice. No significant differences in the expression levels of *Bmal1* was observed in the suprachiasmatic nuclei (the master circadian clock) or hippocampus of KO compared with WT mice. No significant differences in alcohol consumption were observed between $Gpr88^{cre/+}$ and $Gpr88^{+/+}$ mice. *Bmal1* KO male mice drink significantly more alcohol and have a higher alcohol preference than WT mice (n=10/group, $P < 0.05$). Bmal1 KO female mice drink less alcohol and have a lower alcohol preference than WT mice (n=10/group, $P < 0.05$). No significant differences in alcohol intake and alcohol preference between HET and WT male and female mice were observed. No significant differences in sucrose intake and sucrose preference were observed between any of the genotypes studied. These preliminary results suggest a sex based difference in alcohol-related *Bmal1* function in the striatum. A protective role of *Bmal1* against alcohol intake was observed only in male and not in female mice. Further studies are being carried out to determine the mechanisms that explain how *Bmal1* exerts its protective function and to understand the sex differences observed in this study.

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

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Program #/Poster #: 410.05/V8

Topic: F.08. Biological Rhythms and Sleep

Support: UTSW Institutional Support
HHMI (H012233)

Title: Genetic dissection of the circadian rhythm of body temperature

Authors: *I.-M. LIAO[1], M. IZUMO[1], B. JEONG[1], J. S. TAKAHASHI[2];
[1]Dept. of Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [2]Chair, Dept. of Neurosci., Univ. of Texas Southwestern Med. Center, Howard Hughes Med. Inst., Dallas, TX

Abstract: Mammals possess endogenous clocks that drive the near 24-hour circadian rhythms of many important physiological processes. These internal clocks are encoded genetically and are crucial for anticipating environmental changes. Disrupted body temperature (Tb) homeostasis can be life-threatening, thus it is critical for mammals to maintain Tb in a stable physiological range. Tb fluctuates with the circadian cycle, and the daily rhythms of Tb may serve as entraining cues for synchronizing peripheral clocks. The suprachiasmatic nucleus (SCN) and the

medial preoptic area (MPOA) of the hypothalamus play roles in regulating Tb rhythm and thermal sensing, respectively; however, the neural mechanisms underlying Tb rhythmicity remain poorly understood. We hypothesize that the SCN drives the rhythmicity of Tb, while the MPOA provides temporal modulation to shape distinct features of Tb rhythm. To search for critical clocks responsible for generating Tb rhythmicity, we used *Cre/LoxP* recombination to selectively delete *Bmal1,* an essential transcription factor for generating molecular circadian rhythms, from the SCN and/or MPOA. In addition, we used a retrograde neural tracer to investigate the anatomical connections between the SCN and the MPOA seeking direct neuronal interactions between the two regions. We found that disruption of forebrain clocks abolishes the circadian rhythm of Tb in mice. In contrast, disrupting SCN clocks led to the gradual dissociation of Tb rhythm. Finally, our initial neural tracing results indicate that the SCN projects directly to the MPOA. In sum, our data suggest that forebrain clocks are indispensable for, while SCN clocks play a partial role in the generation of Tb rhythms. Further study of forebrain clocks outside of the SCN and functional analyses of the SCN-MPOA neurocircuit are required to understand how individual components work together in a neural network to generate Tb rhythmicity.

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

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Program #/Poster #: 410.06/V9

Topic: F.08. Biological Rhythms and Sleep

Support: MRC-funded research programme MC_U142684173

Title: MYBBP1a - ZFHX3 complex acts a repressor on core circadian gene expression

Authors: *M. YIN[1], S. MILITI[2], H. HILTON[1], G. BANKS[1], C. ESAPA[1], P. NOLAN[1]; [1]Medicail Res. Council Harwell Inst. UK, Didcot, United Kingdom; [2]Ludwig Inst. for Cancer Res., Oxford, United Kingdom

Abstract: Abstract
The transcription factor, ZFHX3 has been identified as a regulator of circadian rhythms in the suprachiasmatic nucleus (SCN) - the site of the master clock of the body. The Short-Circuit missense mutation in ZFHX3 (*Sci*) displays a short circadian period phenotype. Although evidence shows that reducing the transcriptional regulation of ZFHX3 disturbs the expression of neuropeptides which are essential to SCN signalling, the role of ZFHX3 in SCN circadian function and the molecular mechanism underlying ZFHX3-dependent networks in circadian regulation is unclear.

Through immunoprecipitation studies we have identified a potential cofactor of ZFHX3, Myb-binding protein 1a (MYBBP1a). This nuclear protein predominantly localizes in the nucleolus and functions as a shutting protein for both nuclear import and export. Although it does not contain obvious DNA-RNA binding motifs, previous studies showed that MYBBP1a associates with a number of transcription factors to regulate gene transcription. Evidence suggests that it may regulate the molecular circadian clock by acting with CRY1 as a co-repressor of *Per2*. Our results show that ZFHX3 is localized in the nucleus and is co-localized with MYBBP1a at the nucleolus. The protein-protein interaction between ZFHX3 and MYBPP1a has also been demonstrated by pull down. Hence, we propose MYBBP1a may interact with ZFHX3 to regulate circadian gene expression. To test our hypothesis, we employed luciferase reporter assays to characterize the role of MYBPP1a/ZFHX3 complex in circadian gene expression. Through such experiments, the results imply that the MYBBP1a may bind to ZFHX3 to repress its activity as a TF. Moreover, I am currently developing a method to further dissect how this complex effect the transactional regulation to provide insight to the mechanisms underpinning the circadian regulation of transcription.

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant ES027544
NIH Grant DK111436
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American Heart Association AHA30970064

Title: Central circadian clock regulates cognitive function and insulin sensitivity

Authors: ***X. LI**[1], W. ZHOU[1], Y. HE[2], T. YANG[1], Y. GONG[1], G. DING[1], S. LEE[1], J. QIAN[1], Y. XU[2], Z. SUN[1];
[1]Dept. of Med., [2]Children's Nutr. Res. Ctr., Baylor Col. of Med., Houston, TX

Abstract: Glucose metabolism and neurocognitive functions is regulated by the central circadian clock in the brain. Elevated basal glucose levels at the onset of the active cycle is associated with elevated sensitivity to insulin in normal conditions. Here we show that the suprachiasmatic nuclei (SCN) of the brain drives the timing of the glucose counter-regulation. Nuclear receptors Nr1d1/2 are key components of the molecular clock machinery. We found in mice that Nr1d1/2

in the SCN GABAergic neurons controls diurnal variation of insulin sensitivity, likely through indirectly regulating the sympathetic nervous system, in a manner that is independent of the circadian consummatory or locomotor behaviors. In addition to glucose metabolism, Nr1d1/2 also regulate learning and memory. These findings provide molecular and cellular insights into the circadian rhythms and chronodisruption in insulin sensitivity and cognitive dysfunction.

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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: NINDS R21NS103180
NIGMS R15GM126545

Title: Generation and validation of a conditional reporter mouse line for studying circadian rhythms

Authors: *C. B. SMITH[1,2], V. VAN DER VINNE[1], A. C. STOWIE[3], M. E. HARRINGTON[4], L. GARBUTT[5], A. J. DAVIDSON[3], R. DALLMANN[5], D. R. WEAVER[1];
[1]Neurobio., [2]Grad. Program in Neurosci., Univ. of Massachusetts Med. Sch., Worcester, MA; [3]Neurosci. Inst., Morehouse Sch. of Med., Atlanta, GA; [4]Neurosci. Program, Smith Col., Northampton, MA; [5]MRC Doctoral Training Program in Interdisciplinary Biomed. Res. and Warwick Med. Sch., Univ. of Warwick, Coventry, United Kingdom

Abstract: Circadian rhythms are the outward manifestation of an internal timing system that measures time in 24-hr increments. The mammalian circadian system is hierarchical, with a pacemaker in the suprachiasmatic nucleus synchronizing cell-autonomous oscillators in peripheral tissues. Much of what we know about rhythmicity in peripheral tissues comes from studies monitoring bioluminescence rhythms in animal models in which luciferase reporter expression is controlled by a clock gene, e.g., PERIOD2::LUCIFERASE (PER2::LUC) knock-in mice. A limitation with these models is that rhythmicity cannot be monitored in specific cell types due to widespread reporter expression. To address this shortcoming, we generated a mouse that expresses luciferase from the *Dbp* locus only after *Cre*-mediated recombination. Crossing the conditional reporter mice with mice expressing *Cre* recombinase in various cell types allowed detection of rhythmic bioluminescence in the expected tissues, *in vivo* and *ex vivo*, as well as in slice cultures containing the SCN. These results were expected given the widespread, highly rhythmic expression of *Dbp* and the specificity of the Cre lines used. Our *Cre*-conditional

knock-in construct also contains sequence encoding a destabilized GFP, flanked by loxP sites, upstream of the *luciferase* gene; current studies are assessing GFP rhythmicity. The phase of bioluminescence rhythms from explants of mouse peripheral tissues indicated that Dbp^{Luc} bioluminescence rhythms have an earlier phase than PER2::LUC rhythms, as expected from gene expression rhythms. Importantly, we confirmed that editing of the *Dbp* locus did not alter the period of circadian locomotor activity rhythms. Thus, this mouse line is useful for studying circadian rhythms in a cell-type specific manner.

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

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Topic: F.08. Biological Rhythms and Sleep

Support: 015CB856400
2015CB553503
81521063
81871071
31230033
91432303
81171251

Title: Clock gene per1 and per2 is involved in the mechanism underlying the rapid antidepressant action of adenosine A1 receptor agonist

Authors: *S.-X. LI;
Natl. Inst. On Drug Dependence,Peking Univ., Beijing, China

Abstract: Objective
To explore the anti-depressant effect and mechanism of adenosine A1 receptor agonist CCPA
Method
We performed behavioral tests at 6h, 12h, 24h, 36h, 48h, and 72h after intraperitoneal injection of adenosine A1 receptor agonist CCPA (0.2mg/kg) in a 28-day chronic unpredictability stress (CUS) rat model of depression, to explore CCPA's rapid anti-depressive effect and its onset and maintenance time. In order to explore the molecular mechanism underlying CCPA rapid anti-depressant avtion, we used western blot to detect the expression of adenosine A1 receptor and it’s downstream molecules in mPFC and hippocampus CA1 in rats. To further verify the role of

per1 gene in rapid anti-depressant action, microinjection of adenosine related virus to knock down per1 gene expression in target brain region, and then conduct behavioral tests.

Result

Compared to the control, the CUS rats exhibited significant depression-like behaviors. Compared with the CUS group, the preference value of sugar water was significantly increased 12 hours after intraperitoneal injection of CCPA 0.2mg/kg ($p<0.05$), and the immobility time in forced swim test was significantly shortened ($p<0.01$).The antidepressant action of CCPA lasted up to 36 hours.

CUS led to a significant decrease in theexpression of *p*-CREB and PER1 protein levels in the hippocampal CA1 brain region ($p<0.05$), and CCPA (0.2mg/kg) reversed the decrease in *p*-CREB and PER1 levels in the hippocampal CA1 brain region. No significant changes in these protein levels were observed in mPFC brain region. AAV-*shper*1 was injected into the CA1 brain region of normal rats, and behavioral test was conducted 21 days later. It was found that the preference value of sugar water was significantly reduced in the AAV-*shper*1 group ($p<0.05$), and the immobility time in the forced swim test was prolonged ($p<0.05$). After intraperitoneal injection of CCPA (0.2mg/kg) at 12h, 24h and 36h, the preference value of sugar water increased significantly ($p<0.05$).

Conclusion

Adenosine A1 receptor agonist CCPA has a rapid antidepressant effect;*Per1* in the hippocampal CA1 is involved in the occurrence of depression-like behaviors and mediates the rapid antidepressant effect of CCPA.

Disclosures: S. Li: None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.10/V13

Topic: F.08. Biological Rhythms and Sleep

Support: CIHR Grant MDP142458

Title: Molecular and behavioural consequences of circadian clock gene deletion within the mouse striatum

Authors: *P. SOLIS, K. SCHÖTTNER, M. A. BUTTON, N. DE ZAVALIA, S. AMIR;
Ctr. for Studies in Behavioral Neurobio. (CSBN), Concordia Univ., Montreal, QC, Canada

Abstract: Circadian clocks generate rhythms ranging from the molecular to behavioral level which are believed to be essential for optimal function of the biological system in a daily changing environment. Indeed, organism-wide or tissue specific disruption of circadian clocks is

associated with a wide range of chronic health problems in humans including mental disorders, emphasizing their clinical relevance. The circadian clock in medium spiny neurons (MSNs) of the striatum is of great interest given the fact that this brain region is associated with mood- and anxiety-related conditions, raising the question if a disruption of the circadian clock may alter these processes and contribute to the incidence of neuropsychiatric disorders. To investigate the role of circadian clock genes in the striatum, conditional Bmal1 or Per2 knockout mice were generated by crossing C57BJ/6 mice homozygous for floxed alleles of the Bmal1 or Per2 locus with C57BJ/6 mice expressing Cre under the control of the Gpr88 promoter. The knockout is confirmed by mRNA expression analysis of the Bmal1 or Per2 floxed locus in homo- and heterozygote mice compared to homozygote wild type (WT) littermates. Further examination by immunohistochemical staining and Western blotting demonstrates that expression of Bmal1 and Per2 is abolished in striatal MSNs of knockout mice. Strikingly, mRNA expression of circadian clock and clock-controlled genes is altered in clock gene knockouts compared to control animals at various time points throughout the 24h day. Subsequently, knockout mice and WT littermate controls were tested to assess the impact of the Bmal1 and Per2 knockout in striatal MSNs on behaviour with focus on circadian as well as mood- and anxiety-related phenotypes. Various parameters of daily locomotor activity rhythms are similar in Bmal1 or Per2 knockouts compared to control mice. Neither Bmal1 nor Per2 knockout male and female mice kept in groups display differences regarding the time spend in the central area of an open field, or immobility time in a tail suspension test compared to WT controls when tested during the light portion of the light/dark cycle. However, female Bmal1 knockout mice show significantly higher levels of locomotor activity in the open field compared to WT control mice. These preliminary results indicate that loss of the circadian clock genes Bmal1 or Per2 in the striatum doesn't affect anxiety- and depressive-like behavior when tested during the light portion of the 24h day. Future studies need to be conducted in order to elaborate if these outcomes are consistent throughout the entire 24h light/dark cycle and under challenging conditions such as stress.

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.11/V14

Topic: F.08. Biological Rhythms and Sleep

Title: A neuronal circuit that modulates rhythmic activity under warm temperature in the fruit fly, *Drosophila melanogaster*

Authors: *A. IYENGAR, V. SHEEBA;
Neurosci. Unit, Jawaharlal Nehru Ctr. For Advanced Scientific Res., Bangalore, India

Abstract: Organisms modulate their behaviour in response to extreme environmental conditions to mitigate their harsh effects. Under stressful conditions like prolonged durations of warmth, organisms may counter excessive heating or desiccation by shifting their activity from the day into the night, when the conditions are likely to be less harsh. Modulation of a rhythmic behaviour such as locomotor activity in response to the environment would require an interaction between at least two neuronal systems: (1) a sensory system to receive input from the environment, and (2) the internal clock to correctly time rhythmic activity in response to this input. Our lab focuses on understanding the neuronal circuit underlying the behavioural modulation under warm ambient condition, using the genetically amenable model system, the fruit fly, *Drosophila melanogaster*. Previous work in the lab identified a thermosensory mutant of the ion channel *Drosophila* Transient Receptor Potential-A1 (dTRPA1) that fails to sense warm temperature and, unlike a wildtype fly, does not shift its activity into the night under warm ambient condition. To identify the neuronal basis for this behaviour, I investigated the role of a specific cluster of the *dTRPA1*-expressing neurons in the fly brain. Since these *dTRPA1*$^{+}$ neurons were known to include some core clock neurons, I further categorised these neurons into clock and non-clock subgroups and evaluated their relative contributions during modulation of rhythmic activity under warm ambient condition. I also explored how information from the *dTRPA1*-expressing neurons is communicated downstream and identified two neurotransmitters as potential communication factors for this circuit.

Disclosures: A. Iyengar: None. **V. Sheeba:** None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.12/V15

Topic: F.08. Biological Rhythms and Sleep

Support: NSERC Tier II Canada Research Chair in Molecular Genetics of Biological Clocks
Foundation for Innovation John R. Evans Leaders fund
NSERC post-graduate scholarships
CIHR operating grants
NSERC operating grants

Title: The role of SOX2 in circadian timekeeping

Authors: *A. H. CHENG[1,3], P. BOUCHARD-CANNON[1,3], S. W. FUNG[3], S. HEGAZI[1,3], C. LOWDEN[1,3], R. W. NESS[3,2], H.-Y. M. CHENG[1,3];
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Abstract: Most aspects of behaviour and physiology exhibit circadian (~24-hour long) rhythms that align with the day-night cycle. In mammals, these overt rhythms are controlled and coordinated by a central circadian pacemaker in the hypothalamus known as the suprachiasmatic nucleus (SCN). Here we report that SRY (sex determining region Y)-box 2 (SOX2), a transcription factor that have been shown to play critical roles throughout central nervous system development, promotes robust and coherent circadian behavior in mice.
We specifically ablated SOX2 in SCN neurons by crossing mice expressing Cre recombinase in GABAergic neurons (*Vgat*-IRES-Cre) with mice carrying the loxP-flanked alleles of *Sox2* (*Sox2*$^{fl/fl}$). This conditional knockout mouse strain (cKO) demonstrated weaker, poorly consolidated rhythms, unstable activity onsets, and greater variability in the phase angle of entrainment under 12-hr light:12-h dark schedule and skeleton photoperiod. Sox2 cKOs also showed significant wheel-running activity in the first 3 hours after light onset, leading to a prolonged active phase (alpha). In reaction to experimental jetlag, most of the Sox2 cKOs exhibited erratic and impaired re-entrainment to the shifted external light cycles. Upon release into constant darkness conditions, Sox2 cKOs exhibited a significantly longer period, in addition to reduced amplitude and prolonged active phase compared to controls. Under constant light conditions of gradually increasing light intensity, a majority of the Sox2 cKOs became arrhythmic under mid- to high light intensity. Collectively, these findings suggest that Sox2 cKOs may have impaired light-induced entrainment and are highly susceptible to constant light-induced arrhythmicity.
In an effort to understand the origins for the profound circadian behavioral phenotypes arising from *Sox2* ablation within the SCN, we analyzed the SCN transcriptomes of Sox2 cKOs and controls at 4 circadian times (CT 0, 6, 12, and 18) using RNA-sequencing. We identified 233 differentially expressed genes (DEGs) in the SCN of Sox2 cKOs with DESeq2. Amongst the 233 DEGs are known regulators of neuropeptide signaling and rhythmic process in the SCN. Further analysis conducted with MetaCycle confirmed that the rhythmic transcriptome of the SCN is perturbed in Sox2 cKOs. Finally, we selected 7 genes from the set of DEGs and used ChIP-qPCR and confirmed the binding of SOX2 near predicted region in SCN tissues.

Disclosures: A.H. Cheng: None. **P. Bouchard-Cannon:** None. **S.W. Fung:** None. **S. Hegazi:** None. **C. Lowden:** None. **R.W. Ness:** None. **H.M. Cheng:** None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.13/V16

Topic: F.08. Biological Rhythms and Sleep

Support: NIGMS R15GM126545
NIEHS R21 ES024684 (DRW)

Title: Measuring circadian bioluminescence from mice lacking a functional central clock

Authors: *B. MARTIN-BURGOS[1], P. MOLYNEUX[1], V. VAN DER VINNE[2], T. LEISE[3], D. R. WEAVER[4], M. E. HARRINGTON[1];
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Abstract: Circadian (approximately 24 h) rhythms arise from intracellular clocks that must be phase coordinated. The suprachiasmatic nucleus (SCN) is the pacemaker or central clock which receives direct input from the eyes and maintains peripheral phase alignment through systemic rhythmicity. Circadian disruption, as experienced in shift work, nocturnal light exposure and aging, impacts health by affecting interactions between peripheral and central clocks. To better understand this process, we used a method developed by our lab that measures PER2::LUC bioluminescence from freely moving mice. We applied this *in vivo* technique to study peripheral rhythmicity of mice lacking a functional central clock (Vgat-Cre^{+} Bmal1$^{fl/fl}$) in the absence of environmental rhythmicity (DD). Locomotor activity data in LD were collected prior to the experiment and during the 7-day *in vivo* bioluminescence experiment (in DD). Three days prior to recording, each mouse received a subcutaneous implant of an osmotic mini-pump delivering a synthetic luciferin, CycLuc1. Records were analyzed using discrete wavelet transforms (DWT). Results were compared to control and to arrhythmic Bmal1$^{-/-}$ mice to determine whether the absence of a central brain clock results in rapid loss of peripheral rhythm amplitude. The locomotor activity of Vgat-Cre^{+} Bmal1$^{fl/fl}$ mice becomes arrhythmic upon placement in DD, yet here we demonstrate that these mice can show rhythmic PER2::LUC bioluminescence arising from peripheral tissues. The circadian component of this rhythm was weaker than that seen in control (Vgat-Cre^{-} Bmal1$^{fl/fl}$) mice but was significantly different from arrhythmic Bmal1$^{-/-}$ mice. Our results suggest that a functional central clock is not required to maintain synchronization of cellular clocks in peripheral tissues. Circadian oscillators in peripheral tissues may be synchronized by prior housing under LD or by our brief anesthesia (circa 15 min) and pump implantation. Discrete wavelet transforms allowed quantification of persistence of rhythmicity in noisy records. Our technique for *in vivo* bioluminescence measures of circadian gene expression in freely-moving mice allows definite demonstration of rhythms in gene expression without invasive surgery or prolonged anesthesia.

Disclosures: B. Martin-Burgos: None. **P. Molyneux:** None. **V. van der Vinne:** None. **T. Leise:** None. **D.R. Weaver:** None. **M.E. Harrington:** None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.14/V17

Topic: F.08. Biological Rhythms and Sleep

Support: NIH 1R01NS082413
T32HL105349
UAB School of Medicine Multi-PI Pilot Award

Title: Time restricted feeding rescues hippocampal disruption after chronic high fat diet feeding

Authors: *J. DAVIS[1], S. YATES[1], J. POLLOCK[2], D. POLLOCK[2], M. YOUNG[2], S. BAILEY[2], K. L. GAMBLE[3];
[1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL; [3]Psychiatry, UAB Med. Ctr., Birmingham, AL

Abstract: Chronic circadian disruption (i.e. shift work, jet lag) is associated with increased risk for developing obesity, diabetes, and cardiovascular dysfunction. Obesity itself increases the risk of numerous additional diseases including diabetes, cardiovascular disease, and cognitive impairment. Given recent establishment of links between the circadian clock and metabolism, and that both sleep-wake status and obesity can have a profound impact on cognitive performance, we hypothesized that calorically dense diets alter the molecular clock and day-night differences in synaptic plasticity and spatial memory. Secondarily, we predict that these effects are due to altered food intake patterns, and could be rescued by restricting food access to an animal's active period. Wild-type C57BL/6J mice were fed HFD or control diet for 18 weeks followed by 2 weeks of time restricted feeding (TRF, with food access only at night). Following TRF, hippocampal extracts were collected to analyze clock gene expression (PER2, BMAL1) via western blot analysis. An additional cohort of Per2-Luciferase mice were fed following the same protocol and upon competition of the TRF, multiple tissues were extracted and cultured to determine the phase of PER2 expression across various tissues. Spatial working memory was assessed using spontaneous alternation performance in a T-maze during the inactive and active periods. Slice electrophysiology was performed on hippocampal slices at CA3-CA1 synapses to assess differences in long-term potentiation (LTP) during both the active and inactive phases. Results indicate that hippocampal cultures from PER2 luciferase HFD fed mice have a slightly shorter period than control animals and that PER2 protein expression revealed a loss of rhythmicity in the hippocampus of HFD fed mice compared to controls (cosinor analysis, $p < 0.05$ for CD and $p > 0.05$ for HFD). Additionally, we found that 2 weeks of TRF rescues day/night differences in HFD animals in spontaneous alternation as well as restores LTP to levels similar to those of control fed animals. In conclusion, it appears that HFD disrupts hippocampal

clock gene rhythms and spatial working memory. Moreover, restricting food access to the night can rescue synaptic plasticity as well as day-night differences in spatial working memory.

Disclosures: J. Davis: None. **S. Yates:** None. **J. Pollock:** None. **D. Pollock:** None. **M. Young:** None. **S. Bailey:** None. **K.L. Gamble:** None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

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Title: A single factor dominates the behavior of rhythmic genes in mouse organs

Authors: Y. CHENG[1], L. ZHANG[2], ***G.-Z. WANG**[1];
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Abstract: Circadian rhythms, regulated by both the internal and external environment of the body, are multi-scale biological phenomena with great complexity. At the molecular level, thousands of genes have been shown to exhibit rhythmic transcription, which are both organ-specific and species-specific, but what determines their rhythmicity remains unclear. Here we show that, for circadian rhythmic genes in mouse organs, there is a strong positive correlation between transcription level and rhythmic amplitude, and transcription level can explain more than 70% of the variation in amplitude. Our results further demonstrate that functionality and tissue specificity are not strong predictors of amplitude, and expression levels of rhythmic genes are linked to the energy consumed. This single determinant led us to assess the significance of rhythmic expression itself on the design of the transcriptional system. The rhythmical regulation of highly expressed genes can effectively reduce the energetic cost in transcription, facilitating the long-term adaptive evolution of the whole genetic system.

Disclosures: Y. Cheng: None. **L. Zhang:** None. **G. Wang:** None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

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Program #/Poster #: 410.16/V19

Topic: F.08. Biological Rhythms and Sleep

Support: 1R21AG050054
NSF1553067

Title: Circadian variation of neurometabolic activity in the prefrontal cortex: Effects of light and aging

Authors: *N. K. WALLACE, I. N. KARATSOREOS, M. SAVENKOVA, F. POLLARD; Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: Neural activity is energetically expensive, but neurons do not keep large reserves of energetic substrates. Thus, the support of neural activity requires that energy needs be met by dynamic interactions between neurons, glia, and blood vessels. One essential metabolic substrate in the brain is lactate, which is produced in astrocytes in response to increased neuronal activity and transported into the extracellular space for neuronal uptake. The goals of this study were twofold; the first goal was determine whether lactate metabolism in the prefrontal cortex (PFC) shows a circadian pattern, and to explore how environmental circadian desynchronization in young adults might affect it. The second goal was to probe how lactate metabolism was affected by normal aging, which is accompanied by blunted and destabilized circadian rhythms. Environmental circadian desynchronization was induced by exposure to a 20h light-dark (T20) cycle for 6 weeks. In all cases, Male, C57BL6/N mice were used. Adults in Control and T20 groups were 6-7 months old, while Aged mice were obtained from the NIA Aging colonies at 15-17 months old and tested at 19-20 months old. Mice were implanted with EEG, EMG (to monitor sleep), and a PFC cannula through which a lactate biosensor was inserted. Our results demonstrate that in Control mice, extracellular lactate in the PFC is rhythmic under both light-dark (LD) and constant dark (DD) conditions. In Aged mice, lactate rhythms in LD were similar to Controls, but significantly blunted in DD. In the T20 group, the PFC lactate rhythm was significantly reduced in amplitude and phase shifted with respect to the LD cycle. Further exploration revealed that the relationship between sleep state and lactate were modified under these conditions. In healthy mice, extracellular lactate increases during wake and REM sleep, when firing rates are high, and decreases during NREM sleep, when neural firing becomes less frequent. This relationship was blunted by T20. To probe underlying mechanisms, we examined expression of lactate-related transcripts in PFC using RT-qPCR. We found that T20 changed the pattern of expression of several genes related to metabolism and plasticity. Aging also changed expression of the enzymes that convert between lactate and pyruvate. While Control mice

showed a daily rhythm in the ratio of LDHA to LDHB expression, this rhythm was abolished in Aged mice. Our findings provide new information on the circadian control of neurometabolism under both normal and circadian desynchronized conditions. These findings point to a potential mechanism by which disrupted circadian clocks can affect neural function, and perhaps cognitive behaviors.

Disclosures: N.K. Wallace: None. **I.N. Karatsoreos:** None. **M. Savenkova:** None. **F. Pollard:** None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.17/V20

Topic: B.06. Synaptic Transmission

Support: Saint Louis University Start-Up Fund
President Research Fund, Saint Louis University
Beaumont Faculty Development Fund, Saint Louis University
Spark Microgrant, Saint Louis University
Sigma Xi Grant-in-Aid of Research

Title: The role of innexin genes in Lymnaea stagnalis

Authors: *B. MERSMAN, S. JOLLY, Z. LIN, F. XU;
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Abstract: Proper synapse formation is required for all brain functions, from simple reflexes to high cognitive tasks including learning and memory. However, most synaptic studies focus on the regulation and formation of chemical synapses, with advances in gap junction-mediated electrical synapse research lagging far behind. Even more, very little information is known about which genes contribute to proper electrical synapse formation throughout nervous system development. The objective of this study was to help shed light into the role of the *innexin* family, the genes coding for gap junction proteins, in the development of an invertebrate nervous system. To study genetic information at a single cellular level, we opted to utilize the mollusc *Lymnaea stagnalis* (*L. stagnalis*), whose physically large, well-defined neuronal networks and published genome allows for a unique opportunity to examine cell-specific expression of genes throughout electrical synapse formation in the developing *Lymnaea* brain. To begin characterizing *innexin*, eight isoforms of the gene, named *Inx1 - Inx8*, were cloned and sequenced. To do this, degenerate primers were created via homology studies of innexin in other invertebrates and utilized in RT-PCR. 3’ and 5’ rapid amplification of cDNA ends (RACE) was used to obtain a full reading frame of *Inx1*. The *Inx1* sequence was then entered in a whole

genome BLAST search to obtain seven other *innexin* isoforms. To study the localization of *innexin* at the cellular level, *in situ* hybridization experiments were completed with DIG-labeled probes targeting the *Inx1* transcript; these results revealed cell-specific localization to the plasma membrane in some known electrical synapse-forming cells while absent in others, implying genetic differences between functionally identical cells. To further confirm cellular differences in *innexin* expression, intron-spanning primers specific to each *innexin* isoform were designed for single cell qPCR. Throughout the central nervous system, electrical synapse-forming cells expressed various levels of each isoform, indicating the genetic specificity involved in proper synapse formation and validating the *in situ* hybridization results. A similar single cell qPCR experiment was then completed on *L. stagnalis* during a wide range of developmental time points; expression patterns of *innexin* isoforms changed with the development of the invertebrate. Taken together, our results indicate an evolving genetic makeup at the cellular level that contributes to proper electrical synapse formation over developmental time.

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.18/V21

Topic: B.04. Ion Channels

Support: NS067404-02
MDA to CKA

Title: Examination of oligodendrocyte connexin32 interactions with wild-type connexin47 and PMLD/SPG44 connexin47 mutants

Authors: G. DUNGAN, *M. FREIDIN, C. K. ABRAMS;
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Abstract: Connexins (Cx) are a family of homologous integral membrane proteins which form gap junctions. Homotypic cell-cell channels are composed of two identical connexon hemichannels, while in heterotypic channels the two hemichannels differ. Oligodendrocytes (Oligos) express Cx29, Cx32, Cx47. Recent functional and EM studies have shown O/O coupling via either Cx32/Cx32 or Cx47/Cx47 homotypic channels. Based on the lack of heterotypic coupling between Cx32 and Cx47 between transfected cells expressing either Cx32 or Cx47, heterotypic coupling in Oligos is unlikely. However, given the substantial overlap in Oligo expression patterns of Cx32 and Cx47, the possibility of functional interactions between these two remains. Astrocytes (As) express both Cx30 and Cx43. Using transfected cells, we and others have shown coupling between As and Oligos (A/O) is likely composed of Cx30/Cx32 and

Cx43/Cx47 heterotypic channels.
Mutations in the genes for human Cx47 (GJC12) and Cx32 (GJB1) lead to neurologic diseases. Mutations in GJC12 cause a severe early onset central nervous system dysmyelinating disorder, Pelizaeus-Merzbacher-Like disease (PMLD1 or HLD2 and the milder, later onset disorder, Hereditary Spastic Paraplegia (SPG44). Mutations in GJB1 are associated the demyelinating peripheral neuropathy, CMT1X. In these disorders, interactions between mutant forms of Cx47 and wild-type Cx32 may contribute to the disease phenotypes. Mice lacking either Cx32 or Cx47 show minimal CNS abnormalities while mice lacking both connexins show a florid and severe CNS phenotype. Thus, it is unlikely that simple loss of function in patients with mutations in Cx32 or Cx47 is sufficient to explain CNS phenotypes. Some or all of the CNS manifestations may arise as a result of pathological interactions between Cx32 WT and mutant forms of Cx47 or between mutant forms of Cx32 and Cx47 WT. We used dual whole cell recordings, immunofluorescence colocalization, and co-immunoprecipitation to evaluate evidence for heteromeric interactions between WT Cx32 and WT Cx47. We use the same techniques to further evaluate whether four mutant forms of Cx47 associated with PMLD1 and SPG44 show interactions with Cx32 which differ from those between WT Cx47 and WT Cx32.

Disclosures: **G. Dungan:** None. **M. Freidin:** None. **C.K. Abrams:** None.

Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.19/V22

Topic: B.06. Synaptic Transmission

Support: R01 NS 31224-24 (JPW)
R01 NIH R01 NS081248 (KUB)

Title: Electrical coupling and NMDA dependent strengthening of electrical synapses in the mouse inferior olive depend on CaMKIIa

Authors: *H. E. SPEED[1], Z. ZHU[1], A. SHARDARBEKOVA[1], S. COULTRAP[2], K. BAYER[2], J. WELSH[1,3];
[1]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA; [2]Pharmacol., Univ. of Colorado - Denver, Denver, CO; [3]Pediatrics, Univ. of Washington, Seattle, WA

Abstract: Subthreshold membrane oscillations in the inferior olive (IO) drive climbing fiber input to the cerebellum and are critical for the timing of coordinated movement. Connexin36 (Cx36) proteins form gap junctions that allow for electrical coupling between IO neurons and synchronous changes in membrane potential. We have previously shown that NMDA receptors (NMDARs) are bidirectional modulators of electrical coupling and that the NMDAR subunit,

GluN1, collocates with Cx36 at gap junctions. Although a shunting effect of NMDAR activation, due to increased input conductance (Gin) and decreased junctional conductance (Gj), may contribute to reductions in electrical coupling, the potentiating effect of NMDAR activation on electrical transmission is mediated by CaMKII-dependent mechanisms. Supporting this hypothesis, we show that the strength of electrical coupling among IO neurons, as measured by the coupling coefficient (CC), is decreased in a floxed CaMKIIα knockout (KO) transgenic mouse as compared to WT (C57BL/6J). We crossed CaMKIIα KO mice with a second mouse line expressing cre under the ptf1a promoter, resulting in a tissue-specific genetic rescue of CaMKIIα activity in the IO. This restored CC to within WT levels (WT: 0.59 ± 0.16%, N = 28; KO: 0.13 ± 0.04%, N = 22; Rescue: 0.83 ± 0.39%, N = 6, mean ± SEM). Likewise, Gj was decreased between connected pairs from KO mice and was restored to WT levels in the IO-specific rescue (WT: 58.38 ± 12.89 pS; KO: 24.66 ± 10.02 pS, Rescue: 51.53 ± 15.69 pS). IO neurons from KO mice depolarized in response to bath application of 5 µm NMDA, indicating the NMDA receptors were present and active. However, CC was largely unaffected by NMDA in neurons from KO mice (Strengthened: N = 2/17, Weakened: N = 2/17). WT neurons showed mixed responses to NMDA (Strengthened: N = 4/20; Weakened: N = 12/20), and neurons from Rescue mice exhibited robust potentiation of CC (Strengthened: N = 3/3). Subthreshold oscillations in membrane potential were less prevalent in KO mice (WT: 87.9%, KO: 23.8%, Rescue: 61.5%), but the amplitude (WT: 2.55 ± 0.21 mV, N = 45; KO: 2.07 ± 0.38 mV, N = 16; Rescue: 7.65 ± 2.13 mV, N = 15) and frequency (WT: 4.95 ± 0.36 Hz; KO: 4.92 ± 0.34 Hz; Rescue: 6.25 ± 0.90 Hz) of those neurons that did oscillate were typical. We conclude that the basal level of electrical coupling among IO neurons and its strengthening by NMDA receptor activation are dependent on CaMKIIα.

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.20/V23

Topic: B.06. Synaptic Transmission

Support: CONACyT- Mexico, grant num 127658
Facultad de Medicina, UNAM, División de Investigación

Title: Neurons and satellite glial cells of the superior cervical ganglia express Connexin 36

Authors: *E. M. PEREZ-ARMENDARIZ[1], L. CRUZ-MIGUEL[2], B. HERNÁNDEZ-TELLEZ[2], A. CASTELL-RODRÍGUEZ[2], E. RIOS-MUÑIZ[2];
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Mexico; [2]Dept. de Biología Celular y Tisular, Univ. Nacional Autónoma de México, Mexico City, Mexico

Abstract: Connexins (cxs) belong to a family of transmembrane homologous proteins that form intercellular channels. Each intercellular channel is formed by two hemichannels or connexons. Depending on the connexin subtype hemichannels may be functional. The superior cervical ganglia (SCG) is the only ganglion in the sympathetic nervous system that innervates the head and neck, where it innervates many organs, glands and parts of the carotid system. SCG are formed by neuronal units surrounded by satellite glial cells and nerve fibers. Electrical coupling among satellite glial cells in SGG. In addition, incidence of coupling was enhanced by Ach. Moreover, electrical coupling between SCG cultured neurons from neonatal rats has been described. However, the molecular substrate for these cell-cell interactions are unknown. Recently, we found that both satellite glial cells and neurons express connexin 36 in dorsal root ganglia. Here we asked whether cervical superior ganglia also express this gap junction protein. For this, cervical superior ganglia rat sections were stained with a previously characterized anti-Cx36 antibody and analyzed by immunohistochemistry. We found that all neurons in SGCs express Cx36. Their levels of expression were similar. Cx36 was also found at membranes of nerve fibers. Satellite glia cells also express Cx36. SGC sections incubated only with secondary antibody were not stained with anti-Cx36. The subcellular distribution of this protein is presently analyzed by inmunolabeling techniques as well as the expression of Cx36 mRNA using qRTPC. In summary, we provide evidence that Cx36 channels may intercommunicate SCG cells.
Acknowlegments: GRANT number CONACyT 127658

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Poster

410. Circadian Aspects of Sleep and Gap Junctions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 410.21/V24

Topic: B.06. Synaptic Transmission

Support: NIMH MH46742
NIH 2T32GM008396-26

Title: Loss of synchronous activity across gap junctions results in a phase-dependent change in coupling conductance magnitude

Authors: *D. R. KICK[1], D. J. SCHULZ[2];
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Abstract: Many rhythms necessary for survival (e.g. chewing, breathing) require precise coordination between the neurons which generate them. In the Cancer borealis cardiac ganglion (CG) motor neuron coordination is facilitated by gap junction mediated electrical coupling. Altered coupling has been shown to be a component of homeostatic compensation, and a product of tetanic nerve stimulation or evoked spiking. However, the salient electrophysiological signal driving gap junction modification has not been determined.
Here, we use two electrode voltage clamp to alter the synchrony and total depolarization of two CG motor neurons which are entirely synchronous at baseline. We separate the effect of desynchronization between neurons from that of depolarization by introducing a phase shift or aberrantly depolarizing the two clamped neurons. We tested phase angles of 0°, 22.5°, 45°, 90°, (0, 1/16th, 1/8th, or 1/4th the period) and increased the amplitude and duration of depolarization, with and without a 22.5° delay.
Using a resampled linear model we find coupling resistance changes due to an interaction of phase and time (empirical p (ep) &lt 0.001). Furthermore, the time course and directionality of the effect appears to change with phase, 22.5° resulting in a decrease of the median delta of -0.6 megaohms (MΩ) by 20 minutes whereas 90° manifested increase in the median delta of +0.5 MΩ by 60 minutes. Our results suggest there may be a cost to network output that is non-linear with respect to phase angle - minimized by increasing coupling when the difference between activity is slight and decreasing when it passes a threshold.
To confirm that the effects seen are due to desynchronization, we compared the effect of two phase angles (0° and 22.5°) with and without elevated depolarization. We find elevated depolarization has no effect on coupling resistance (ep &gt 0.9), whereas phase does (ep &lt 0.01). Taken in conjunction with the above, we conclude that desynchronization rather than depolarization per se induces modulation of coupling strength. While the gap junctions are unaffected, depolarization amplitude appears to alter coupling indirectly by altering membrane resistance over time (ep &lt 0.01).
We extend these findings by using dynamic clamp to assess the consequences of coupling strength and desynchronization to motor neuron output. We achieve this by artificially coupling motor neurons of two active CGs, permitting full control over coupling conductance and a wide range of phase angles. Taken together our work suggests a specific signal which drives coupling change and suggests why non-linear regulation with respect to desynchronization may occur.

Disclosures: D.R. Kick: None. **D.J. Schulz:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.01/V25

Topic: G.01. Appetitive and Aversive Learning

Support: DFG CRC 1193
DFG SPP 1665
Max Planck Society

Title: A critical role for neocortical processing of threat memory

Authors: *T. DALMAY[1], E. ABS[1], R. B. POORTHUIS[1,2], S. ONASCH[1], Y. R. LOZANO[3], P. TOVOTE[3], J. GJORGJIEVA[1,4], J. J. LETZKUS[1];
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Abstract: Memory for cues associated with threat is critical for survival, and a leading model for elucidating how sensory information is linked to adaptive behavior by learning. While the brain-wide circuits mediating auditory threat memory have been intensely investigated, it remains unclear whether auditory cortex is critically involved. Here, we address the role of auditory cortex and the adjacent temporal association area (TeA) in auditory threat memory using a combination of optogenetic activity manipulations in defined cortical areas and output pathways, viral tracing, pathway-specific *in vivo* 2-photon calcium imaging, and computational analyses of population plasticity. Intriguingly, our results reveal that for both acquisition and retrieval of threat memory, auditory cortex is selectively required for memory to complex stimuli, while stimulus processing in TeA is vital for all forms of cued threat memory. Additionally, we found that inhibition of ventral auditory cortex and TeA resulted in much stronger memory retrieval deficits than inhibition of primary auditory cortex. Retrograde tracing revealed that this medio-temporal impact gradient is paralleled by the organization of projections to the lateral amygdala (LA), an area known to be crucial for threat memory, with the majority of amygdala-projecting neurons in temporal neocortex located in TeA. Consistent with a critical role for information transfer from temporal neocortex to the amygdala in memory retrieval, inhibition of temporal neocortex axon terminals in the amygdala produced a robust retrieval deficit. Finally, *in vivo* 2-photon calcium imaging specifically of amygdala-projecting neurons in auditory cortex before and after threat conditioning revealed that this projection pathway governs threat memory expression through a balanced form of population plasticity selectively supporting the discrimination of salient sensory stimuli. In summary, our results demonstrate a striking impact of CS complexity on the role of auditory cortex, and identify temporal neocortex and its projection to the LA as critical substrates for acquisition and expression of auditory threat memory. Information transfer from temporal neocortex to the LA thus plays a critical role in cued threat memory.

Disclosures: **T. Dalmay:** None. **E. Abs:** None. **R.B. Poorthuis:** None. **S. Onasch:** None. **Y.R. Lozano:** None. **P. Tovote:** None. **J. Gjorgjieva:** None. **J.J. Letzkus:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.02/V26

Topic: G.01. Appetitive and Aversive Learning

Support: KAKENHI 18H05213
KAKENHI 19H04994

Title: Cell assemblies representing contextual emotional memory in the basolateral amygdala

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Abstract: The basolateral amygdala (BLA) is required for the expression of fear learning and memory, but it is not clear how contextual emotional memories are represented in BLA neurons. Recent recordings of neural ensembles in rats learning the location of an aversive air puff in a linear track have revealed coordinated reactivations of hippocampal-BLA cell pairs during sleep following learning [1]. Sequential firing of neuronal ensembles plays an active role in hippocampal memory processing, but it is not known whether sequential firing of cell assemblies exists in the BLA and, if so, whether this represents any aspect of contextual emotional memory. By using edit distance [2], a metric in computer science to measure similarity between two strings, we have recently developed a machine learning method to detect the recurring firing patterns of neuronal ensembles. With this method, we identified subgroups of BLA neurons that are repeatedly activated in fixed sequential firing patterns near the location of air puff. The recurring activation was stronger for the run direction involving the air puff than for the "safe" direction where no air puff was presented. Moreover, when the location of the air puff was changed and animals learned the new location, the reactivation of the previous subgroup of BLA neurons largely decreased, while a different subgroup became activated over the course of training at the new air puff location. These results suggest that contextual emotional memory is encoded as sequential firing of cell assemblies in the BLA.
[1] G. Girardeau, I. Inema and G. Buzsáki, Nat Neurosci 20, 1634-1642, 2017. [2] K. Watanabe, T. Haga, D. R. Euston, M. Tatsuno, T. Fukai, bioRxiv 202655.

Disclosures: K. Watanabe: None. **G. Girardeau:** None. **G. Buzsaki:** None. **J.P. Johansen:** None. **T. Fukai:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.03/V27

Topic: G.01. Appetitive and Aversive Learning

Support: VA RR&D Career Development Award (CDA-2, RX-001479-01A2)
NIH/NINDS (1RO1-NS-101108-01)
DoD/CDMRP ERP (W81XWH-15-ERP-IDA)

Title: TBI increases gamma in amygdala-prefrontal cortex circuitry and impairs extinction of fear memories

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Abstract: Traumatic Brain Injury (TBI) has been implicated in memory deficits and anxiety disorders such as Post-Traumatic Stress Disorder (PTSD), and is commonly seen in Veterans returning from combat. To investigate the effect of TBI on substrates of fear memory, we subjected rats to a lateral fluid percussion injury (FPI) and chronically implanted high-density silicon electrodes in the basolateral amygdala (BLA) as well as bipolar electrodes in medial prefrontal cortex (mPFC) and ventral hippocampus (vHC). We recorded during an anxiety and fear behavior paradigm 2 weeks post injury using elevated plus maze (PM), open field (OF), and fear conditioning (FC). The FC paradigm consisted of acquisition, where rats learned to associate white noise (CS+) with a mild footshock, followed by two days of extinction, where rats were presented the white noise and a novel tone (CS-) without the footshock. Although we found no difference in anxiety behaviors and fear acquisition between FPI and sham surgery rats, FPI animals extinguished fear at a slower rate and showed a higher fear response on day 2 of extinction. We tested an additional cohort of rats 6 months post injury. Sham and FPI rats at 6 months showed no difference in the rate of fear acquisition, but both groups were faster compared to rats tested at 2 weeks. FPI animals at 6 months also had slower fear extinction than the 6 month sham animals, and both groups extinguished fear slower than 2 week FPI rats, suggesting an aging effect. In the 2 week animals, we found that FPI led to an increase in the power of baseline gamma oscillations (45-110Hz) in BLA, which further increased following FC in comparison with sham animals. During extinction, this increased local BLA gamma began to return to baseline levels. BLA gamma is thought to arise from interactions of interneurons with pyramidal cells, so this increase may result from a compensatory interneuronal response to increased pyramidal cell activity. Additionally, FPI led to an increased mPFC-BLA gamma

coherence post FC, which also lowered during extinction, and an increase in phase-amplitude coupling of BLA gamma to low frequencies in mPFC, which shifted to theta after acquisition. These changes to the amygdala-prefrontal interactions could represent dysfunction in top-down extinction pathways, leading to impaired extinction. We also have mirrored our paradigm in a pig model, using heart rate as an indicator of fear state. We found that heart rate changes during extinction follows the traditional extinction curve during and between trials. Combined with future recordings from these animals, this paradigm will help present a thorough elucidation of the limbic circuitry affected by TBI and PTSD.

Disclosures: M.D. Sergison: None. **C.D. Adam:** None. **C. Cottone:** None. **N. Maheshwari:** None. **A.V. Ulyanova:** None. **H.I. Chen:** None. **V.E. Johnson:** None. **J.A. Wolf:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.04/V28

Topic: G.01. Appetitive and Aversive Learning

Support: the National Natural Science Foundation of China (no. 81701312 and 81521063)

Title: A projection from ventral hippocampus to basolateral amygdala is involved in the destabilization of remote fear memory

Authors: *Y. HAN[1], J. SHI[2], L. LU[3];
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Abstract: Background: Fear response often occurs after reactivation of fear memories by exposure to unconditioned stimulus (UCS) or fear-associated conditioned stimuli (CSs). Fear memories that were reactivated by fear-associated CSs undergo reconsolidation and interfering with this reconsolidation inhibit subsequent fear response. Our previous study demonstrated the efficacy of a novel UCS-based memory reconsolidation interference procedure for inhibiting nicotine craving induced by exposure to diverse nicotine-associated CSs and nicotine itself. In present study we intend to investigate the neural circuits underlying the destabilization after UCS-based retrieval. **Methods**: Brain activity was assessed by c-fos immunofluorescence after CS or UCS retrieval 1 d or 14 d after fear conditioning. Designer receptors exclusively activated by designer drugs (DREADDs), anisomycin or propranolol were used to explore the involvement of ventral hippocampus (vHipp) and basolateral amygdala (BLA) in the reconsolidation of recent and remote fear memory. **Results**: We found that CS and UCS retrieval induce different patterns of brain region activation and this pattern change with time. Both CS and UCS retrieval 1 d after learning induced the activation of prelimbic cortex (PrL), dorsal hippocampus and BLA.

Fourteen days after conditioning, CS retrieval activated PrL and infralimbic cortex, while UCS retrieval activated PrL, vHipp, BLA and central amygdala. Propranolol microinjections into the BLA or anisomycin microinjections into the vHipp disrupted the UCS retrieval-mediated reconsolidation of remote fear memory, and this memory impairment effects persisted for at least two weeks with no recovery after reminder shock. Moreover, inactivation of vHipp, but not PrL projections to BLA by DREADDs after UCS retrieval impaired remote fear memory.
Conclusion: Our results suggest that a projection from ventral hippocampus to basolateral amygdala may be involved in UCS retrieval-induced reconsolidation of remote fear memory.

Disclosures: Y. Han: None. **J. Shi:** None. **L. Lu:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.05/V29

Topic: G.01. Appetitive and Aversive Learning

Support: KIST Grant 2E29180
Samsung Science and Technology Foundation 2I23560

Title: Fear induced synaptic reorganization in the BLA-mPFC connectivity

Authors: *J. SONG, J. KIM;
Ctr. for Functional Connectomics, KIST Korea Inst. of Sci. & Tech., Seoul, Korea, Republic of

Abstract: The neural connection between the basolateral amygdala (BLA) and the medial prefrontal cortex (mPFC) is important for the acquisition and extinction of fear. Previously, it is demonstrated that fear conditioning results in synaptic changes of mPFC neurons, such as the elimination of individual spines and alterations in the synaptic efficacy (1,2). However, detailed descriptions of input- and cell type-specific changes in single neurons remains elusive.
In this study, taking advantage of our newly developed mGRASP as a synapse detector, we sought to delineate the fear-induced cell-type-specific changes in the BLA→mPFC synaptic connectivity. We found that fear conditioning induces layer- and cell type-specific decreases in the anatomical measures of the BLA→mPFC synaptic connectivity.Our observation suggests that the cell-type- and location-dependent differences in synaptic decreases in the BLA→mPFC connectivity may result in the changes of net E/I balance in the mPFC microcircuits. We further investigated the changes in the synaptic connectivity in the reciprocal projection (i.e. mPFC→BLA) and discuss its implication in fear-induced responses.
1. Lai et al. "Opposite effects of fear conditioning and extinction on dendritic spine remodelling." *Nature* 483.7387 (2012): 87.

2. Klavir et al. "Manipulating fear associations via optogenetic modulation of amygdala inputs to prefrontal cortex." *Nature neuroscience* 20.6 (2017): 836.

Disclosures: J. Song: None. **J. Kim:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.06/V30

Topic: G.01. Appetitive and Aversive Learning

Support: FAPESP 2016/13027-2
FAPESP 2016/25755-2
AFIP
CNPq

Title: Functional disconnection between ventral hippocampus and prelimbic cortex impairs the acquisition of contextual fear conditioning with temporal discontinuity

Authors: *T. B. DOS SANTOS, A. E. WALLAU, J. C. K. SOARES, M. M. DE OLIVEIRA; Psychobiology, Univ. Federal de São Paulo, Sao Paulo, Brazil

Abstract: A previously experienced context can be associated separated by a time interval of 5s from a posterior aversive stimulus. This task was called contextual fear conditioning with temporal discontinuity (CFC-5s) and differentially required prelimbic cortex (PL) activity. However, it is unclear the effect of this time factor in the interplay among brain regions to form the memory trace. Because there are ascending ipsilateral connections from ventral CA1 hippocampus (CA1v) to PL; PL is related to the maintenance of stimuli over time and CA1v to contextual fear conditioning (CFC), their functional interaction could be necessary for the acquisition of CFC-5s, and so, in a distinctive manner compared to delay associations, as standard CFC. We investigated the effect of pharmacological functional disconnection of CA1v and PL in CFC-5s and CFC tasks. Male Wistar rats of 11-week-old were submitted to ipsi or contralateral cannula implantation in CA1v and PL, counterbalanced by side. After 1-week recovery, rats were infused with muscimol (IPSI or CONT groups) or PBS (PBS group, counterbalanced) 30min before training. In CFC-5s task, rats were exposed for 5min to the context and then allocated in transition boxes for 5s. Next, they were placed in the context receiving one footshock immediately (0.8mA, 1s). In CFC task, rats received a footshock after 5min in the context. Test was performed 48h later and freezing responses were measured during 5min. Rats were submitted 7d later to re-training and 48h later to re-test, similarly to the described, but without any infusions. Data were analyzed by Generalized Estimating Equations adjusted for Gamma distribution followed by Bonferroni pairwise contrast. In CFC-5s, PBS and

IPSI groups had significantly higher freezing responses in test compared to their values in training and higher freezing responses compared to CONT group in test. In CFC task, all groups had significantly higher freezing responses compared to their values in training and none differed in the test. There was no difference between groups in re-training or in re-test in both tasks, indicating that the impairment observed was due the disconnection. Time affects the neurobiology of CFC. The functional connection between CA1v-PL is necessary for acquisition of CFC-5s, but not CFC.

Disclosures: T.B. dos Santos: None. **A.E. Wallau:** None. **J.C.K. Soares:** None. **M.M. de Oliveira:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.07/V31

Topic: G.01. Appetitive and Aversive Learning

Support: NIMH Grant R01MH112141

Title: Distinct roles of the anterior and posterior retrosplenial cortices in encoding, but not retrieval, of trace fear memory

Authors: *S. TRASK[1], S. E. PULLINS[2], F. J. HELMSTETTER[3];
[1]The Univ. of Wisconsin - Milwaukee, Milwaukee, WI; [2]Psychology, Univ. of Wisconsin - Milwaukee, Milwaukee, WI; [3]Deptartment of Psychology, Univ. of Wisconsin Milwaukee Dept. of Psychology, Milwaukee, WI

Abstract: The retrosplenial cortex (RSC) has a prominent role in memory and has dense reciprocal connections with other brain regions important for memory formation and retrieval including the hippocampus and prefrontal cortex. Trace fear conditioning, in which a neutral conditional stimulus (CS) is followed by a brief stimulus-free interval before a footshock (the unconditional stimulus or UCS), is a powerful method used to study memory in rats and can be separated into encoding (e.g., original learning) and retrieval (later performance to the CS) phases. Importantly, this type of learning incorporates information about the environment in which learning takes place ("where" information) with information about the CS ("what" information). While most research on encoding and retrieval of trace fear memory has focused on the hippocampus and prefrontal cortex, recent data has begun to suggest a critical role for the RSC in trace memory encoding. In two experiments, we tested the hypothesis that the posterior region of the retrosplenial cortex (pRSC, which is closely interconnected with the hippocampus) would have a selective role in both encoding and retrieval of context-related information while the anterior portion (aRSC) would be important for processing event-related (e.g., CS-UCS)

information. Using optogenetics, either the aRSC or pRSC was silenced precisely during the CS-UCS period on training trials during memory encoding (Experiment 1) or during the CS period of retrieval testing (Experiment 2). In Experiment 1, we found that silencing the aRSC during acquisition resulted in a selective impairment in responding to the CS during retention testing, whereas silencing the pRSC during acquisition resulted in impaired responding to the training context. However, in Experiment 2 silencing of either region resulted in impaired CS freezing. Preliminary data quantifying immediate early gene (e.g., zif268) expression in RSC support effective and anatomically selective inhibition of neural activity. Together, these results suggest that while encoding of trace fear conditioning relies on distinct "where" and "what" circuits, both regions are needed later expression of that memory. While the role of the retrosplenial cortex has been demonstrated in other paradigms examining contextually-bound learning, these results suggest distinct roles for the anterior and posterior regions of the retrosplenial cortex in the encoding, but not retrieval, of trace fear memory.

Disclosures: S. Trask: None. **F.J. Helmstetter:** None. **S.E. Pullins:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.08/V32

Topic: G.01. Appetitive and Aversive Learning

Support: Whitehall Foundation Research Grant 2014-08-67
National Science Foundation IOS:1558121

Title: Ventral hippocampus dynamically regulates encoding of shock-related information in the basolateral amygdala during trace fear conditioning

Authors: *M. R. HERBST[1], R. C. TWINING[1], M. R. GILMARTIN[2];
[2]Biomed. Sci., [1]Marquette Univ., Milwaukee, WI

Abstract: The association of a neutral conditional stimulus (CS) and aversive footshock unconditional stimulus (UCS) in fear conditioning critically depends on the amygdala. However, if the CS and UCS are separated in time, additional brain areas are needed, including the prelimbic cortex (PL) and the ventral hippocampus (VH). We have identified a putative role for the PL in providing sustained firing during the CS-UCS interval (Gilmartin & McEchron, 2005; Gilmartin et al., 2013); however, the contribution of the VH to memory formation and learning-related neuronal firing remains unclear. Here, we first characterized neuronal responses in the VH during trace fear conditioning (TFC) using *in vivo* electrophysiological recordings of area CA1 of the VH. The majority of recorded VH neurons (90%) encoded the shock UCS, with the predominate response being a sustained increase in neuronal spiking (72%). Since the VH sends

direct monosynaptic connections to multiple targets required for trace fear memory, these results suggest that the VH may provide shock-related information to these structures, perhaps in parallel. We have shown previously that input from the VH to the PL during trace fear conditioning is needed for contextual, but not cued, fear memory. In the current study we silenced VH terminals in the basolateral amygdala (BLA) in rats using projection targeting optogenetics (AAV-ArchT) while simultaneously recording BLA neurons during TFC. Silencing occurred briefly during each CS-UCS pairing, beginning 20-sec prior to CS onset and terminating 20-sec after UCS offset. In controls, the shock caused predominantly sustained increases in BLA neuronal spiking (35.4% excitatory; 6.3% inhibitory). However, silencing VH input to BLA during these trials not only reduced shock-related excitatory encoding (8.3%) but, in some cases, inverted it (47.2% inhibitory). The loss of VH input to BLA led to deficits in contextual fear and increased fear to the cue in a shifted context. These initial findings suggest that VH modulates the encoding of shock information in the BLA during training to differentially affect cued and contextual fear.

Disclosures: **M.R. Herbst:** None. **R.C. Twining:** None. **M.R. Gilmartin:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.09/V33

Topic: G.01. Appetitive and Aversive Learning

Support: Whitehall Foundation Research Grant 2014-08-67
National Science Foundation IOS:1558121
The Charles E. Kubly Mental Health Research Center

Title: Prefrontal neuronal encoding of threat-predictive cues across the estrous cycle

Authors: M. LAVIOLA, M. HERBST, R. C. TWINING, ***M. R. GILMARTIN**;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: The association of a neutral conditional stimulus (CS) and aversive footshock unconditional stimulus (UCS) that are separated in time, as in trace fear conditioning, requires activity in the prelimbic area (PL) of the medial prefrontal cortex. We have previously shown that a subset of PL cells shows sustained firing in response to the CS and that optogenetic silencing of prefrontal activity during the trace interval between the cue and shock prevents learning (Gilmartin & McEchron, 2005; Gilmartin et al., 2013). Recently, we have uncovered sex differences in the prefrontal cortical contribution to trace conditioning (Kirry et al., 2018; 2019). In one study, the estrous cycle gated the memory-impairing effects of a muscarinic antagonist in the PL (Kirry et al., 2019), which suggested that circulating ovarian hormones may

modulate prefrontal encoding during aversive learning. Here we recorded neuronal activity in the medial prefrontal cortex during the acquisition and extinction of trace fear conditioning. The estrous cycle of female Long-Evans rats was tracked for two cycles and then half of the rats started training on the day of proestrus and the other half started training on the day of metestrus. Training occurred over two days and testing in a shifted context occurred when the rats returned to their initial training cycle stage. Initial results (n = 3/group) revealed similar sustained activation to the CS but divergent encoding of the UCS during day 1 of training. Proestrus females exhibited a robust increase in firing in response to the UCS, and metestrus females exhibited a modest response and even a decrease in firing. Neuronal encoding on day 2 of training and subsequent conditional fear to the cue and context at test was similar between groups. Follow-up experiments will determine whether these divergent patterns of PL encoding of the UCS mediate cycle differences in cued fear retention after only one day of training, that can be overcome with additional training. These findings will reveal how prefrontal encoding of threat-related stimuli does and does not change across the estrous cycle, shedding light on how neuromodulation of prefrontal activity differentially affects the formation of fear memories between sexes and across the estrous cycle.

Disclosures: **M.R. Gilmartin:** None. **R.C. Twining:** None. **M. Herbst:** None. **M. LaViola:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.10/V34

Topic: G.01. Appetitive and Aversive Learning

Support: National Science Foundation IOS:1558121
Whitehall Foundation Research Grant 2014-08-67

Title: Silencing afferent input to prefrontal cortex during trace fear conditioning

Authors: *R. C. TWINING, A. J. KIRRY, K. LEPAK, M. R. GILMARTIN;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: The prelimbic medial prefrontal cortex (PL) receives afferent input from the amygdala, insula, mediodorsal thalamus (MD), and the ventral hippocampus (VH). It is an integral component of a neural system that mediates cognitive and emotional processes and regulates a wide range of adaptive behaviors (Hiser and Koenigs, 2018). In humans, dysfunction of the mPFC and its functional connections is associated with anxiety disorders and post-traumatic stress disorder. In animal models, the mPFC is critical for cognitive and emotional aspects of conditional fear expression, extinction, and renewal, the encoding of aversive

locations, prospective or temporal encoding, and spatial working memory (Ramanathan et al., 2018; Marek et al., 2018; Sharpe and Killcross, 2015, Spellman, 2015; Sotrez-Bayon, 2012); however, it is not required during acquisition of standard fear conditioning when an auditory conditional stimulus (CS) co-terminates with a shock unconditional stimulus (UCS). The PL is required for the acquisition of cued fear only when a temporal gap or trace interval is imposed between the CS and UCS. Indeed, approximately 30% of PL neurons exhibit sustained increases in learning-related neuronal spiking that bridges the trace interval (Gilmartin & McEchron, 2005) and optogenetic silencing of this activity blocks the formation of a trace fear memory (Gilmartin, 2013). While the PL mediates cued and contextual fear during TFC, it is unknown which afferent inputs support learning or learning-related neuronal spiking. Given the similar density of monosynaptic input to PL from the VH and MD and their demonstrated importance for spatial working memory, we hypothesized that either or both inputs would be required for trace fear memory and might regulate learning-related PL activity. Here we injected AAV9/CAG-ArchT-GFP into the VH or MD (750 or 500 nl/side) and implanted optic fibers 0.5 mm above the terminals in PL or cell bodies in the MD. We silenced during a 2-trial TFC protocol and repeated for 3 days. Cued and contextual fear was tested without silencing after each 2-trial block. So far, we have shown that silencing VH-PL impaired context fear but could be restored if the VH-PL was silenced again during retrieval. Interestingly, monosynaptic VH input to PL is not required for cued fear. Therefore, despite the dependence of cued fear on both the PL and VH, either indirect or parallel afferents mediate trace fear memory formation. Since the MD inputs sustain PL neuronal activation across temporal delays during spatial working memory tasks (Bolkan, 2017), the ongoing study will determine the extent to which trace fear memory depends on MD neuronal activity and its inputs to PL.

Disclosures: **R.C. Twining:** None. **A.J. Kirry:** None. **M.R. Gilmartin:** None. **K. Lepak:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.11/V35

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant GM109817 awarded to AMK
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NIH Grant MH114961 awarded to AMP
NIH Grant – UTEP RISE Program GM069621

Title: Atlas-based mapping of Fos-immunoreactive neurons in the medial prefrontal cortex and ventral hippocampus in association with context fear in the juvenile and adult male rat

Authors: *A. PINEDA SANCHEZ[1], A. ENRIQUEZ[1], K. NEGISHI[1], A. J. SANTARELLI[2], A. M. KHAN[1], A. M. POULOS[2];
[1]Biol. Sci., Univ. of Texas at El Paso, El Paso, TX; [2]Psychology, State Univ. of New York, Univ. At Albany, Albany, NY

Abstract: The hippocampus and the medial prefrontal cortex (mPFC) are important brain regions for contextual fear retrieval in rats. We have reported (Santarelli *et al*., 2018; *Neurobiol Learn Mem*) greater numbers of Fos-immunoreactive (Fos-ir) neurons in the juvenile mPFC and adult ventral hippocampus (vH) for rats that acquired a contextual fear response, which was not evident in infants (P19). Here, we focused on atlas-based mapping for the precise spatial distributions of these Fos-ir patterns. Two-channel immunofluorescence images of Nissl-stained tissue immunolabeled for Fos-ir were examined in juvenile and adult rats for one of three different behavioral conditions: home cage and non-shock controls; or a delayed shock experimental group. Nissl cytoarchitecture was parcellated, assigned locations within a rat brain atlas (L. W. Swanson (2018) *Brain Maps 4.0*, *J Comp Neurol*) to plot mPFC and vH Fos-ir patterns, and the boundaries established from the Nissl channel overlaid onto the second channel showing Fos-ir. Each Fos-ir neuron was then annotated onto a data layer and the final drawings transferred to the corresponding atlas templates. For Fos-ir neuronal distributions in the vicinity of the mPFC, we identified Swanson atlas level 8 gray matter structures as harboring significant numbers of neurons, especially along medially located subregions of the mPFC (dorsal part of the anterior cingulate area, and prelimbic and infralimbic areas), as well as olfactory-associated regions, orbitofrontal subregions, tenia tecta, and claustrum. For the ventral hippocampus, robust Fos-ir neurons were identified across groups at atlas level 39, specifically within dorsal and ventral aspects of the subiculum, CA1, and CA3 regions; and within the dentate gyrus. The careful delineation of the Fos-ir neuronal distributions in these animals is a critical step for our ongoing analysis of neuronal activation associated with context fear across developmental ages and will facilitate detailed quantitative analyses across behavioral conditions in the near future. These data can then, in turn, be contextualized in relation to other datasets that have also been mapped within the same spatial reference.

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Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

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Program #/Poster #: 411.12/V36

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01MH114961-01

SUNY Start up funds

Title: Inactivation of ventral hippocampus and medial prefrontal cortex in juvenile and adult rat during context fear retrieval

Authors: A. J. SANTARELLI[1], L. M. COLON[2], ***A. M. POULOS**[3];
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Abstract: In adult rodents the ventral hippocampus (vH) and medial prefrontal cortex (mPFC) contribute to the retrieval of contextual fear memories. However, in juvenile rats it remains to be known about the relative contributions of these regions in contextual fear conditioning. In the current study, we sought to determine the necessity of the vH and mPFC in the retrieval contextual fear memories in juvenile and adult Long-Evans male rats by temporary inactivation of these regions. During surgery, bilateral cannulae were stereotaxically positioned within vH or mPFC. Following recovery, juvenile and adult rats underwent standard contextual fear conditioning procedures. Prior to testing of context fear, GABA-A agonist, muscimol or saline were infused within vH or mPFC. Our preliminary results indicate a differential effect of infusion upon age and site of infusion on observed freezing during context fear testing. These results may suggest that there may be age-dependent differences in the functional contribution of vH and mPFC in contextual fear conditioning.

Disclosures: A.J. Santarelli: None. **L.M. Colon:** None. **A.M. Poulos:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.13/V37

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01MH114961-01
SUNY Start up funds

Title: Neuronal tract tracing of developmental fear-related neural circuitry in male and female rats

Authors: *N. ODYNOCKI, L. M. COLON, A. M. POULOS;
Univ. at Albany State Univ. of New York, Albany, NY

Abstract: Much of our current understanding of the neural circuits in context fear conditioning has been established in the adult male rat. The basolateral amygdala complex (BLA) and the

major sources of its afferents - the medial prefrontal cortex (mPFC) and CA1 region of the ventral hippocampus (vCA1) - represent a major portion of the neural circuit underlying the processing, storage and retrieval of conditional fear responses. We and others have previously demonstrated in developing male rats that the connectional and functional status of many of these afferents undergoes a protracted development. In contrast, little is known regarding the neuroanatomical status of the BLA afferents and efferents in the developing female rat. Here we use both the anterograde and retrograde tracers, *Phaseolus vulgaris*-leucoagglutinin (PHA-L) and Fluorogold (FG), respectively, to elaborate the distributions, densities and inter-connectivity of the BLA, mPFC and vCA1 in juvenile and adult male and female Long-Evans rats. We have begun initial injection site mapping as part of a larger project to map developing BLA afferents and vCA1 and mPFC efferents. In experiment 1, FG iontophoretic injections were targeted within the basolateral amygdalar nuclei of female rats. In experiment 2, PHA-L iontophoretic injections were targeted within vCA1 and mPFC regions of male and female rats. After four days, animals were perfused, brains were extracted, sectioned and Nissl-stained. The cytoarchitecture of specific coronal sections was parcellated and aligned to a rat brain atlas (L.W. Swanson, 2018 *Brain Maps 4.0, J Comp Neurol*). These parcellations were used to inform the bounded limits within which PHA-L and FG injection deposits were confined. The BLA injection sites were confirmed within the rostral BLA and resulted in FG labeling within the ventral hippocampus CA1 and medial prefrontal cortical regions. A similar approach as that used to map the injection sites is being used as part of our ongoing analyses to map the distribution and/or density of FG-labelled neurons and PHA-L-labelled terminals within prelimbic and infralimbic areas, ventral CA1 and anterior and posterior portions of the BLA. Collectively, these studies will provide high-spatial resolution maps of key neural projection patterns that likely contribute to conditional fear responses.

Disclosures: **N. Odynocki:** None. **L.M. Colon:** None. **A.M. Poulos:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.14/V38

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01MH117852
NIH Grant R01MH065961

Title: The bed nucleus of the stria terminalis regulates context-dependent flight behavior in rats

Authors: *M. TOTTY[1,2], N. WARREN[1], K. R. RAMANATHAN[1,2], R. RESSLER[1,2], S. MAREN[1,2];
[1]Psychological and Brain Sci., [2]Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: Fadok and colleagues (2017) have developed a modified Pavlovian fear conditioning procedure in which a serial conditioned stimulus (SCS) consisting of serial presentations of pure tone (7 kHz) and white noise (1-20 kHz), followed by a footshock unconditioned stimulus (US). After conditioning, mice exhibit freezing to the tone, but transition to flight (e.g. escape jumps and increased movement speed) during the noise. The transition from freezing to flight behavior is gated by the central nucleus of the amygdala (CeA), and flight responses are only elicited within the conditioning context (Fadok et al., 2017). Here, we replicate these behavioral findings in male and female Long-Evans rats and further investigate how flight responses are contextually regulated. After SCS conditioning, rats either received unsignaled footshocks in a novel context (Shock) or were exposed to the same novel context for an equal amount of time (No-Shock). The next day, Shock animals displayed flight responses to SCS-alone presentations within the unsignaled footshock context, whereas No-Shock animals did not. We therefore conclude that flight responses are dependent upon contextual fear, irrespective of where SCS conditioning occurs. The bed nucleus of the stria terminalis (BNST), central amygdala (CeA), and the ventral hippocampus (VH) have been implicated in contextual fear. In the second experiment, we show that muscimol inactivation of either the BNST or the CeA, but not the VH, diminishes flight responses in the conditioning context. These findings advance our understanding of the neural circuitry underlying the contextual regulation of active defensive behavior by demonstrating that flight responses are dependent upon contextual fear and that this effect is mediated by the BNST.

Disclosures: M. Totty: None. **N. Warren:** None. **K.R. Ramanathan:** None. **R. Ressler:** None. **S. Maren:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.15/V39

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01MH065961

Title: Overexpression of microRNA-33 in the bed nucleus of the stria terminalis blocks state-dependent learning of contextual fear in rats

Authors: *R. S. BLAIR[1], G. M. ACCA[2], S. MAREN[1,2], N. NAGAYA[1,2];
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Abstract: Sex steroids can modulate emotional behavior in both rodents and humans. We have previously shown that allopregnanolone (ALLO), a progesterone metabolite, can induce state-dependent contextual fear when infused into the bed nucleus of the stria terminalis (BNST) of male rats. Because ALLO is a strong potentiator of $GABA_A$ receptors, the state dependence it

confers may involve regulation of GABAergic tone. In mice, intra-hippocampal infusion of gaboxadol induces state-dependent contextual fear by suppressing miR-33, a microRNA that regulates GABA-related proteins. To determine if miR-33 contributes to ALLO-induced state-dependent learning, we bilaterally injected a virus designed for overexpression (GFP rno-mir-33 AAV) into the BNST of adult males; blank virus (AAV-mir-GFP-Blank) was injected into the control group. Two weeks post-injection, animals received intra-BNST infusions of either ALLO (8 μg/μl) or vehicle (VEH; 30% β-cyclodextrin). Within 10 min, they were subjected to Pavlovian fear conditioning consisting of 5 tone (2kHz, 10 s, 80 dB)-footshock (2 s, 1 mA) pairings. On subsequent days, retention tests for context (10 min) and tone (4 CS-alone trials in a novel context) followed infusions of ALLO only. VEH-trained, ALLO-tested controls displayed low levels of contextual freezing compared to ALLO-ALLO controls, consistent with state-dependent learning. In contrast, VEH-ALLO rats from the miR-33-injected group displayed contextual freezing similar to ALLO-ALLO rats of both groups, consistent with a lack of state-dependent learning. Cue-induced freezing was not affected by viral transduction or hormone treatment. These findings suggest that overexpression of miR-33 in the BNST can block induction of state-dependent contextual fear. Thus, ALLO-induced state-dependent fear learning may involve miR-33-regulated, GABA-mediated molecular pathways within the BNST.

Disclosures: **R.S. Blair:** None. **G.M. Acca:** None. **S. Maren:** None. **N. Nagaya:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.16/V40

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH065961
NIH Grant MH117852
Mcknight memory and cognitive disorders award

Title: Nucleus reuniens influences medial prefrontal cortex and hippocampal neuronal activity during retrieval of extinguished fear memories

Authors: *K. R. RAMANATHAN[1], J. JIN[1], K. DEISSEROTH[2], S. MAREN[1];
[1]Dept of Psychological and Brain Sci. & Inst. for Neurosciences, Texas A&M Univ., College Station, TX; [2]Bioengin & Psych, Stanford Univ. Dept. of Psychology, Stanford, CA

Abstract: Coordinated activity between the medial prefrontal cortex (mPFC) and hippocampus (HPC) is essential for encoding and retrieving spatial, working and contextual memories. The nucleus reuniens (RE) is a ventral midline thalamic nucleus that has a role in synchronizing activity in the HPC and mPFC. In Pavlovian fear conditioning, we have recently showed that RE

inactivation impairs both the acquisition of hippocampal-dependent contextual fear memories as well as the extinction of fear to an auditory conditioned stimulus (CS). We hypothesized that the extinction deficit may be due to RE inactivation impairing behaviorally relevant neural activity in the mPFC and HPC. To test this idea, we examined the influence of RE inactivation on the induction of c-fos in mPFC and HPC by an extinguished conditional stimulus (CS). Consistent with our hypotheses, we found that inactivation of RE impaired the expression of extinction and this was associated with decreased c-fos expression in both the mPFC and HPC. We are currently exploring the functional role for RE projections to mPFC or HPC (or both) in extinction retrieval using an intersectional optogenetic strategy. Taken together, these data show that RE has a crucial role regulating neuronal activity in the mPFC and HPC that promotes successful retrieval of extinguished fear memories.

Disclosures: **K.R. Ramanathan:** None. **J. Jin:** None. **K. Deisseroth:** None. **S. Maren:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.17/V41

Topic: G.01. Appetitive and Aversive Learning

Support: R01MH065961
R01MH117852
F31MH112208
McKnight Foundation Memory and Cognitive Disorders Award
Brain & Behavior Research Foundation NARSAD Distinguished Investigator Grant

Title: Locus coeruleus norepinephrine drives stress-induced increases in basolateral amygdala firing and impairs extinction learning

Authors: *O. W. MILES[1], T. F. GIUSTINO[2], K. R. RAMANATHAN[2], M. S. TOTTY[1], S. MAREN[1];
[1]Psychological and Brain Sci., [2]Texas A&M Univ., College Station, TX

Abstract: Stress impairs extinction learning and these deficits depend, in part, on stress-induced norepinephrine release in the basolateral amygdala (BLA). For example, systemic or intra-BLA administration of propranolol reduces the immediate extinction deficit (IED), an impairment in extinction learning that occurs when extinction trials are administered soon after fear conditioning. Here, we explored whether locus coreuleus norepinephrine (LC-NE) regulates stress-induced changes in spike firing in the BLA and consequent extinction learning impairments. Rats were implanted with recording arrays in the BLA and, after recovery from

surgery, underwent a standard auditory fear conditioning procedure. Fear conditioning produced an immediate and dramatic increase in the spontaneous firing of BLA neurons that persisted (and in some units increased further) up to an hour after conditioning. This stress-induced increase in BLA firing was prevented by systemic administration of propranolol. Conditioning with a weaker footshock caused smaller increases in BLA firing rate, but this could be augmented by chemogenetic activation of the LC. Conditioned freezing to a tone paired with a weak footshock was immune to the IED, but chemogenetic activation of the LC prior to the weak conditioning protocol increased conditioned freezing behavior and induced an IED. These data suggest that stress-induced activation of the LC increases BLA spike firing and causes impairments in extinction learning. Stress-induced increases in BLA activity mediated by LC-NE may be a viable therapeutic target for individuals suffering from stress- and trauma-related disorders.

Disclosures: O.W. Miles: None. **T.F. Giustino:** None. **K.R. Ramanathan:** None. **M.S. Totty:** None. **S. Maren:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.18/V42

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant RO1MH065961
NIH Grant RO1MH117852
BBRF NARSAD

Title: Dorsal hippocampus mediates covert retrieval of a contextual fear memory

Authors: *R. RESSLER[1,2], T. D. GOODE[3], C. EVEMY[1], A. MARTINEZ[1], S. KIM[1], S. MAREN[1,2];
[1]Psychological and Brain Sci., Texas A&M Univ., College Station, TX; [2]Inst. for Neurosci., College Station, TX; [3]Massachusetts Gen. Hosp., Boston, MA

Abstract: Memories enter a liable state after retrieval, and administration of protein synthesis inhibitors interferes with the reconsolidation of reactivated memories. Although inhibition of protein synthesis within the amygdala interferes with the reconsolidation of fear to a first-order auditory conditioned stimulus (CS), indirectly reactivated fear memories are not sensitive to protein synthesis within the amygdala. Given that higher order S-S associations are thought to depend on the hippocampus, we conducted a series of experiments to examine the role of the dorsal hippocampus (DH) in the reconsolidation of indirectly retrieved fear memory. Previous reports suggest backward (BW) conditioning, a procedure in which the unconditioned stimulus directly precedes the CS is mediated by contextual fear. To confirm this, we tested whether

extinction of the conditioning context selectively reduced fear to a BW CS. Accordingly, animals were fear conditioned using either forward (FW) or BW trials (context A). Next, animals underwent context extinction (A) or novel context exposure (B). Finally, animals were tested for fear to the CS in a third novel context (C). Results revealed that CS-elicited fear was attenuated in BW-conditioned but not FW-conditioned animals. In a separate cohort, we also examined whether the opposite was true. That is, does extinction of an auditory CS in a novel context (B) reduce fear to the conditioning context (A) selectively in BW-conditioned animals. Similar Exp. 1, impairments in contextual fear retrieval following CS extinction were selective to animals that had received BW conditioning. In the final experiment, animals were implanted with bilateral cannulae aimed at DH and after recovery were conditioned to a FW or BW CS as in Exp.1 and 2. 24 hours later rats received a single CS presentation in a familiar context (B), which we hypothesized would reactivate the memory of the conditioning context (A) in BW but not FW-conditioned animals. To target the reconsolidation of this reactivated context memory we infused a protein synthesis inhibitor (rapamycin) or vehicle into the DH immediately following the retrieval session. Lastly, we assessed fear in the conditioning context (A). Consistent with our hypothesis, intra-DH infusion reduced fear selectively in animals that were conditioned to a BW CS. These results provide evidence that the BW, but not FW, CS reactivated a hippocampal representation of the original conditioning context, and that reconsolidation of this memory required hippocampal protein synthesis. This has important implications for novel therapeutic approaches to target and selectively erase traumatic memories.

Disclosures: **R. Ressler:** None. **T.D. Goode:** None. **C. Evemy:** None. **A. Martinez:** None. **S. Kim:** None. **S. Maren:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.19/V43

Topic: G.01. Appetitive and Aversive Learning

Support: R01 MH065961
RO1MH117852

Title: Signaled active avoidance performance is context-dependent

Authors: *C. R. OLEKSIAK, J. M. MOSCARELLO, S. MAREN;
Psychological and Brain Sci. and Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: After Pavlovian fear conditioning, the expression of conditioned fear is context-independent: rats that learn an association between a conditioned stimulus (CS) and an aversive unconditioned stimulus (US) in one context will show robust fear responses, such as freezing, to

the CS in a different context. It is unclear whether other learned defensive responses, such as active avoidance behavior, are context-independent. Here we examined whether two-way signaled active avoidance behavior in rats is affected by a context shift. Sprague-Dawley rats received four days of signaled avoidance conditioning which consisted of thirty tone warning signals (2 kHz, 80 dB, 15 s) paired with footshock (0.7 mA, 0.5 s) per day. Rats could avoid the footshock US and terminate the warning signal by shuttling from one side of the apparatus to the other prior to US onset. After conditioning, the rats had two counterbalanced tests in either the conditioning context or in a shifted context. In both tests, 10 tones were presented absent shock and avoidance responses did not terminate the tone (i.e. both tests were 'reinforcement-free'). The rats performed significantly fewer avoidance responses in the shifted context compared to the original context. This was accompanied by an increase in freezing to the warning signal in the shifted context. Hence, unlike Pavlovian fear conditioning, active avoidance conditioning is context-dependent. Future work will examine the neural circuits mediating the context-dependence of avoidance responses.

Disclosures: C.R. Oleksiak: None. **J.M. Moscarello:** None. **S. Maren:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.20/V44

Topic: G.01. Appetitive and Aversive Learning

Title: The role of hippocampus to amygdala projection in two way active avoidance

Authors: *K. SUNG[1], J. YU[2], J. PYO[2], J.-H. KIM[2];
[1]Div. of Integrative Biosci. & Biotech., [2]Dept. of Life Sci., POSTECH, Pohang, Korea, Republic of

Abstract: Fear and defensive behavior triggered by fear are critical for survival of animal. For a decade, studies about neural circuit of fear are based on pavlovian fear conditioning paradigm whereas avoidance related fear circuit is overlooked.In prior studies, it is revealed that amygdala, medial prefrontal cortex, and nucleus accumbens are involved in avoidance learning & performance. But it is not well known about hippocampus in active avoidance. There were some reports that hippocampus involve in active avoidance performance. But it is not known how hippocampus contribute in avoidance learning and performanceIn our study, we found that hippocampal neurons are activated during active avoidance training & test by c-fos staining. And we found that hippocampal amygdala projection is necessary for active avoidance learning by optogenetically inhibiting hippocampal amygdala projection during avoidance learning.

Disclosures: K. Sung: None. **J. Yu:** None. **J. Pyo:** None. **J. Kim:** None.

Poster

411. Fear and Aversive Learning and Memory: Circuits I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 411.21/V45

Topic: G.01. Appetitive and Aversive Learning

Support: NSF IOS1353137
NSF T32DA037202
NIMH K01MH115158
STAMPS Family Scholarship

Title: Reduced renewal of conditioned suppression following lesions of the dorsal hippocampus

Authors: *A. TAVAKKOLI, D. I. FOURNIER, D. J. BUCCI, T. P. TODD;
Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Extinction of fear to a conditioned stimulus (CS) is a context-dependent; removal from the extinction context results in renewal of conditioned fear to the CS (Bouton & Bolles, 1979). Prior experiments have demonstrated a critical role for the dorsal hippocampus (DH) in fear renewal. However, the majority of these studies examined renewal in conditioned freezing procedures. The role of the DH in renewal is less clear in other procedures, such as conditioned suppression. For example, pre-training fornix lesions or pre-training neurotoxic lesions of the entire hippocampus have no impact on ABA renewal of conditioned suppression (Frohardt et al., 2000; Wilson et al., 1995). Likewise, post-extinction lesions of the DH do not weaken ABA renewal of conditioned suppression (Todd et al., 2017). Since all prior conditioned suppression experiments examined ABA renewal, which involves a return to the original conditioning context, the current study examined the impact of pre-training DH lesions on ABC renewal. ABC renewal isolates the contextual retrieval of extinction because it does not involve a return to the original conditioning context. All rats received light-shock pairings in Context A, followed by the extinction of the light CS in Context B. In experiment 1, rats received DH lesions prior to conditioning, while in experiment 2 they were lesioned after extinction. In both experiments, sham lesioned rats showed reduced fear to the CS presentation in Context B, followed by a renewal of fear when the CS was presented in an equally familiar Context C. Although Sham lesioned rats demonstrated ABC renewal, DH lesioned rats did not. Summation testing revealed that the extinction context was not a conditioned inhibitor, and likely controlled extinction responding by modulating the light-shock association. Overall, these experiments suggest that the DH is necessary for renewal when it occurs in a relatively neutral context.

Disclosures: A. Tavakkoli: None. **D.I. Fournier:** None. **D.J. Bucci:** None. **T.P. Todd:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.01/V46

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant DA020041

Title: MDMA and memory: A dose-effect analysis on Pavlovian fear conditioning

Authors: *M. M. PANTONI, S. G. ANAGNOSTARAS;
Dept. of Psychology, UC San Diego, La Jolla, CA

Abstract: ±3,4-methylenedioxymethamphetamine (MDMA) is a recreational drug that is also being pursued as a therapeutic for PTSD and other mood and anxiety disorders. Despite strong evidence of its therapeutic potential, these pursuits are hindered by evidence that MDMA produces robust neurotoxicity and cognitive deficits at high doses. These findings, however, may not generalize to typical recreational or therapeutic use of low-dose MDMA. To date, there is little research on the cognitive effects of low/moderate doses of MDMA (less than 3 mg/kg) - the doses that users typically take. In the present study, we examined the effects of MDMA on learning and memory across a range of doses using a Pavlovian fear conditioning paradigm. Hybrid C57BL/6Jx129S1/SvImJ mice were randomly assigned to groups by MDMA dose. Mice were trained on-drug with a single tone-shock pairing and then tested off-drug one week later for long-term context and tone fear memory. We assessed the effects of doses of 0.1 to 8 mg/kg MDMA relative to vehicle control on immediate memory and long-term contextual and cued memory. With increasing interest in therapeutic uses of MDMA, this research will help determine if low doses are therapeutically viable in terms of adverse effects on learning and memory.

Disclosures: M.M. Pantoni: None. **S.G. Anagnostaras:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.02/W1

Topic: G.05. Anxiety Disorders

Support: Austrian Science Fund FWF W1206-B18
Austrian Science Fund FWF I2433-B26
Austrian Science Fund FWF SFB-F4410

Title: Dopamine and the hunger hormone ghrelin: Novel targets to rescue impaired fear extinction?

Authors: *E. M. FRITZ[1], A. PIERRE[2], M. KHARITONOVA[1], A. SAH[1], O. BUKALO[3], D. DE BUNDEL[2], A. HOLMES[3], N. SINGEWALD[1];
[1]Dept. of Pharmacol. and Toxicology, Inst. of Pharmacy, CMBI, Univ. of Innsbruck, Innsbruck, Austria; [2]Dept. of Pharmaceut. Chemistry, Drug Analysis and Drug Information, Ctr. for Neurosciences, Vrije Univ. Brussel, Brussels, Belgium; [3]Lab. of Behavioral and Genomic Neurosci., NIH/NIAAA, Rockville, MD

Abstract: A considerable number of patients suffering from anxiety disorders and PTSD do not show long-term benefit from currently available treatments such as exposure-based behavioral therapy (EBT). It has been proposed that in those patients fear extinction, the central mechanism underlying EBT is impaired, leaving them with refractory symptoms and a high risk of relapse. This insufficient treatment response is well-modeled in the 129S1/SvImJ (S1) mouse strain, which exhibits impaired fear extinction following Pavlovian fear conditioning. We have previously shown that enhancing dopaminergic (DA) signaling by L-DOPA treatment rescues the deficient extinction phenotype of S1 mice.
In order to elucidate the underlying neurocircuitry we are now employing optogenetics and viral tracing techniques to target the midbrain DA system. Our preliminary findings suggest a major role for mesocortical projections from the ventral tegmental area (VTA) to the infralimbic cortex (IL), as selective inhibition of these projections decelerates extinction learning in usually extinction-competent DAT-Cre (C57BL/6J) mice. To forge new therapeutic paths, we are also exploring non-pharmacological measures to enhance endogenous DA signaling. The hunger hormone ghrelin is known to promote mesocorticolimbic DA release, so we utilized an overnight fasting paradigm to activate the ghrelin-DA axis. Using ELISA, we assessed whether ghrelin release is intact in extinction-impaired S1 mice and found total ghrelin plasma levels comparable to those of C57BL/6J mice. Active ghrelin levels, however, were higher in S1 mice under fasted as well as non-fasted conditions. qPCR revealed that ghrelin receptor (GHSR) mRNA expression in arcuate nucleus and VTA is influenced differently by fasting in both mouse strains. Fear extinction in GHSR-KO mice was not altered, suggesting that intact ghrelin signaling is no requisite to extinguish fear. Nevertheless, in S1 mice overnight fasting before fear extinction supported the formation of long-lasting extinction memories. Protection from fear reinstatement was accompanied by higher expression of Zif268, a marker for neuronal activity, specifically in layer 5/6 of the IL, which receives a majority of VTA DA projections.
Overall, our data further strengthens the notion of the IL as a critical player in mediating dopamine's extinction-promoting effects. What role alterations of the ghrelin/GHSR system play in impaired fear extinction, however, remains to be elucidated. We propose enhancing DA signaling (pharmacologically or non-pharmacologically) as a powerful tool to augment EBT in patients with treatment-resistant anxiety disorders.

Disclosures: **E.M. Fritz:** None. **A. Pierre:** None. **M. Kharitonova:** None. **A. Sah:** None. **O. Bukalo:** None. **D. De Bundel:** None. **A. Holmes:** None. **N. Singewald:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.03/W2

Topic: G.05. Anxiety Disorders

Support: CAPES, Brazil.

Title: Role of μ-opioid and CB1 receptors of the dorsal periaqueductal gray matter in the modulation of aversive and antinociceptive responses induced by fear

Authors: *J. M. ZANOVELI, M. M. GODOI, J. M. CUNHA;
Federal Univ. of Parana, Curitiba, Brazil

Abstract: A wealth of evidence indicates that the activation of CB1 and μ-opioid receptors (MOR) in the dorsal periaqueductal gray matter (dPAG) inhibits more intense fear-like responses. Given that the aversive dPAG stimulation also induces an antinociceptive effect, we propose to investigate the role of cannabinoid and opioid system in dPAG on antiaversive and antinociceptive effects mediated by the dPAG chemical stimulation. For that, the basal thermal threshold was evaluated using the tail-flick test. In the next day, the animals received intra-dPAG injection of CB1 receptor-selective agonist ACEA (0.5 pmol/0,2 μL), CB1 receptor antagonist AM251 (100 pmol/0,2 μL), MOR agonist DAMGO (0.5 pmol/0,2 μL), MOR antagonist CTOP (0,1 nmol/0,2 μL) or its vehicle (0,2 μL) followed by the intra-dPAG injection of N-methyl-D-aspartate (NMDA, 1 nmol/0,2 μL) to evaluate aversive responses - freezing and crossing behaviors - in free-moving animals. Immediately after, the thermal threshold was evaluated again. All procedures were approved by the Research Ethics Committee for the Use of Animals of the Biological Sciences Sector of the Federal University of Paraná (#1188). The CB1 receptor agonist and MOR agonist, but not its respective antagonists, prevented the NMDA-induced aversive responses. While the CB1 agonist did not alter the antinociceptive effect induced by aversive chemical stimulation in the dPAG with NMDA, the MOR agonist induced a more marked antinocicpertive effect when compared to vehicle-treated animals chemically stimulated with NMDA. Our findings indicate a beneficial effect of the activation of CB1 receptor and MOR into dPAG on aversive behavioral consequences of chemical stimulation of dPAG, highlighting that the activation of MOR in the dPAG enhances the fear-induced antinociception.

Disclosures: **J.M. Zanoveli:** None. **M.M. Godoi:** None. **J.M. Cunha:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.04/W3

Topic: G.05. Anxiety Disorders

Support: MOST 106-2320-B-007 -006 -MY3

Title: Nucleus reuniens in Pavlovian trace fear conditioning

Authors: *Y.-J. LIN, C.-H. CHANG;
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Abstract: The Nucleus Reuniens (RE) of the midline thalamus interconnects the hippocampus (HPC) and the medial prefrontal cortex (mPFC). A considerable amount of studies has suggested that the RE plays a critical role in emotion, memory, and various cognitive tasks. However, much less is known about RE contribution in different learning phases of Pavlovian fear conditioning. In Pavlovian fear conditioning, an initially neutral conditioned stimulus (CS), such as tone, is associated with an aversive unconditioned stimulus (US), such as foot shock. The temporal relationship between the CS and the US is critical and leads to different experimental procedures: Delay conditioning consists of co-termination of CSs and USs, while trace conditioning consists of pairings of CS and US that are separated in time by a stimulus-free "trace" interval. These procedures engage different underlying neural mechanisms. Researches have indicated that the HPC and mPFC are involved in trace, but not delay, fear conditioning. Given the role of RE in mediating this neurocircuitry, we hypothesized that RE inactivation leads to a learning deficit only in trace, but not delay, conditioning. First, we sought to determine if RE was activated in different learning phases of trace and delay fear conditioning in rats using cfos expression as an activation index. We found that in general, RE was recruited in the encoding phase of trace and delay fear memory, but not the retrieval phase. Next, we pharmacologically inactivated RE to investigate RE contribution in each learning phases of trace and delay conditioning. Interestingly, our data suggested that pre-conditioning inactivation of RE impaired the acquisition of the trace fear; however, inactivation immediately after conditioning and before retrieval test did not impair the performance, indicating that RE was only necessary during memory acquisition. By contrast, RE inactivation in neither of the time periods affected delay fear learning. In addition, when we brought the RE off-line the entire encoding and retrieval process of trace fear, no learning deficit was observed, suggesting a state-dependent retrieval of trace fear memory. Together, our data revealed the important role of RE in the learning process of trace fear conditioning.

Disclosures: Y. Lin: None. **C. Chang:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.05/W4

Topic: G.05. Anxiety Disorders

Support: Austrian Science Fund FWF (SFB F4410, W-1206 SPIN)

Title: Dopaminergic strategies to rescue deficient fear extinction

Authors: S. B. SARTORI[1], T. M. V. KEIL[1], K. K. KUMMER[2], M. KRESS[3], K. EBNER[1], ***N. SINGEWALD**[1];
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Abstract: Existing treatments of anxiety- and trauma-related disorders, including exposure-based behavioral therapy (EBT) show only partial long-term therapeutic effects. One reason is that the central process underlying EBT, namely the formation of a new, fear inhibitory extinction memory is often deficient in these disorders. A promising option for improvement is the pharmacological boosting of exposure-based therapy (Singewald et al, 2015). We have shown previously that the dopamine bioprecursor L-DOPA facilitates fear extinction in extinction-intact humans and extinction-deficient 129S1/SvImJ (S1) mice (Haaker et al. 2013, Whittle et al. 2016). This was associated with enhanced extinction-induced activity in the infralimbic cortex (IL). We now used a combination of *in vitro* and *in vivo* techniques including microdialysis, multi-electrode array (MEA) recordings and immunohistochemical mapping (utilising e.g. pERK and pCREB as markers for functional dopamine receptor activation) and revealed evidence of blunted dopamine neurotransmission in the infralimbic cortex (IL) of S1 as compared to extinction-competent C57BL/6 mice. Compensation of this deficiency by pharmacologically enhancing local dopamine availability in the IL was sufficient to behaviorally rescue the extinction deficit persistently and protect from the return of fear. While we excluded a possible contribution of adrenoceptors, the exact receptor(s) or combinations of receptors mediating these effects are now being revealed by microinjections of selective dopamine receptor ligands. Taken together, we revealed dysfunctional dopaminergic signaling in extinction-deficient mice and identified the IL as a brain region critically involved in the rescue of deficient fear extinction by dopamine. The results suggest the dopaminergic system as a promising target for the development of pharmacological adjuncts for EBT to overcome extinction resistance and to protect from fear relapse in extinction-impaired individuals.
Acknowledgement: Supported by the Austrian Science Fund FWF (SFB F4410, W-1206 SPIN)

Disclosures: S.B. Sartori: None. **T.M.V. Keil:** None. **K.K. Kummer:** None. **M. Kress:** None. **K. Ebner:** None. **N. Singewald:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.06/W5

Topic: G.05. Anxiety Disorders

Support: National Institute on Alcohol Abuse and Alcoholism Grant AA025368, AA026949, AA026675
National Institute on Drug Abuse Grant DA045897
NARSAD Young Investigator Award, Brain and Behavior Research Foundation, #23603

Title: β-arrestin-dependent opioid signaling positively contributes to reduced conditioned fear and anxiety-like behavior

Authors: *M. KO[1], T. CHIANG[1], A. A. MUKADAM[1], G. E. MULIA[1], J. A. CHESTER[2], R. M. VAN RIJN[1];
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Abstract: G protein-coupled receptors (GPCRs) are key drug targets for various neuropsychiatric disorders due to their active role in neuronal signal transduction. To develop GPCR-based drugs with low on-target adverse effects and high effectiveness, it is imperative to identify intracellular messenger systems linked to the therapeutic effects. Here, we aim to dissect the distinct role of downstream GPCR signal transduction in the modulation of anxiety and fear with an emphasis on ligand bias related to β-arrestin-dependent signaling. To address our goal, we utilized the δ-opioid GPCR (δOR), which is implicated in the modulation of emotional behaviors, and two specific δOR agonists that can mediate downstream signaling, primarily ERK1/2, through G protein (TAN-67) and β-arrestin (SNC80). We found that systemic administration of TAN-67 increased whereas SNC80 decreased conditioned fear-related behavior in mice and the behavior was inversely correlated with ERK1/2 activation. The SNC80-induced reduction in fear-related behavior was still present in the β-arrestin 2 global knockout mice; however, we did note that ERK1/2 activation by SNC80 was abolished in the striatum and nucleus accumbens of β-arrestin 1 knockout mice suggestive of a role of this isoform in the modulation of fear. SNC80 also significantly reduced anxiety-like behavior in an elevated plus maze and a dark-light transition test in wild-type but not in β-arrestin 2 knockout mice, implicating β-arrestin 2 in the modulation of anxiety-like behavior. We observed that ERK1/2 activity by SNC80 in the hippocampus and amygdala relied on the presence of β-arrestin 2. Moreover, we were able to attenuate the anxiolytic-like effects of SNC80 by systemic administration of the MEK inhibitor SL327, which indirectly blocked ERK activity in the tested

regions. Thus, it appears that the β-arrestin 2-mediated ERK1/2 activation is an integral component of δOR modulation of anxiety-like behavior. Overall, our results reveal a region-specificity to β-arrestin isoform function in modulating ERK signaling, which appears differentially associated with modulation of anxiety- and conditioned fear-related behavior. Our findings may aid in the development of 'precision medicine' for treating mood disorders.

Disclosures: M. Ko: None. **T. Chiang:** None. **A.A. Mukadam:** None. **G.E. Mulia:** None. **J.A. Chester:** None. **R.M. Van Rijn:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.07/W6

Topic: G.07. Other Psychiatric Disorders

Support: Queensland University of Technology

Title: Olfactory fear conditioning enhanced neuroplasticity and neurogenesis in the hippocampus and the olfactory bulb of adults rat

Authors: M. HAKIM[1,2,3], A. BATTLE[1,3,2], L. R. JOHNSON[3,4], ***F. CHEHREHASA**[1,2,3]; [1]Queensland Univ. of Technology, Sch. of Biomed. Sci., Brisbane, Australia; [2]Inst. of Hlth. and Biomed. Innovation, Queensland Univ. of Technol., Brisbane, Australia; [3]Translational Res. Institute, QUT, Brisbane, Australia; [4]Univ. of Tasmania, Sch. of Med., Launceston, Australia

Abstract: Post-traumatic stress disorder (PTSD) is a memory-related disorder characterised by emotional responses of intense fear as a result of intrusive memory recall. While odours are especially effective at triggering memories of high emotional saliency and intensity more than other sensory cues, the neurocircuitry of olfactory fear memory is not fully understood yet. Here, we investigated the neurocircuitry of olfactory fear memory acquisition and consolidation. We used Pavlovian fear conditioning in adults rats to determine whether recollection of olfactory memories changes the proliferation of newborn neurons or leads to neuroplasticity of neurons in the olfactory pathway, amygdala and the hippocampus which are associated with fear memory. We used the plasticity marker CREB and EdU (5-ethynyl-2'-deoxyuridine) labelling to study neuroplasticity and neurogenesis of different brain regions after olfactory fear memory. Our results showed that the olfactory fear conditioning resulted in a significant increase in number of CREB positive neurons in the medial and cortical subnuclei of the amygdala and the piriform cortex. We also found that there was a significant increase in number of EdU positive neurons in the dentate gyrus of the hippocampus and the glomerular layer of the olfactory bulb 24 hours and 14 days post-conditioning. These findings contributed to the complete understanding of the

neurocircuitry of olfactory fear conditioning and the role of neurogenesis in olfactory fear memory consolidation.

Disclosures: **M. Hakim:** None. **A. Battle:** None. **L.R. Johnson:** None. **F. Chehrehasa:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.08/W7

Topic: G.07. Other Psychiatric Disorders

Support: Avielle Foundation Basic Neuroscience Research Grant

Title: Role of the gut microbiome in mouse territorial aggression

Authors: *C. KWIATKOWSKI[1], S. KASZUBINSKI[2], C. WAELCHLI[1], K. MOON[3], A. L. EAGLE[3], C. MANNING[1], A. MOESER[4], M. E. BENBOW[5], A. ZEOLI[6], A. ROBISON[3]; [1]Neurosci., [2]Integrative Biol., [3]Physiol., [4]Vet. Med., [5]Entomology, [6]Sch. of Criminal Justice, Michigan State Univ., East Lansing, MI

Abstract: Violence is a widespread public health and justice system problem with far-reaching consequences for victims, offenders, and their communities. Aggression, the cognitive and behavioral antecedent to violent action, is mainly understood in terms of the psychosocial risk factors that increase the likelihood of aggressive behavioral strategies. In order to gain a better understanding of the individual-level biological mechanisms that precipitate violence, the current study examines the relationship between the gut microbiome and behavioral aggression in mice. Territorial male CD1 retired breeder mice were screened for aggressive versus passive social interactions using an intruder paradigm with younger male C57Bl6J mice, and aggression was scored based on attack latency, bout number, and bout length. The gut microbiomes of aggressive and non-aggressive CD1 mice were assessed using 16s RNA sequencing of extracted fecal matter to identify correlations between microbial strain abundance and aggressive behavior, and the abundances of several bacterial strains were found to correlate with aggression. In addition, aggressive and non-aggressive mice underwent microbial elimination using chronic antibiotic treatment and subsequent transplantation of the gut microbiome from donor mice expressing an aggressive behavioral phenotype to determine whether gut microbiome directly drives aggressive behavior. Concurrently, activity of the prefrontal cortex (PFC), habenula, amygdala, and nucleus accumbens (NAc) was measured post mortem using immunohistochemistry for the immediate early genes FosB and cFos, thereby identifying a potential role of the gut microbiome in modulation of brain regions involved in aggression and reward. This study elucidates peripheral molecular mechanisms that drive altered brain function

and behavioral aggression, contributing to a more comprehensive biopsychosocial explanation and potential intervention for violence.

Disclosures: **C. Kwiatkowski:** None. **S. Kaszubinski:** None. **C. Waelchli:** None. **K. Moon:** None. **A.L. Eagle:** None. **C. Manning:** None. **A. Moeser:** None. **M.E. Benbow:** None. **A. Zeoli:** None. **A. Robison:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.09/W8

Topic: G.05. Anxiety Disorders

Support: This work has received support from the Fondation pour la Recherche Medicale to DP (DPP20151033983) and from ANR–10–LABX–54 MEMOLIFE and ANR–10–IDEX–0001–02 PSL* Research University.

Title: Cerebello-thalamo-prefrontal control of fear memories

Authors: *I. A. GEORGESCU, J. L. FRONTERA, H. BABA AÏSSA, C. MAILHES-HAMON, C. LENA, D. POPA;
Inst. de Biologie de l'Ecole Normale Superieure, Paris, France

Abstract: Fear conditioning is mediated by excitatory and inhibitory connections between multiple areas. The role of brain structures such as the amygdala and the prefrontal cortex (PFC) in this form of learning is well established, but recent data indicate an involvement of the cerebellum in emotional disorders. Using a combination of neuroanatomy, behavior, chemogenetic and electrophysiology approaches, we have been studying the involvement of the cerebello-thalamo-PFC pathway in fear conditioning and extinction.
Through stereological injections of retrograde viral tracers in PFC and amygdala, and anterograde virus in the deep cerebellar nuclei, we analyzed the possible link between these areas. We found fibers from the cerebellar fastigial nucleus and neurons projecting to amygdala and PFC in the parafascicular (PF) and mediodorsal (MD) nucleus of the thalamus indicating an anatomical coupling if the cerebellum to these thalamic nuclei.
We also found neurons in the MD activated with a short latency by optogenetic cerebellar nuclei stimulations suggesting the existence of a functional pathway from the cerebellum to MD. MD and PF are known to be involved in fear learning and extinction.
We investigated the effects of transient silencing or excitation of neuronal activity of cerebellar projections to MD and PF thalamic nuclei, during fear conditioning and extinction in mice. We virally expressed inhibitory or excitatory DREADDs in the fastigial nucleus, combined with the injection of retrograde CAV-Cre in the target areas, and we examined the effects during fear

learning and extinction training after clozapine-N-oxide (CNO) injection. We have found changes in the extinction learning induced by the fastigial-MD pathway. These results indicate that the cerebellum exerts a powerful effect on limbic circuits during fear memories.

Disclosures: I.A. Georgescu: None. **J.L. Frontera:** None. **H. Baba Aïssa:** None. **C. Mailhes-Hamon:** None. **C. Lena:** None. **D. Popa:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.10/W9

Topic: G.05. Anxiety Disorders

Title: The endocannabinoid system as a predictor of fear extinction learning - An fMRI study

Authors: *J. SPOHRS[1], M. ULRICH[2], L. BINDILA[3], P. PLENER[1], G. GROEN[2], B. ABLER[2]; [1]Dept. of Child- and Adolescent Psychiatry and Psychotherapy III, [2]Dept. of Psychiatry and Psychotherapy III, Ulm, Ulm Univ., Ulm, Germany; [3]Facility of Endocannabinoids/Lipidomics, Mainz Univ., Mainz, Germany

Abstract: With the legalisation of medical cannabis, its potential to treat fear- and anxiety-related disorders has become of great interest. With a focus on animal models in previous research, translational approaches in human research are mandatory. Lutz et al. (2015) have postulated that endocannabinoids (anandamide (AEA) and 2-arachidonoylglycerol (2-AG)) play a neuromodulatory role in fear-related processes. AEA is degraded by fatty acid amide hydrolase (FAAH), and the single-nucleotide polymorphism (SNP) rs324420 in the FAAH-coding gene has been demonstrated to modulate fear extinction learning (Dincheva et al. 2015).
In the present functional magnetic resonance imaging (fMRI) study, 52 healthy men underwent a Pavlovian fear conditioning paradigm to investigate the neural signals during fear learning (day 1), extinction learning (day 2), and retention (day 3). To elucidate the role of AEA and 2-AG, blood samples were analysed using liquid chromatography-mass spectrometry, and FAAH genotyping was performed.
Replicating the results of meta-analyses (Fullana et al., 2015, 2018), we observed brain activation in the anterior insula (AI), dorsal anterior cingulate cortex (dACC), and ventral striatum related to extinction learning. Across participants, baseline AEA-, but not 2-AG-levels, significantly correlated with the degree of extinction learning in the dACC and the right AI (whole-brain analysis, voxel-level $p < 0.001$, cluster-level $p < 0.05$, FWE-corrected). Using the AI as a seed region, we analysed baseline resting-state fMRI data to explore AEA's modes of action. Highly significant functional coupling, negatively correlating with the degree of extinction, was found exclusively with the right hippocampus (whole-brain analysis, voxel-level $p < 0.001$; cluster-level $p < 0.05$, FWE-corrected). As with fMRI activations, baseline AEA-, but

not 2-AG- levels, were negatively correlated with resting state insula-hippocampal coupling indices (peak voxel at [28, -24, -18]; z-score = 3.35; p = 0.002, FWE-corrected for search volume), suggesting that individual AEA-availability could have modulated the neural network mediating extinction learning. The neural extinction signal did not significantly differ between FAAH genotypes.
Thus, our translational research set-up confirmed the hypothesis of an essential role of endocannabinoids - particularly AEA - in the neurobiology of anxiety-related disorders in humans. As a clinical proof of concept, patients with stress or anxiety disorders and lower AEA levels might be pre-treated with AEA-enhancing drugs to promote extinction learning prior to cognitive behavioural therapy interventions.

Disclosures: **J. Spohrs:** A. Employment/Salary (full or part-time):; University Ulm, Department of Child- and Adolescent Psychiatry and Psychotherapy III. **M. Ulrich:** None. **L. Bindila:** None. **P. Plener:** None. **G. Groen:** None. **B. Abler:** None.

Poster

412. Fear Conditioning, Extinction, and Aggression

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 412.11/W10

Topic: G.07. Other Psychiatric Disorders

Support: CONICYT BECA DOCTORADO NACIONAL

Title: 5-HT2A and cfos expression within the orbitofrontal cortex of isolation reared aggressive mice

Authors: *A. AGURTO[1], P. R. MOYA[2];
[1]Univ. De Valparaíso, Valparaiso, Chile; [2]Physiol., Univ. de Valparaiso, Valparaiso, Chile

Abstract: Aggression and violence are problematic symptoms by themselves and pervasive components in psychiatric disorders as diverse as Schizophrenia, drug addiction, Alzheimer and depression, negatively impacting on the psychological lives of patients, family and caregivers. The serotonergic (5-HT) system and frontal cortex have both been involved in the modulation of aggressive behavior in humans and rodent models, of which, the 5-HT1A, 5-HT1B and 5-HT2A receptors are the most studied. However, human and rodent studies on the 5-HT2A receptor involvement in aggression are contrasting, and the lack of specific neuroanatomical correlates as well as the diverse pathologies or models underlying each study of aggressive behavior have made difficult to establish the role of 5-HT2A receptor in modulation of aggression. Using the isolation-reared model of aggression in male CF1 mice, we measured 5-HT2A receptor and c-fos expression through immunohistochemical labelling within the orbitofrontal cortex (OFC) subregions. Blind analysis showed higher 5-HT2A receptor expression in ventral and lateral

OFC of aggressive mice compared to non-aggressive controls while no significant changes were detected in other OFC subregions or control cortices. In addition, c-fos labeled neurons with 5-HT2A receptor expression in the lOFC was 59+/-35% in aggressive mice and 15+/- 9.8% in non-aggressive mice. Ongoing experiments are underway to observe the effects of local administration of 5-HT2A receptor antagonists in the OFC on aggressive behavior and pattern of c-fos activation in other brain areas involved in aggression. Our results suggest that regarding 5-HT2A mediated activity, the ventral and lateral OFC are the most important areas in controlling aggression and that 5-HT2A receptors within the lOFC have a potential role in modulating aggression in this mouse model.

Disclosures: **A. Agurto:** None. **P.R. Moya:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.01/W11

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: A convolutional neural network based deep learning model to predict depressive patients with suicide attempts using brain structural imaging

Authors: *F.-T. WONG[1,3], V. C.-H. CHEN[2,4], Y.-H. TSAI[5], J.-C. WENG[1,4];
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Abstract: The fact that an underestimated figure that a million people reported dying by suicide every year shows the importance of suicide prevention and detection. In this study, a deep learning algorithm was employed to predict people with suicide attempt based on their brain structural imaging. The subjects in our generalized q-sampling imaging (GQI) dataset consisted of 3 groups, which are 64 healthy controls (HC), 51 depression patients (DP), and 33 depression patients with suicide attempts (SA). In the GQI dataset, indices of generalized fractional anisotropy (GFA), isotropic value of the orientation distribution function (ISO), and normalized quantitative anisotropy (NQA) were separately trained in different deep learning models. The training and testing 2-dimensional images were generated from a 3-dimensional volume of each subject, and images sliced from 3 views, i.e. axial, sagittal, and coronal view, were concatenated. The training images were augmented by randomly rotating the image twice. The architecture of the convolutional neural network (CNN) contained three convolution and max-pooling layers. After flattening the output of the last max pooling layer, there followed 4 fully connected layers and a dropout layer. Linear Rectification Function (ReLU) was implemented as an activation

function in each of the aforementioned convolutional layers. The classification results were obtained through a softmax layer. Adam optimizers were employed in the training process, and parameters were updated with cross-entropy loss scores weighted by the sample size. The 5-fold cross-validation accuracy scores of classifications of SA and DP respectively against HC were 0.88 and 0.52 in ISO. In NQA, the accuracy scores were 0.73 and 0.51, respectively. We also applied the same CNN architecture to indices of amplitude of low-frequency fluctuations (ALFF) and regional homogeneity (ReHo) in resting-state functional magnetic resonance imaging (rs-fMRI) and arterial spin labeling (ASL)-based rs-fMRI, as well as voxel-based gray/white matter volume. However, the classification accuracy scores in the other indices were under 0.7. The results suggested that, compared with other brain imaging indices, GQI-ISO retained more distinctive features for our implemented CNN for prediction of suicide attempts. In addition, accuracy scores between SA and DP in ISO revealed a gradient pattern, which could imply that there was a gradient pattern in terms of distinctive neural signatures between the aforementioned groups.

Disclosures: F. Wong: None. **V.C. Chen:** None. **Y. Tsai:** None. **J. Weng:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.02/W12

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: Risk factors for major depressive disorder in general population: A systematic review and meta-analysis

Authors: *D. LIU[1], G. LI[1], L. LI[3], Y. LIU[2];
[1]Sch. of Mental Hlth., [2]Shandong Key Lab. of Behavioral Medicine, Sch. of Mental Hlth., Jining Med. Univ., Jining, China; [3]Jining Taibaihu New District Construction Bureau, Jining, China

Abstract: Motivation: The aim of this study is to conduct a systematic review and meta-analysis of risk factors for major depressive disorder (MDD) among general populations.
Methods: Four English databases (Web of science, Embase, Pubmed, psyINFO) and three Chinese databases (Weipu, CNKI, Wanfang) were searched for cohort or longitudinal studies. Random effect models were used for the combined effect calculations. Funnel plot and Begg's test were conducted to investigate the potential publication biases. All statistical analyses were conducted by STATA Version 14.
Results: A total of 84 studies were included in systematic review. Based on quality assessment with the Newcastle-Ottawa Scale, 71 studies which were more than 5 scores, covering 1,825,324 populations, were included for meta-analysis. The range of followed-up period was from 11 months to 50 years and the median was 5.5 years. Six strongest predictors were as follows:

Anxiety (RR: 1.72, 95% CI: 1.56-1.87), High Life stress (RR: 1.47, 95% CI: 1.28-1.66), Female (RR: 1.46, 95% CI: 1.14-1.77), Physical disease history (RR: 1.42, 95% CI: 1.35-1.50), High work stress (RR: 1.18, 95% CI: 1.03- 1.34), and Highly educated (RR: 0.82, 95% CI: 0.64-1.00). Other risk factors (Obesity, Current smoker, Former Smoker, Low social support, Loss a job, Have a new job, Family history of mental disorders, Parental divorce and self-mental disorder history) and protective factors(Physical activity and Low work stress) were also reported. The incidence rate of MDD was 9% (95% CI: 7%-10%) based on 60 related studies. With subgroups analysis, the intercontinental incidences varied widely. The incidence of MDD of North America was 13% (95% CI: 11%-16%), followed by Asia (9%, 95% CI: 7%-10%) and Europe (6%, 95% CI: 5%-7%). There were gender differences of incidence of MDD. Incidence of MDD in females (11%, 95% CI: 9% -13%) was higher than that of males (5%, 95% CI: 2%-7%).
Conclusion: It is crucial to summary a theoretical framework according these risk factors of MDD. It would provide clues for public health strategy development and individual prevention measures. More researches are needed to confirm the causalities of these risk factors and MDD.

Disclosures: D. Liu: None. **G. Li:** None. **L. Li:** None. **Y. Liu:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.03/W13

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: Predictive factors for major depressive disorder in the elder population: A systematic review and meta-analysis

Authors: *G. LI[1], D. LIU[1], L. LI[2], Y. LI[1], Y. WU[1], Y. LIU[1];
[1]Jining Med. Univ., Ji ning, China; [2]Jining Taibaihu New District Construction Bureau, Ji ning, China

Abstract: Motivation: The aim of this study is to conduct a systematic review and meta-analysis of predictive factors for major depressive disorder (MDD) in the elder population.
Methods: Four English databases (Web of science, Embase, Pubmed, psyINFO) and three Chinese databases (Weipu, CNKI, Wanfang) were searched for cohort or longitudinal studies. Random effect models were used based on significant heterogeneity. Funnel plots and Begg's test were both conducted to investigate the potential publication biases. Stata version 14.0 was used for all statistical analyses.
Results: A total of 89 studies were included in this systematic review. Based on quality assessment with the Newcastle-Ottawa Scale, 39 studies which were more than 5 scores, covering 226,248 elderly participants, were included for meta-analysis. The strongest five predictors were as follows: physical function limitation (RR=2.08, 95% CI: 1.37-2.79), sleep

disturbance (RR=2.07, 95% CI: 1.16-2.97), cognitive function limitation (RR=1.97, 95% CI: 1.31-2.64), obesity (RR=1.12, 95% CI: 1.03-1.20) and physical activity (RR=0.88, 95% CI: 0.76-0.99). Other risk factors (current smoker, alcohol user, family history of mental disorders, marital status and self-mental disorders history) and protective factors (social contact) were also reported. The range of followed-up period of all 39 studies was 1-21 years and the median was 4 years.
Conclusion: It is crucial to summary a theoretical prevention framework according these risk factors of MDD among the elderly. It provided clues for the policy-makers to prevent late-life MDD and to facilitate MDD remission among the elderly. More researches were needed to confirm the causalities of these risk factors and MDD in the elder population.
Keywords: Predictive factors; Major depressive disorder; Elder population

Disclosures: G. Li: None. **D. Liu:** None. **L. Li:** None. **Y. Li:** None. **Y. Wu:** None. **Y. Liu:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.04/W14

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: Mapping brain structural abnormality in depressed patients with suicide ideation using GQI

Authors: *M.-T. CHEOK[1], V.-H. CHEN[2,3], Y.-H. TSAI[4], J.-C. WENG[1,3];
[1]Dept. of Med. Imaging and Radiological Sci., [2]Sch. of Med., Chang Gung Univ., Taoyuan, Taiwan; [3]Dept. of Psychiatry, [4]Dept. of Diagnos. Radiology, Chang Gung Mem. Hosp., Chiayi, Taiwan

Abstract: Over 300 million people are suffering from various degrees of depression worldwide. The order of depression severity is a significant risk factor for suicidal behavior. The integrity of white matter is considered to be associated with depression psychopathology. In light of this, we used generalized q-sampling imaging (GQI) to investigate the white matter integrity in major depressive disorder (MDD) patients with suicide ideation.
In our study, 3T MRI (Verio, Siemens) was used to acquire GQI images of 52 healthy control (HC) and 35 MDD patients with suicide ideation (ID), and sort out as the first comparison. The second comparison is related to the HC and 48 MDD without suicide ideation (non-ID) patients. Lastly, the third comparison is between 35 ID patients and 48 non-ID patients. Eddy current correction with FSL (FMRIB Software Library) and spatial normalization with Statistical Parametric Mapping (SPM) were performed. The GQI indices, including normalized quantitative anisotropy (NQA) and generalized fractional anisotropy (GFA), were calculated using DSI

Studio. Paired sample t-test was used to detect the significant changes with SPM. Additionally, age, gender, and education years were varied across the subjects and used as covariates in this study.
In the first comparison, we found significant white matter integrity decreased in the corpus callosum, right cingulate gyrus and right superior temporal gyrus in ID compared with HC. In the second comparison, a significant reduction in right caudate and corpus callosum was found in the non-ID compared with HC. More severe demyelination in callosum, left anterior cingulate, and left medial frontal gyrus impairments were observed in ID compared with non-ID in the third comparison.
We found significant differences of white matter integrity between the MDD and HC group in the corpus callosum, cingulate and the frontal area, which are responsible for emotional processing, stress response. These results supported the severity of white matter demyelination directly affect the severity of MDD. It is also worth noted that the demyelination is severer at the anterior part of corpus callosum and left cingulate gyrus in suicide ideation patients. Corpus callosum plays a pivotal role in the integration of interhemispheric information, including higher cognitive functions and impulsive action. Anterior cingulate involved in the cognitive and emotional regulation of behavior, especially the left anterior cingulate was associated with improved response stability. All of these results indicated that suicide ideation affects brain structure and could be used as imaging biomarkers for clinical diagnosis.

Disclosures: M. Cheok: None. **V. Chen:** A. Employment/Salary (full or part-time):; Chang Gung University. **Y. Tsai:** A. Employment/Salary (full or part-time):; Chang Gung University. **J. Weng:** A. Employment/Salary (full or part-time):; Chang Gung University.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.05/W15

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: A prospective cohort study investigating factors associated with depression in Chinese training residents

Authors: *F. SHEN[1], Z. LIU[2], Y. ZHOU[3], W. LI[4];
[1]Bio-X Institute, Shanghai Jiao Tong Univ., Shanghai, China; [2]Bio-X Institutes, Shanghai Jiao Tong Univ., Shanghai, China; [3]Bio-X Institutes,Shanghai Jiao Tong Univ., Shanghai, China; [4]Shanghai Jiao Tong Univ., Shanghai City, China

Abstract: IMPORTANCE. High rates of depression is commonly reported in medical internships. Identifying associated risk factors could help to improve the diagnosis and intervention of depression.

OBJECTIVE. To identify factors associated with depression or depressive symptoms before and within internship training.
METHODS. Participants were recruited from multiple hospitals in China and the United States. Medical students who were going to start their internship, were invited to enroll this survey. Demographic characteristics were collected and the depressive symptoms of PHQ-9 scale were assessed for subjects before internship. During internship, subjects were assessed the depressive symptoms using PHQ-9 in every quarter. Besides, many other factors were included as baseline (before internship) or within-internship factors based on statistical models.
RESULTS. The mean score of PHQ-9 depressive symptoms reported by subjects significantly increased from 3.98 in pre-internship to 7.14 during internship in China, and it increased from 2.64 to 5.73 in the United States. The proportion of participants who met PHQ-9 criteria for depression increased from 8.58% to 23.43% after internship in China, meanwhile it augmented from 3.98% to 35.61% in the United States. Further, factors of lower baseline scores of depressive symptoms and personal history of depression before internship, increased work time and lack of sleep within internship, and high perceived stress before and during internship, were associated with a greater increase of depressive symptoms scores in both China and the United States. The depressive symptoms scores showed age-related only in China, whereas gender, marital,
neuroticism and early family environment were identified as associated factors only in the United States. Interestingly, Chinese interns reported less medical errors than that of American interns.
CONCLUSION. In the present study, we conduct a prospective cohort study of intern health in China and the United States. We assessed and investigated depression related factors using mood scale and questionnaire survey before and within internship, and identified several factors associating with the development of depression. These findings may help to improve the early diagnosis and intervention, reduce medical errors and prevent the suicide of interns.

Disclosures: F. Shen: None. **Z. Liu:** None. **Y. Zhou:** None. **W. Li:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.06/W16

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: Mindfulness intervention in autism increases middle cingulate cortex activity during self-reflection which predicts depression alleviation

Authors: *B. A. PAGNI[1], M. WALSH[1], E. FOLDES[1], A. SEBREN[1], M. DIXON[1], L. BAXTER[2], C. RIECKEN[1], C. HAYNES[1], A. MACOMBER[1], A. CLARK[1], C. WEBB[1], M.

CATCHINGS[1], J. ALVAR[1], B. B. BRADEN[3];
[1]Arizona State Univ., Tempe, AZ; [2]Neuroimaging, Barrow Neurolog. Inst., Phoenix, AZ; [3]Dept. of Speech and Hearing Sci., Arizona State Univ. - Tempe Campus, Tempe, AZ

Abstract: Adults with autism spectrum disorder (ASD) experience high rates of depression and anxiety. There is some evidence that Mindfulness-Based Stress Reduction (MBSR) is effective in reducing depression and anxiety, however, the neural mechanisms and benefit beyond support groups are unknown. Functional MRI (fMRI) research shows adults with ASD do not activate the ventral medial prefrontal cortex (vmPFC) and middle cingulate cortex (MCC) during self-reflection as seen in neurotypicals. Mindfulness training regulates self-reflection neural activation in NT adults, making this a likely mediator of symptom-reducing effects in adults with ASD. We investigated whether MBSR would increase blood-oxygen-level dependent (BOLD) response in regions activated during self-reflection, compared to a support/education control group, and if the BOLD signal change correlated with changes in depression and anxiety. Twenty-eight adults (nine women; mean age=31.8±12.9, range=18-64; mean IQ=106±18.5, range=70-139) were randomly assigned to a MBSR group (n=15) or a support group (n=13). All participants met ASD criteria on the ADOS-2 and both groups met for two-hours once/week for eight weeks with homework. Pre- and post-intervention fMRI scans were collected for the self-reflection task where participants: (1) reflected on whether or not the word displayed was a trait they possessed (self-condition), and (2) made a judgement about the positive valence of words (word-condition). Self-reported symptoms were assessed via the Beck Depression Inventory-2 and the State-Trait Anxiety Inventory. Within group comparisons were performed with paired t-tests and correlations with Pearson product-moment. The MBSR group demonstrated significant depression reduction with a moderately large effect size ($t(14)=3.31$, $p=0.005$, $d=0.66$), while the control group approached significance ($t(12)=1.82$, $p=0.09$, $d=0.40$); neither intervention significantly reduced anxiety symptoms. We found increased activation in right MCC ($p=0.018$, small-volume family-wise error corrected [FWE]) in the Self>Word contrast after MBSR intervention that negatively correlated with depression alleviation ($r(11)=-0.49$, $p=0.04$). There were no changes in vmPFC for the MBSR group or either region for the control group. Seed-to-voxel connectivity analysis revealed that MBSR increased functional connectivity between right MCC and pre/postcentral gyrus (p=0.001, FWE). This study suggests that MBSR may be effective for reducing depression in adults with ASD over and above a support/education group, and the neural mechanism may be increased right MCC activation during self-reflection.

Disclosures: **B.A. Pagni:** None. **M. Walsh:** None. **E. Foldes:** None. **A. Sebren:** None. **M. Dixon:** None. **L. Baxter:** None. **C. Riecken:** None. **C. Haynes:** None. **A. Macomber:** None. **A. Clark:** None. **C. Webb:** None. **M. Catchings:** None. **J. Alvar:** None. **B.B. Braden:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.07/DP10/W17

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Science Foundation Ireland Stokes

Title: Changes in brain activation to angry faces in adolescent participants with depression symptoms

Authors: A. ABDULSADYKOVA, ***A. L. BOKDE**;
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Abstract: Introduction: Depression has been associated with altered processing of emotional face stimuli compared to healthy groups. The hypothesis is that the 'sub-clinical depression' group would show altered activation to emotional faces compared to healthy control group in the prodromal stages of the disease. Data & Methods: The brain activation, measured using fMRI, for angry and neutral faces were evaluated in 425 adolescents from over 2000 recruited for the IMAGEN study (Schumann et al., 2010). The symptoms of depression were quantified using the Development and Well-Being Assessment (DAWBA) (Goodman et al., 2000) at baseline (BL) and follow-up 2 (FU2). There were 3 groups: clinical depression (scores 4 or 5 on DAWBA), subclinical depression (scores 1- 3), and healthy controls (score 0). There were 41, 179 and 205 participants in the clinical, subclinical and healthy groups, respectively. The average age at BL was 14.39 ± 0.5 years and at FU2 were 18.9 ± 0.68 years. The groups were matched for age and sex ratio (40% male). A 'face network' and a 'depression network' were defined using meta-analysis through neurosynth.org website. The results presented are the angry versus neutral face contrast and the ANCOVA statistical models include center, age, sex as covariates of no interest (additional covariates are mentioned in results). Statistical results are Bonferroni corrected. Results: Activation differences at FU2, controling for FU2 age and BL depression score, there were statistically significant effect of group in right IFG_1 (IFG_1, coordinates -18, -11, -26), and left IFG_2 (IFG_2, coordinates 9, -38, -20). Posthoc t-test revealed higher activation in the right IFG_1 in healthy group (EMM= 0.79; SEM=0.04) than in subclinical depression group (EMM=-0.61; SEM=0.036), at p=0.018. For IFG_2 ROI, healthy controls showed higher neural activity (EMM=0.156; SEM=0.05) than participants with clinical depression (EMM= -0.093; SEM=0.08), p=0.026. An ANCOVA model applied to brain activation at BL, found differences in activation in right hippocampus the clinical depression group (EMM=-0.185; SEM=0.071), than in the subclinical depression group (EMM=0.038; SEM=0.04; p=0.019) and the healthy

group (EMM=0.064; SEM=0.044; p=0.009). In the right IFG_1 brain region the activityt was higher in subclinical depression (EMM=0.118; SEM=0.043) than in clinical depression group (n=41; EMM=-0.128; SEM=0.076), p=0.015. Discussion & Conclusion: The symptoms of depression are associated with changes in brain activation in hippocampus and right IFG about 4 years preceding the appearance of the symptoms.

Disclosures: A. Abdulsadykova: None. **A.L. Bokde:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.08/W18

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: National Institute of Mental Health MH 048063

Title: Stimulus valence contextually modulates network profiles of the hippocampus and dlPFC in episodic memory: Assessing borderline personality disorders with co-morbid depression

Authors: *T. J. ATTISHA[1], E. L. KALLABAT[1], T. D. MERAM[1], P. H. SOLOFF[2], A. Z. CHOWDURY[1], V. A. DIWADKAR[1];
[1]Psychiatry & Behavioral Neurosci., Wayne State Univ. Sch. of Med., Detroit, MI; [2]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Background: Memory processing is affected by emotion, especially in disorders of emotion dysregulation, including borderline personality disorder (BPD) and major depressive disorder (MDD). MDD is frequently comorbid with BPD (Yoshimatsu et al., 2014), but how comorbidity affects the brain's connectivity profiles is unknown. Because episodic memory formation is driven by the hippocampus and prefrontal cortex (Preston & Eichenbaum, 2013), we studied how *stimulus valence* differentially affected network profiles of the hippocampus (during memory encoding) and/or the dorsolateral prefrontal cortex (dlPFC) (during recognition of encoded associations) in BPD patients with or without co-morbid depression (BPD_{MDD+}, BPD_{MDD-}), and healthy controls (HC).
Methods: Task-based fMRI data collected in 36 BPD patients and 27 HC were analyzed (BPD_{MDD+}=12; 3.0T Siemens Trio). During the task, memory for images (drawn from the International Affective Pictures System (IAPS) (Lang et al., 1993) presented during Encoding epochs was tested in subsequent Recognition. Standard methods (SPM 8) were used to process fMRI data. Psychophysiological Interaction (PPI) (Friston et al., 1997, $p<.05$, cluster level) estimated network profiles of the Hippocampus and dlPFC. A second level analyses was modeled with two factors: Group (BPD_{MDD+}, BPD_{MDD-}, HC) and stimulus valence (positive, negative and neutral). Inter-group differences were investigated under each valence.

Results: (a) Encoding and Recognition of positive and neutral valenced images induced similar effects: BPD_{MDD+} displayed *hyper-modulation* by the hippocampus and dlPFC (BPD_{MDD+} > BPD_{MDD-}; BPD_{MDD+}> HC) across the memory network. (b) Negatively valenced images: BPD_{MDD+} displayed both *hyper-modulation* (BPD_{MDD+}>BPD_{MDD-}), and *hypo-modulation* when compared to HC (BPD_{MDD+} < HC).
Discussion: Our results suggest that the effects of comorbid depression (BPD_{MDD+}) are complex and non- "linear" and that stimulus valence exerts complex modulatory effects on network function. An absence of negative valence induces *hyper-modulatory* profiles (positive and neutral) of the hippocampus and dlPFC in BPD_{MDD+}, consistent with models suggesting that depression drives inefficiency in brain networks (Wagner et al., 2006; Zhi et al., 2018). However, negative valence appears to interact with the depressive phenotype, inducing *hypo-modulatory* effects. This shift is a class of "affective interference" observed in disorders of emotion regulation (Soloff et al., 2015). Studying the effects of the comorbidity may help unravel the complex relationships between dysfunctional brain network profiles and clinical traits.

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Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.09/DP11/W19

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

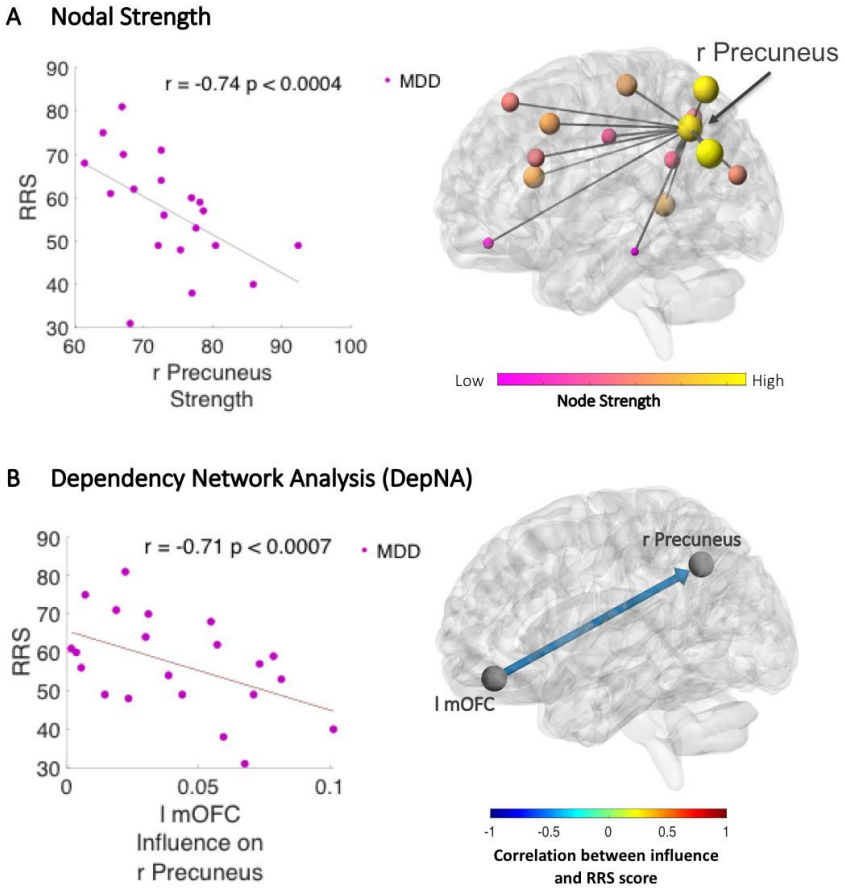
Support: NIH R01 MH109544Icahn

Title: Correlates of brain network hierarchies with rumination in major depressive disorder

Authors: *Y. JACOB, L. S. MORRIS, K.-H. HUANG, M. SCHNEIDER, G. VERMA, J. W. MURROUGH, P. BALCHANDANI;
Icahn Sch. of Med., New York, NY

Abstract: Major depressive disorder (MDD) patients exhibit higher rumination levels; repetitive thinking and focus on negative states. Rumination is known to be associated with aberrant activity in the brain default mode network (DMN) regions, yet, the underlying whole-brain network topological organization remains unclear. Implementing a graph-theory analysis we tested whether whole brain network connectivity hierarchies during fMRI resting state are associated with rumination. We hypothesized that the functional network topological

organization will distinguish between MDD patients and healthy controls (HC) and will also associate with individual differences in self-reported rumination scores (RRS). Applying this data-driven approach on 21 MDD patients and 21 HC, we found that MDD patients exhibited the same hierarchy in the network as HC during rest. However, within the MDD group, lower strength of a DMN related region - the right precuneus - was associated with greater rumination scores (r=-0.77, p<0.0002, q *FDR*<0.05) (Figure 1A). There was also a negative trend within the HC group (r=-0.34, p=0.18). We then applied the Dependency Network Analysis (DepNA), a newly developed graph theory method to quantify networks' node importance according to its connectivity influence. The DepNA revealed that within the MDD group, greater rumination tendency was associated with decreased influence of the left medial orbito-frontal cortex (mOFC) on the right precuneus (r=-0.71, p<0.0007, q *FDR*<0.05)(Figure 1B). Lastly, we used an information theory entropy measure that quantifies the cohesion of each network's correlation matrix. We show that the subjects with higher rumination scores exhibit higher entropy levels within the DMN i.e. decreased overall connectivity within the DMN (r=0.47, p<0.05). These results emphasize the general DMN involvement during self-reflective processing related to maladaptive rumination among MDD. More specifically, the influence of the mOFC on the Precuneus might serve as a target for clinical neuromodulation treatment.



Disclosures: **Y. Jacob:** None. **L.S. Morris:** None. **K. Huang:** None. **M. Schneider:** None. **G. Verma:** None. **J.W. Murrough:** None. **P. Balchandani:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.10/W20

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: No anhedonia for music in major depressive disorder

Authors: *A. POINCOT, S. SPIVACK, D. TOSSAVAINEN, S. E. MCCLELLAND, G. LENNER, L. CRANMER, P. WALLISCH;
New York Univ., New York, NY

Abstract: There is a prevalent view that major depressive disorder (MDD) is accompanied by anhedonia, the inability to experience pleasure from everyday activities that used to be enjoyable. We tested this hypothesis in a high-powered sample of participants exhibiting a wide range of depression scores as operationalized by the Beck Depression Inventory (BDI). These participants listened to a representative corpus of music samples and rated them in terms of their appraisal. We found no statistical association between BDI scores and music appraisal ratings, with an empirical correlation coefficient indistinguishable from zero. This challenges prevalent notions that MDD is associated with anhedonia and supports prior work suggesting a dissociation between mood and musical appreciation, leaving the possibility of rescue from MDD through individually targeted music therapy.

Disclosures: A. Poincot: None. **S. Spivack:** None. **D. Tossavainen:** None. **S.E. Mcclelland:** None. **G. Lenner:** None. **L. Cranmer:** None. **P. Wallisch:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.11/W21

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: Cortical and subcortical deficits observed in individuals with seasonal affective disorder

Authors: *N. B. RODRIGUES[1], W. SWARDFAGER[2], A. LEVITT[3], P. GIACOBBE[3], N. LIPSMAN[4];

[2]Dept. of Pharmacol. and Toxicology, [1]Univ. of Toronto, Toronto, ON, Canada; [3]Psychiatry, [4]Neurosurg., Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada

Abstract: The heterogeneity of Major Depressive Disorder (MDD) has made it notoriously difficult and expensive to investigate changes to neural structure between episodes of depression and remission. Further, it remains undetermined whether patients suffering from Seasonal Affective Disorder (SAD), a subtype of MDD, have volumetric cortical and subcortical deficits. As depressive episodes and euthymic periods can be reliably predicted in SAD based on the season, the disorder offers a unique naturalistic model to investigate whether neural structures change between states. The aim of this exploratory study was to characterize atypical cortical or subcortical volumetric properties during periods of depression and euthymia. This study recruited ten participants with a confirmed diagnosis of winter-SAD and ten healthy controls between the ages of 18 to 65. There were no statistically significant differences in gender, age, ethnicity or body mass index between the two cohorts. Participants were scanned using a 3T MRI to generate high resolution T1-weighted structural images (192 volumes, $1mm^3$ voxels). The volumes were reconstructed, preprocessed, segmented and parcellated using the FreeSurfer v6.0 automated pipeline. The thickness, volume and surface area for cortical regions and the volume of subcortical regions were extracted and analyzed using a general linear model, covarying for age and intercranial volume, during the winter and summer. In order to correct for multiple comparisons, the family-wise error was set at $p < 0.005$. There was no significant difference in volume or surface area, however, there was a significant reduction in the cortical thickness of the superior frontal gyrus in both the left and right hemisphere of SAD patients in the winter and summer months compared to healthy controls ($p < 0.005$). Additionally, there was a statistically significant volumetric reduction in the left and right pallidum in SAD patients compared to healthy controls in the summer and winter ($p < 0.005$). This study presents novel evidence that patients suffering from SAD have structural abnormalities to the bilateral superior frontal cortex, a region associated with modulating impulse control and high order executive function, and the bilateral pallidum, which is a central node in the motivation and reward limbic pathway (de Boisgueheneuc et al., 2006; Hu et al., 2016). These results are further corroborated by cognitive studies that reported cognitive impairments persist during depressive and remitted periods in SAD patients (Hjordt et al., 2017). Taken together, this study helps to characterize why certain cognitive deficits may not ameliorate in MDD.

Disclosures: N.B. Rodrigues: None. **W. Swardfager:** None. **A. Levitt:** None. **P. Giacobbe:** None. **N. Lipsman:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.12/W22

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Loyola University Intramural Funds

Title: Elevated glutamate transporter expression in females with depression

Authors: B. E. POWERS[1], ***M. SODHI**[2];
[1]Mol. Pharmacol. and Therapeut., [2]Loyola Univ. Chicago, Maywood, IL

Abstract: Suicide leads to 40,000 deaths per year in the US and is particularly prevalent in patients with major depressive disorder (MDD). The pathophysiological mechanisms leading to MDD and suicide are unclear. Accumulating evidence indicates that abnormal function of the glutamate system contributes to the efficacy of antidepressant treatments, and that antidepressant efficacy includes modification of the activity of the dorsolateral prefrontal cortex (DLPFC). We have previously shown increased glutamatergic gene expression in the DLPFC of females but not males with MDD. Here, we have tested the hypothesis that abnormal expression of the genes encoding the glutamate transporters and the transporters of monoamine neurotransmitters occurs in the DLPFC in MDD and suicide. We extracted RNA from the gray matter of the DLPFC from three groups of subjects: MDD suicides (MDD-S, n=51), MDD patients who did not complete suicide (MDD-NS, n=28), and controls (CTRLs, n=32). Laboratory staff were blind to diagnosis during the experiments. We used QPCR to measure the expression of genes encoding transporter proteins within the glutamate (EAAT1, EAAT2, VGLUT1, VGLUT2) and monoamine systems (SERT, NET, DAT, PMAT, VMAT). We used multivariate analysis of covariance to test if these gene expression markers were associated with MDD or suicide. Our results show that females but not males with MDD had higher expression levels of all glutamate transporters relative to CTRLs ($P<0.05$). MDD-S groups of both sexes had higher VGLUT2 expression ($P<0.05$). Analyses of monoaminergic genes revealed lower VMAT1 expression ($P=0.002$) in MDD males, and conversely higher VMAT2 in MDD females ($P=0.004$). MDD females also had higher TPH2 and NET expression ($p<0.05$). Therefore, we report sex differences in the expression of glutamate transporters and some monoaminergic genes in the DLPFC in MDD. Most of these findings are novel, but lower EAAT1 expression in MDD-S replicates previous studies. Altered glutamate transport may contribute to lower DLPFC activity, poor problem solving and impaired executive function observed in patients with severe depression and suicidal behavior.

Disclosures: B.E. Powers: None. **M. Sodhi:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.13/W23

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: National Natural Science Foundation of China [Grants No.81621003]

Title: Mutations of enzymes involved in dietary carbohydrate digestion system in Chinese patients with major depressive disorder

Authors: *N. ZHANG[1], J. LI[2], S. BI[3], W. KUANG[1];
[1]West China Hospital, Sichuan Univ., Chengdu, China; [2]West China Hosp., Chengdu, China; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Major depressive disorder (MDD) is a neuropsychiatric disorder with the high prevalence and life-threatening risk, and is characterized by a variety of symptoms, such as sadness, anhedonia and decreased interest in daily activities. Genetic analysis is a powerful tool to identify risk variants and to enhance understanding of the etiology of MDD, leading to improvement of early diagnosis and development of more effective therapies. This study was carried out for genetic analysis of Chinses patients with MDD. The study was approved by the West China Hospital Clinical Trials and Biomedical Ethics Committee of Sichuan University, and a total of 121 patients and 78 healthy individuals were recruited from Mental Health Center of West China Hospital. Patients (age between 18 and 65) with MDD were diagnosed according to the DSM-IV (American Psychiatric Association, 2000) by board certified and experienced psychiatrists. Peripheral blood samples were collected from patients and their DNAs were extracted from blood samples. The quality and quantity of DNA extracts were first assessed, and DNA samples with concentrations >20 ng/μL and 260/280nm absorbance ratios between 1.8 and 2.0 were selected for subsequent sequencing analysis. Whole exome sequencing was carried out with high-throughput second-generation sequencing technique using Illumina HiSeq platform PE150. Only sequence reads with $Q_{phred}30$ (indicating an error rate of <0.1%) were used for further analysis. DNA samples collected in parallel from healthy participants served as normal controls.
Series of mutations in enzymes involved in dietary carbohydrate digestion system were identified in all patients, but were not observed in healthy controls. Though multiple mutations were identified, the number of mutations was not correlated to the severity of this disease. Our preliminary data suggested a potential role of enzymes for dietary carbohydrate digestion in MDD pathology. Further analysis will combine other neuropsychological and psychoradiological assessments in search for the relationships between its genotype and endophynotype of MDD.

Disclosures: N. Zhang: None. **J. Li:** None. **S. Bi:** None. **W. Kuang:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.14/W24

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: Intervention to decrease the depression of the elderly people

Authors: *C. E. HERNÁNDEZ HERNÁNDEZ[1], B. A. FLORES FLORES[2], K. CHÁVEZ RUBIO[3], A. B. LÓPEZ ESCUDERO[3], M. LAZCANO ORTÍZ[3], V. RAMÍREZ GUTIÉRREZ[3], E. H. CRUZ MERA[3], R. C. JIMENEZ SANCHEZ[3], J. ARIAS RICO[3], K. JUÁREZ CORTES[3], O. A. JARAMILLO-MORALES[3];
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Abstract: Introduction: The depression is a disease or mental disorder that is characterized for a Deep sadness, soulish decay, low selfsteem, loss of interest for everything and decrease of the psychic functions. According to World Health Organization (OMS) in the old adult, the depression affects to more of 350 millions of people in the world, contributing to raise the morbi-mortality, which decreases the life quality. The depression isn't something normal in the process of aging and the majority of the old adults feel satisfied with their lifes. However, is a problem of mental health. The **object** is determine the impact of a intervention to decrease the depression in the old adults. The **methodology** of the estudy is of type quasiexperimental. The population were 20 residents of the house of the third age. The sample was 6 old adults identified with some level of depression, applied a pre-test and post-test with a intervention through a program of activities with 13 sessions with a duration of 5.5 hours a week, doing differents activities physical and recreational. In the **results** the avergae of age was of 74.17 years old (DE= 6.274; 66-80). The gender that predominates is male (67%), the civil status of higher frequency free union and widower. In the pre-test s found 4 people (67%) with moderate depression and 2 people (33%) with severe depression, while that in the post-test was deleted the degree of depression in 3 people (50%), 2 (33%) moderate depression and just 1 person (17%) remained with several depression. It **conclude** that the depression in the old adult, in terms of prevalence, is every time higher; also is a disease that induce a important inability, what facilitates the presence of new pathologies or the aggravation of the already existing. Is essential implement programs multidisciplinary to the integral approach of the old adults that can attend differents aspects how their nutrition, medical treatment, so that they are avoided some status of depression and improves the life quality of the old adult.

Disclosures: C.E. Hernández Hernández: None. **B.A. Flores Flores:** None. **K. Chávez Rubio:** None. **A.B. López Escudero:** None. **M. Lazcano Ortíz:** None. **V. Ramírez Gutiérrez:** None. **E.H. Cruz Mera:** None. **R.C. Jimenez Sanchez:** None. **J. Arias Rico:** None. **K. Juárez Cortes:** None. **O.A. Jaramillo-Morales:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.15/W25

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIH grant ZIA-MH002957-01

Title: A longitudinal fMRI study of reward processing in adolescents with depression

Authors: *S. M. JACKSON, P. VIDAL-RIBAS, D. M. NIELSON, G. O'CALLAGHAN, A. STRINGARIS;
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Abstract: Background: Diminished striatal activity during reward anticipation is related to Major Depressive Disorder (MDD) and has been shown to precede the onset of MDD. However, it is unclear whether reward processing aberrations are a cause or consequence of depressive symptoms. Additionally, we want to learn how such reward processing aberrations can predict clinical course.
Methods: Participants were 155 adolescents aged 11-17, 99 MDD (59.5% female), 10 subthreshold MDD (sub-MDD) (80.0% female), and 45 healthy volunteer (HV) (65.2% female). Volunteers were assessed for diagnoses and inclusion criteria: IQ > 70; and a lack of mania, schizophrenia, substance abuse, or medical illness that could cause MDD symptoms. Eligible participants were invited to complete the Monetary Incentive Delay task during fMRI scans. These scans were collected again in MDD participants after 4 months, 8 months, and in all participants after 12 months. We collected the Mood and Feelings Questionnaires (MFQ) at each scan, providing a self-report of depressive symptoms. The clinical assessment was repeated at 12 months. Reliability of the MFQ was assessed using intraclass correlation (ICC) and hierarchical linear models were used to examine changes in MFQ score over time. A contrast for the neural activity during anticipation of winning rewards versus a control cue was analyzed across time and subjects.
Results: The MFQ ICC from baseline (n=76) to 4 months (n=52), 8 months (n=24), and 12 months (n=13) was 0.477, 0.62, and 0.45, respectively. For changes in MFQ as modelled by MFQ ~ Time * Sex + (Time|Subject), there was a main effect of time as well as a time by sex interaction; over time, depressive symptoms improved significantly more in males than in females. Across time and subjects, there was a significant cluster of activity in the ventral striatum (VS) with a focal point in the caudate ($F=20.97$, $p<0.01$, cluster corrected).
Conclusions: The reliability of the MFQ over time is promising for understanding the course of depression. Furthermore, the model of MFQ provides a basis for exploring predictions of clinical course. Finally, the significant cluster of activity in the VS suggests that the anticipation of reward results in activation in this region robustly over time and across subjects.

Disclosures: S.M. Jackson: None. **P. Vidal-Ribas:** None. **D.M. Nielson:** None. **G. O'Callaghan:** None. **A. Stringaris:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.16/W26

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: National Institute of Mental Health MH 048063

Title: Affective interference on OFC and dACC brain network profiles in borderline personality disorder: An fMRI study

Authors: *E. L. KALLABAT[1], T. J. ATTISHA[1], T. D. MERAM[1], P. H. SOLOFF[2], A. Z. CHOWDURY[1], V. A. DIWADKAR[1];
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Abstract: Introduction: Borderline Personality Disorder (BPD) is often characterized by co-morbid major depressive disorder (MDD), yet the co-morbidity's effects on the brain's functional profiles is unknown. Previous studies provide evidence for dysfunction of brain network profiles of the dACC and OFC in both BPD and MDD (Kaiser et al., 2015; Soloff et al., 2017; Wolf et al., 2012). We assessed the effects of affective context on neural processing during a Go No-Go task, and dysfunction in evoked network profiles of the dACC and the OFC in subjects that have BPD with co-morbid depression (BPD_{MDD+}) compared to those without co-morbid depression (BPD_{MDD-}; HC). Network analyses focused on a) dACC given its role in cognitive control and mediation of allocation of resources during complex processing, and b) on the OFC given its role in regulation of affect and impulse control.
Methods: 42 BPD subjects (BPD_{MDD+}=30) and 27 HC subjects underwent fMRI (3T) while performing the Go No-Go task. The task required subjects to respond ("Go") or withhold a response ("No-Go") based on target class (facial valence). In each block of trials, faces were shown briefly (500ms) in a jittered event-related design. fMRI data were processed using standard methods in SPM12. dACC and OFC network profiles were estimated using Psychophysiological Interaction (PPI) (Friston et al., 1997). First level PPI maps for each subject were forwarded to a second level analyses with two factors: Group (BPD_{MDD+}, BPD_{MDD-}, HC) and Valence, permitting assessment of group differences under each valence class ($p<.05$).
Results: From dACC seed, BPD_{MDD+} were characterized by increased modulation (compared to BPD_{MDD-} and HC), and BPD_{MDD-} were characterized by decreased modulation (<HC) when responses were gated by *negative* valence. BPD_{MDD+} were characterized by increased modulation (> BPD_{MDD-}) but decreased modulation (< HC), and BPD_{MDD-} were characterized by decreased modulation (< HC) when responses were gated by *positive* valence. And from OFC seed, BPD_{MDD+} were characterized by increased modulation (compared to BPD_{MDD-} and HC), and

BPD_{MDD-} were characterized by decreased modulation (< HC) when responses were gated by *negative* and *positive* valence.
Discussion: The use of valence to gate impulse control induced the following general trends in network profiles: a) $dACC_{(-ve)}$: BPD_{MDD+}>HC>BPD_{MDD-} and b) OFC $_{(-ve, +ve)}$: BPD_{MDD+}>BPD_{MDD-}>HC. These results suggest that the contextual responses of the dACC and the OFC are different in BPD patients with co-morbid MDD, with the OFC being more susceptible to affective interference, consistent with the region's dual commitments to affective regulation and impulse control.

Disclosures: E.L. Kallabat: None. **T.J. Attisha:** None. **T.D. Meram:** None. **P.H. Soloff:** None. **A.Z. Chowdury:** None. **V.A. Diwadkar:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.17/W27

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: NIH R01-MH098260

Title: Inflammation moderates changes in resting state connectivity following SSRI treatment in geriatric depression

Authors: *D. SEOK, E. LUNING-PRAK, I. ASELCIOGLU, G. GREEN, Y. SHELINE; Univ. of Pennsylvania, Philadelphia, PA

Abstract: *Introduction:* Previous studies have identified increased levels of serum cytokines like C-reactive protein (CRP) and disrupted patterns of resting state fMRI functional connectivity (FC) in major depressive disorder (MDD), particularly in the elderly. While treatment of MDD with selective serotonin reuptake inhibitors (SSRIs) has been shown to reduce serum concentrations of inflammatory cytokines, it remains unclear (1) whether inflammation related dysfunctions in resting state connectivity improve following treatment and (2) how improvements in symptoms track with changes in FC. *Methods:* 26 adults aged 47-91 diagnosed with MDD according to criteria set by the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) were recruited into a double-blind, placebo controlled study and were randomized to treatment with the SSRI escitalopram (ESC) or placebo for 8 weeks. Symptom severity was assessed using the Montgomery-Åsberg Depression Rating Scale (MADRS). Prior to randomization and again following treatment, participants had blood drawn for CRP levels and underwent MRI scanning on a Siemens Prisma 3 T scanner with a 64-channel head coil. *Results:* A subset of patients (n = 9/26), exhibited high levels of CRP (> 3mg/L), although mean CRP did not differ from controls. At baseline, serum CRP concentration was correlated (in voxel-wise,

whole brain functional connectivity analyses) with reduced FC (corrected $p < 0.05$) between a region in the ventromedial prefrontal cortex (vmPFC) and a number of *a priori* seeds, including the left amygdala and bilateral regions of the caudate and putamen. At follow-up, baseline CRP no longer predicted disrupted connectivity between the vmPFC and the limbic or striatal seeds. Moderation analysis revealed that patients with higher baseline CRP (who had reduced FC at baseline) had the greatest improvements in connectivity at follow-up. In patients with higher inflammation, ESC treatment significantly improved MADRS scores ($p = 0.013$) as well as FC between the vmPFC and caudate and putamen seeds. Further, greater increases in corticolimbic and corticostriatal FC were correlated with greater reductions in symptoms. *Conclusion:* The results from this study reveal different treatment trajectories for patients with elevated inflammation, suggesting that decreased corticolimbic and corticostraital connectivity may serve as a target for anti-inflammatory treatments in populations vulnerable to elevated inflammation, such as the elderly.

Disclosures: D. Seok: None. **E. Luning-Prak:** None. **I. Aselcioglu:** None. **G. Green:** None. **Y. Sheline:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.18/W28

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: Menninger Clinic
NIH Grant D43

Title: Dimensional characterization of brain volumetry, connectivity and behavior expression in hospitalized patients by affective and anxiety symptoms, their utility to predict clinical outcomes

Authors: *M. J. USCAMAYTA AYVAR[1], J. M. TORANZO[2], J. ARNEDO[3], I. ZWIR[4], S. N. GOSNELL[5], G. A. DE ERAUSQUIN[6], R. SALAS[7];
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Abstract: Mental disorders rank second among the causes of disability worldwide for the year 2020, behind ischemic heart disease, but ahead of all other diseases (OMS). However, current nosological systems have been unsuccessful in unraveling causal explanations, and are inadequate in predicting treatment or prognosis at the individual level. The objective of this work

is to explore the heterogeneity of affective-anxious symptoms syndromes from the perspective of the underlying affected brain networks, specific circuits and volumetric differences associated with behavioral and self-evaluation variables. We applied the GFM-NMF methodology to examine a sample of hospitalizaed patients from the Menninger clinic (Houston, TX). Structural and diffusion-weighted brain images were processed using Freesurfer and Tracula. We searched for biclusters reflecting different volumetric and tractography patterns shared by distinct subsets of patients. Then we evaluated the significance of each bicluster by comparing the differential tractography profile within a bicluster with that exhibited by every other individual not present in the bicluster. Lastly, we cross-correlated the uncovered biclusters with collected descriptions of clinical features of the patients including affective symptoms severity, personality traits, and identified clusters of individuals with high level of sharing of multiple clinical traits and structural connectivity. The results show that these clusters (representing specific biotypes) are highly predictive of clinical outcomes (response to treatment and duration of admission). These biotypes may suggest distinct etiologies in patients diagnosed with affective or anxious symptoms, characterized by differences in brain connectivity leading to distinct symptoms and clinical outcomes.

Disclosures: **M.J. Uscamayta Ayvar:** None. **J.M. Toranzo:** None. **J. Arnedo:** None. **I. Zwir:** None. **S.N. Gosnell:** None. **G.A. De Erausquin:** None. **R. Salas:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.19/W29

Topic: G.05. Anxiety Disorders

Support: CONACYT 1840
CONACYT 931448

Title: Risk factors and their relationship with affective disorders in students

Authors: Y. GONZALEZ-MARES[1], J. MORALES-MAVIL[2], B. BERNAL-MORALES[3], F. CARDEÑA-RODRIGUEZ[4], *T. CIBRIAN-LLANDERAL[1];
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Abstract: Previous studies revealed that 8% to 12% adolescents experience mood disorders, and which had interfered with adolescent daily life and they social function was affected. Early stress exposure has a wide range of effects impacting cognition, making early trauma a risk factor for

development of mental disorders later in life. The aim of this study was to evaluate anxiety and depression status and its related factors among students aged 15-20 years from Xalapa, Veracruz, Mexico. This was a cross-sectional observational, descriptive study. A sample of students were recruited from two high schools. Problem Oriented Screening Instrument for Teenagers (POSIT), Childhood Adverse Experiences (CAE), Beck Anxiety Inventory (BAI), and Beck Depression Inventory (BDI), were used to measure risk factors, the anxiety and depression symptoms, and some demographic characteristics of students was determined. The preliminary results shown a total of 274 students included in our study (57.6% women, 42.3% men); 17.9% women and 7.7% men presented moderate-severe depression, observing a significant association between female sex and the BDI score (P = 0.015). The association between the presence of risk was made based on the score obtained in the POSIT with moderate-severe depression levels, obtaining an association of variables in 24.5% of adolescents (P = 0.000). Likewise, a statistically significant association was found between 8.4% of the population that presented levels of moderate-severe anxiety and that in turn presented 4 or more CEA (P = 0.021). Later, the score obtained in the POSIT showed that 39.8 % of women and 22.3% of men who presented risk in this instrument was associated with levels of moderate-severe anxiety (P = 0.000) and, finally, the moderate-severe levels of anxiety was associated moreless with women (39.8%) in comparasion with men (22.3%; P = 0.006).
Conclusions: the findings demonstrate that exist a significant association between the presence of risk factors in adolescence, having 4 or more adverse experiences during childhood and that the symptoms related to moderate-severe levels of anxiety and depression are associated with the women, this in high school students in the city of Xalapa, Veracruz.

Disclosures: Y. Gonzalez-Mares: None. **J. Morales-Mavil:** None. **B. Bernal-Morales:** None. **F. Cardeña-Rodriguez:** None. **T. Cibrian-Llanderal:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.20/W30

Topic: G.05. Anxiety Disorders

Support: CONACYT 813363

Title: Prevalence of mathematical anxiety in university students

Authors: *S. ZAMORA LUGO[1], M. CADENA-BARAJAS[3], T. CIBRIAN-LLANDERAL[2]; [1]Doctorado en Neuroetología, [2]Neurofisiologia y Neurobiologia de la Conducta, Inst. de Neuroetologia, Univ. Veracruzana, Xalapa, Mexico; [3]Facultad de Estadistica e Informatica, Univ. Veracruzana, Xalapa, Mexico

Abstract: Mathematical Anxiety (MA) corresponds to the idea that for some people the handling of numbers or situations related to mathematics provokes an emotional response that interrupts performance. Research reports that MA has a negative impact on daily activities. It is known that when exposed to extenuating physical or social challenges such as mathematical processing, the organism gives an endocrine response commanded by the activation of the Hypothalamic Hypophysis Adrenal axis, a circuit responsible for the release of corticotropin which in turn stimulates the adrenal cortex for the release of cortisol. The aim of this study was to describe of Mathematical Anxiety Index (MAI) by areas of knowledge and their comorbidity with the scores of the Beck Anxiety Inventory (BAI). An observational, retrospective, descriptive and transversal study was carried out. A structured questionnaire was carried out in three sections, in the first the participants read and approved the informed consent and general data of Sex, Age and Study Area were collected, Biological Sciences (BS), Health Sciences (HS), Administrative economic (AE), Humanities (Hu) and Technique (Te). Subsequently, the participant answered the MAI questionnaire, which measures the assessment of 20 propositions using a Likert scale to measure attitudes, emotions, and beliefs towards mathematics, the scale goes from Never (1) to Always (5), the MAI is obtained in a range from 0 to 1, the 0 represents an absence of anxiety and the 1 maximum mathematical anxiety. Finally, the BAI was presented to the participant, a Likert-type scale that self-reportedly measures anxiety traits. The questionnaire was applied to 140 students with an average age of 21.5 years (SD=3.4), 50% corresponded to women. The project was submitted and approved by the research ethics committee of the Veracruz Institute of Mental Health, the ethical principles of this research will be based on the guidelines of the Declaration of Helsinki. The analysis of variance confirms that there is a significant influence on the AMI scores ($F_{1,4} = 10.08$, $p = 0.001$) per study area. Multiple comparisons by the Tukey test showed a significant difference between the BS and HS groups ($p = 0.02$), BS and AA ($p = 0.001$) and Hu and AE ($p = 0.01$). In the BAI it is confirmed that there is a significant influence in the IAM scores ($F_{1,2} = 8.95$, $p = 0.03$) by the Sex factor, where a significant difference was found ($p = 0.009$) in the group with low BAI score, observing higher scores in women. The students of the area of Humanities and Biological Sciences, present higher Mathematical Anxiety Index, even when in their areas of study, do not take classes that involve numerical-mathematical processing

Disclosures: S. Zamora Lugo: None. **M. Cadena-Barajas:** None. **T. Cibrian-Llanderal:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.21/W31

Topic: G.05. Anxiety Disorders

Support: CONACYT 863433

Title: Allostasis and the relationship with anxiety in adolescents: A Bayesian approach

Authors: *M. A. PEREZ HERNANDEZ[1], N. ISMAIL[2], B. BERNAL-MORALES[3], M. CADENA-BARAJAS[5], I. CIBRIÁN-LLANDERAL[4];
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Abstract: Background. This study analyzing the probability of occurrence of allostasis, such as physical ailments during adolescence and the development of disorders such as anxiety; Several studies have shown evidence between the allostatic load at an early age generates a long-term vulnerability to develop different psychopathologies. The present study is important because it generates a greater interest in the prevention of mental disorders. **Objetive.** The objective of this study is to observe the probability of occurrence of variables considered chronic stress in adolescence and the severe presence of anxiety and depression. **Method.** Participants were choosen between 18-40 years from Instituto Veracruzano de Salud Mental (IVSM) who presented a diagnosis of anxiety. We applied Beck's anxiety inventory and a questionary about the factors associated with allostasis in adolescence. Descriptive, observational and transversal study. Sampling for convenience. 30% of women and 17% of men presented moderate and severe anxiety. The research protocol and informed consent were prepared according to the principles of the Helsinki code declaration and approved by the IVSM Ethics Committee. The analysis was carried out using software WEKA. **Results:** To explore the causality in the data set, a probabilistic analysis was carried out using Bayesian networks. The results show that in women there are more than 65% chance of suffering from severe anxiety in the presence of chronic stressors in adolescence such as nausea, diarrhea and weakness; in the case of males, none of the probabilities exceeded 50%, the highest being associated with chronic chest pain, which obtained 43%. **Conclusions**. This work opens new ways to prevent anxiety in adulthood with the detection of symptoms in adolescence and a novel instrument is implemented in the field of psychology, the Bayesian networks.

Disclosures: M.A. Perez Hernandez: None. **N. Ismail:** None. **B. Bernal-Morales:** None. **M. Cadena-Barajas:** None. **I. Cibrián-Llanderal:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.22/W32

Topic: G.05. Anxiety Disorders

Support: CONACyT 719882

Title: Psychoaffective evaluation of formal and informal caregivers of patients with chronic diseases

Authors: *E. ACOSTA-MARI[1], A. CABALLERO-VELARDE[1], E. MONTES-VILLASEÑOR[2], V. VILLANUEVA-HERNANDEZ[3], T. CIBRIAN-LLANDERAL[1]; [1]Univ. Veracruzana, Xalapa, Mexico; [2]Ctr. Estatal de Cancerología, Xalapa, Mexico; [3]Inst. Veracruzano de Salud Mental, Xalapa, Mexico

Abstract: Stress and overload caused by providing care to a patient or family member can contribute to the origin of affective disorders, immunological alterations, cardiovascular disease and early death. To evaluate the mental health state in relation to the levels of Stress, Anxiety, Depression, Overload and Empathy in formal caregivers (CF) and informal caregivers (CI) in an oncologic center (CeCan) and a psychiatric center (IVSM). An observational, descriptive, transversal, prospective and correlational study was carried out. Sociodemographic data of 22 CF and 22 CI were collected. The Perceived Stress Scale (PSS), the Hospital Anxiety and Depression Scale (HADS), the Davis Interpersonal Reactivity Index (IRI) and the Reading the Mind in the eyes (RME) test were used for the psycho-affective evaluation. They were evaluated 22 IC, 11 from IVSM and 11 from CeCan, the most prevalent gender was feminine in 73.3%, 33.3% of the sample obtained went to junior high school, 40% reached the high school level, 26% graduate or MD. 43.3% are exclusively dedicated to housework while only 10% have full-time work activities, 56% are married or in free union, 86% are brothers, son or mother of the patient. Within the group of FC, 22 participants were evaluated, 11 from IVSM, 11 from CeCan, 71% female, 38% of those evaluated belong to the Psychology service, 28.6% general doctors while 19% doctors with specialty, 38.5% work more than 40h. In the analysis of the psycho-affective evaluation, the median test was used to compare two groups, which reported statistically significant differences in the evaluation of the PSS with an mean of 27.04 (SD: 1.65) and FC of 18.38 (SD: 1.44) (P = 0.031), in the subscale of depression of HADS the IC group obtained a mean of 7.00 (SD: 1.14) while in the CF group it was 2.85 (SD: 0.57) (P = 0.001). In the RME test, the mean of IC was 19.80 (SD: 1.14) while in the FC group it was 35.04 (SD: 1.79) (P = 0.000), and the interpersonal reactivity index of Davis in the subscales of empathic concern (EC) with a mean of 22.80 (DE: 1.01) in IC and of 25.33 (DE: 0.71) in FC and personal distress (PD) with a mean in IC of 16.71 (SD: 0.96) (P = 0.064) and 13.00 (SD: 0.92) in FC. The analysis of the results shows that the CF group obtained a higher mean score in the RME and the EC subscale, which implies higher levels of cognitive empathy, which could be related to a higher academic level. The IC group obtained a higher mean score on the perceived stress scale, on the depression sub-scale and lower skill in tasks involving empathy.

Disclosures: E. Acosta-Mari: None. **A. Caballero-Velarde:** None. **E. Montes-Villaseñor:** None. **V. Villanueva-Hernandez:** None. **T. Cibrian-Llanderal:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.23/W33

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Title: Alterations of the postsynaptic plasticity genes Homer1a and mGluR5 in the dorsolateral prefrontal cortex, amygdala and hippocampus of suicide completers

Authors: *M. GARCIA-GUTIERREZ, A. VIUDEZ MARTINEZ, J. MANZANARES;
Inst. De Neurociencias, Univ. Miguel Hernandez-CSIC, San Juan de Alicante, Spain

Abstract: Converging evidences suggested that the Homer1 family of scaffold proteins, mainly located in the postsynaptic density (PSD) at glutamatergic synapses, appears to play a relevant role in the development of certain neuropsychiatric disorders, such as depression. In this respect, Homer1a, it has been proposed as one of the main isoforms involved in the response to stressful situations and to pharmacological and non-pharmacological antidepressant treatments. This isoform is an essential element for the proper control of glutamatergic neurotransmission regulating the trafficking of metabotropic glutamate receptor mGluR5 from the cell body to dendrites and axons, contributing to control synaptic plasticity. Considering the potential implications of Homer1a for neuropsychiatric disorders, exploring its role in suicide is of special relevance. Here, we analyzed the gene expression of Homer1a and mGluR5 by real-time PCR in the dorsolateral prefrontal cortex (DLPFC), amygdala (AMY) and hippocampus (HIPP) of 26 suicide victims with no clinical psychiatric history or treatment with anxiolytics or antidepressants, and 25 corresponding controls. All samples were matched, as much as possible, for age (C: 48.4±12 years; S: 45.6±15.5 years) and postmortem interval (PMI, C: 21±14.5 h; S: 16.8±5 h). Statistical analyses revealed that there were no significant differences in the DLPFC to any of both genes when comparing S and C groups. Interestingly, S exhibited higher levels of Homer1a and mGluR5 in the AMY (+23% and +29%, respectively) and HIPP (+28% and +29%, respectively) compared to C. These findings provided evidences about a potential association between Homer1a and mGluR5 and suicide. The alterations of Homer1a and mGluR5 gene expression in brain areas close related with emotional response (AMY and HIPP) may represent an opportunity to stimulate the development of further studies designed to evaluate the role of these targets as biomarkers for suicide prevention.

Disclosures: M. Garcia-Gutierrez: None. **A. Viudez Martinez:** None. **J. Manzanares:** None.

Poster

413. Depression in Patient Subpopulations

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 413.24/W34

Topic: G.04. Mood Disorders – Depression and Bipolar Disorders

Support: CIHR

Title: Cell type specific transcriptomic alterations in the prefrontal cortex of abused suicides

Authors: *D. M. ALMEIDA, G. G. CHEN, J. THEROUX, Z. AOUABED, M. DAVOLI, N. MECHAWAR, G. TURECKI;
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Abstract: Introduction: The transcriptome of a cell constitutes an essential piece of cellular identity and accounts for the multifaceted complexity and heterogeneity of cell types within the mammalian brain. Thus, while a wealth of studies have investigated transcriptomic alterations underlying the molecular neurobiology of childhood abuse (CA) and suicide, the use of bulk-tissue homogenates may have masked their ability to determine cell-type specific dysfunctions. Here we employ a cell-type specific investigation of transcriptomic alterations, in prefrontal infragranular pyramidal neurons, associated specifically with a history of CA. **Methods:** Laser captured microdissection (LCM) was used to isolate prefrontal (BA 10) infragranular pyramidal neurons from post-mortem human brain. Subject groups included individuals who died by suicide with and without a history of severe CA, and non-psychiatric controls. RNA sequencing libraries were constructed using SMARTseq v4 cDNA synthesis and Nextera XT indexing. The abused suicide and control groups were used for sequencing. Libraries were sequenced on the HiSeq 4000 using PE 100bp sequencing at a depth of ~40M reads per subject. Validation included all three clinical groups and employed a high throughput qPCR approach. **Results:** We achieved a mapping efficiency of ~80% and captured a wide distribution of transcripts. Differential gene expression analysis revealed significant (FDR <0.10) dysregulation of protein coding transcripts in abused suicides compared to controls (74 downregulated and 45 upregulated). Weighted Gene Co-Expression Network Analysis (WGCNA) was employed to identify a module that negatively associated with CA. Gene ontology (GO) analysis of the members within this module revealed an enrichment of genes related to glutamatergic synapse functioning and nervous system development. During our validation stage, three genes were found to be specifically altered in abused suicides versus non-abused suicides, and controls. These genes included UBE2R2, ACBD4 and UNG; the first of which was found to be a hub gene (highly interconnected gene) within the aforementioned WGCNA module. **Conclusions:** By employing LCM followed by RNA sequencing our work has uncovered cell-type specific changes associated with a history of CA. Our future research will include cell-type specific

whole genome bisulfite sequencing (WGBS) to explore whether epigenetic processes might explain our expression findings.

Disclosures: D.M. Almeida: None. **G.G. Chen:** None. **J. Theroux:** None. **Z. Aouabed:** None. **M. Davoli:** None. **N. Mechawar:** None. **G. Turecki:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.01/W35

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIAAA grant AA006420
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NIH/NIAAA grant AA026999
NIH/NIAAA grant AA007456

Title: Blocking hypocretin receptors in the infralimbic cortex of Wistar rats reduces footshock stress-induced ethanol seeking in EtOH-dependent rats

Authors: *J. KIM, R. MARTIN-FARDON;
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Abstract: This study's aim was to assess the role of hypocretin (Hcrt) in the infralimbic (IL) cortex during stress-induced ethanol (EtOH)-seeking behavior following chronic intermittent EtOH (CIE) vapor inhalation in male and female Wistar rats. The study tested whether bilateral intra-IL administration of TCS1102 (TCS), a dual Hcrt-r1/r2 antagonist (0 and 15µg/side), would prevent footshock stress (FS)-induced reinstatement of EtOH seeking behavior at three abstinence (Abst) timepoints following EtOH dependence: acute (A-Abst; 8h), late (L-Abst; 2 weeks), and protracted (P-Abst; 6 weeks). Rats were first trained to orally self-administer (SA) 10% EtOH. Half were then made dependent (EtOH-D) by CIE (14h ON, 10h OFF) vapor exposure for 8 weeks while the other half were exposed to air (EtOH-ND). During weeks 4-6 rats could self-administer EtOH three times/week during acute withdrawal (i.e., 8h following daily CIE vapor cessation). Extinction (EXT) training began at the end of week 6 while rats were still exposed to CIE vapor at the 8h withdrawal timepoint. EXT training continued daily for two weeks. EXT sessions were the same as SA sessions except EtOH delivery was withheld. Once the animals' behavior was extinguished, A-Abst testing occurred (8h following the final CIE vapor session). All subsequent EXT sessions and two remaining tests (L-Abst and P-Abst) were conducted in the absence of CIE.
During CIE, EtOH-D rats escalated EtOH intake, which was corroborated by relatively higher

blood alcohol levels and somatic withdrawal signs compared to EtOH-ND rats. EtOH-D rats also required more EXT sessions to reach EXT criteria compared to EtOH-ND rats, demonstrating a stronger propensity to seek EtOH. Overall (males + females), antagonizing IL Hcrt receptors did not suppress FS-induced EtOH seeking in EtOH-ND rats at any Abst timepoint. On the contrary, TCS induced a consistent reduction in FS-induced EtOH seeking in EtOH-D rats at all Abst timepoints. There were also observable sex differences. Among EtOH-ND rats, TCS did not prevent FS-induced reinstatement in females at any timepoint but prevented reinstatement in males at P-Abst. Among EtOH-D rats, males showed reduced FS-induced reinstatement during A- and P-Abst but not L-Abst while females displayed reduced EtOH seeking at all timepoints. These data suggest that FS-induced EtOH seeking behavior in EtOH-D rats is at least in part due to dysregulated Hcrt transmission in the IL, as shown by the increased efficacy of TCS in preventing FS-induced EtOH-seeking behavior in EtOH-D rats. This distinction may be sex-mediated, given the differences noted between males and female rats in either dependence condition.

Disclosures: **J. Kim:** None. **R. Martin-Fardon:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.02/W36

Topic: G.08. Drugs of Abuse and Addiction

Support: MOST 107-2410-H-431-004

Title: Ethanol simultaneously produces rewarding conditioned place preference and aversive conditioned taste aversion in the animal model: A paradoxical effect hypothesis of abused drugs

Authors: *Y.-Q. LIU, A. C. W. HUANG;
Fo Guang Univ. Dept. of Psychology, Yilan Jiaoxi, Taiwan

Abstract: Since 1997, Grigson and her colleagues provided numerous evidence to show the conditioned suppression is due to the reward comparison between the tastant conditioned stimulus (CS) and abused drugs, unconditioned stimulus (US), in termed as the reward comparison hypothesis. However, our laboratory offered conflict data to challenge the reward comparison hypothesis. Recently, we offer alternative viewpoint that the paradoxical effect hypothesis of abused drugs was to revise the reward comparison hypothesis (1997). This hypothesis focused on simultaneously reward and aversion induced by abused drugs in drug addiction. On the initial experiment, rats were conducted an adaptation for 7 days. After that, all of rats were subjected to all compartments 10min for conditioned place preference (CPP) and lickometer 15min for conditioned taste aversion (CTA) served as the baseline. On odd days of

the conditioning phase, the ethanol group was allowed 15 min to drink 0.1% saccharin solution (CS1) and then injected (i.p.) ethanol solution (20%, 2 ml/kg; US). Then, rat was placed in another compartment (CS2) of the CPP box for 30 min. For even days, all rats did not receive the CTA treatment and stayed in the home cage. They were injected (i.p.) a normal saline to place in the other compartment for 30 min. The conditioning phase was 5-ethanol and 5-saline intermittent injections. For CTA test, a 2 x 3 mixed two way ANOVA showed a significant difference occurred in the factor of group ($F1, 7 = 6.21$, $p < 0.05$). Non-significant differences occurred in sessions ($F5, 35 = 0.66$, $p > 0.05$) and the interaction of group and sessions ($F5, 35 = 1.04$, $p > 0.05$). For CPP tests, there was not a significant difference for the unpaired side and the paired side in the saline group ($t = -0.61$, $p > 0.05$). The ethanol group appeared a significant difference between the unpaired and paired sides ($t = -3.65$, $p < 0.05$). In conclusion, this dose of ethanol (20%, 2 ml/kg) could simultaneously produce an aversive effect in conditioned taste aversion and a rewarding effect in conditioned place preference. The present findings challenge the reward comparison hypothesis and support the paradoxical effect hypothesis of abused drugs. The present data should be discussed in the further studies.

Disclosures: Y. Liu: None. **A.C.W. Huang:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

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Program #/Poster #: 414.03/W37

Topic: G.08. Drugs of Abuse and Addiction

Support: Swedish Research Council (Grant 2013-7434 to M. Heilig)

Title: Effect of positive allosteric modulation of $GABA_B$ receptors on pathological alcohol choice

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Abstract: Alcohol addiction is characterized by a progressive shift of decision making, in which alcohol is increasingly chosen over healthy non-drug rewards. Only a subset of people transition from recreational to addictive alcohol use. By contrast, in commonly used animal models, nearly all rats learn to self-administer addictive drugs, including alcohol and animals have no alternative to drug use. Using an exclusive choice-based method to identify rats that continue to self-administer alcohol at the expense of a high-value natural reward, a sweet solution, we recently found that only about 15% of outbred rats choose alcohol over an alternative high-value reward. Furthermore, these animals display a constellation of behavioral traits that resembles those currently considered diagnostic for alcohol addiction. Among several dysregulations in

GABAergic pathways, we found low amygdala expression of the GABA-transporter GAT-3 in vulnerable rats. Low GAT-3 expression resulted in impaired GABA-clearance, suggesting that rescuing impaired GABA-clearance due to suppressed GAT-3 expression might be a successful therapeutic approach in alcohol addiction.
Recent observations indicate that presynaptic $GABA_B$ receptors inhibit GABA-release within the CeA. In a first experiment, we therefore evaluated the potential of ADX71441 (3 or 10 mg/kg, I.P), a novel positive allosteric modulator of $GABA_B$ receptors that has entered Phase 1 clinical testing, to rescue pathological alcohol choice over high value alternative rewards in male Wistar rats (n=32). We found that the $GABA_B$ PAM potently, dose-dependently and selectively normalizes choice preference in the minority of rats that choose alcohol over a natural reward. This effect is achieved at doses that are not associated with sedation or adverse effects. In a second experiment, we asked whether ADX71441 could also rescue choice preference for alcohol over a sweet solution in rats when this preference is induced as a result of alcohol dependence. To this end, we characterized a population of male rats for alcohol vs sweet choice preference (n=63), and exposed them to the alcohol vapor inhalation procedure. We found that a history of physical alcohol dependence increased the proportion of rats that choose alcohol over an alternative reward to about 45% of the population and therefore assessed the effect of the $GABA_B$ PAM on dependence-induced alcohol choice. Together, our results indicate that targeting $GABA_B$ receptors is a valid strategy to rescue impaired GABA-clearance in pathological alcohol choice and extend previous data indicating that $GABA_B$ PAMs merit being tested clinically as a therapeutic for alcohol addiction.

Disclosures: **E. Augier:** None. **G. Augier:** None. **M. Heilig:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

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Program #/Poster #: 414.04/W38

Topic: G.08. Drugs of Abuse and Addiction

Support: NIMH Grant MH077908

Title: Decreased reward-related brain function to monetary rewards predicts shorter time to substance use disorder occurrence

Authors: *C. P. BART[1], R. NUSSLOCK[2], T. H. NG[1], M. K. TITONE[1], A. L. CARROLL[2], K. S. F. DAMME[2], C. B. YOUNG[3], C. ARMSTRONG[2], J. M. CHEIN[1], L. B. ALLOY[1]; [1]Temple Univ., Philadelphia, PA; [2]Northwestern Univ., Evanston, IL; [3]Standford Univ., Stanford, CA

Abstract: Substance use disorders (SUDs) are a significant global health concern, account for a substantial disease burden, and are associated with reward sensitivity abnormalities. Reward sensitivity is supported by a fronto-striatal neural circuit including the orbitofrontal cortex (OFC), ventral striatum (VS), and dorsal striatum (DS), among other regions. Considerable research has examined reward circuity once addiction has set in, but there is a dearth of research examining if neural reward processing dysfunction constitutes a pre-existent risk factor for SUDs. Also, there is debate about whether SUDs arise from hyper- or hypo-reward sensitivity. The current study aimed to address these gaps in the literature, by examining whether a hyper- or hypo-reward sensitivity in reward-related brain regions prospectively predicted substance use (SU) course. Seventy-nine right-handed individuals (*Mage*=21.52, *SD*=2.19 years, 53% female, 57% White) completed a Monetary Incentive Delay (MID) fMRI task, and a diagnostic interview to assess for SUDs. fMRI images were spatially normalized to MNI space and smoothed using 6mm FWHM Gaussian kernel. We used a general linear model in SPM8 to deconvolve the hemodynamic signal during MID anticipation phase across six trial types (i.e., Win $0.00, Win $1.50, Win $5.00, Lose $0.00, Lose $1.50, Lose $5.00). We generated first-level voxel-wise *t*-statistics per participant to contrast reward vs. non-reward trials. Parameter estimates were then extracted from a priori reward-related regions-of-interest (anatomically defined as bilateral OFC and DS using Harvard Oxford Atlas and Wake Forest Toolbox, respectively). In line with the reward hyposensitivity model, we found that decreased activity in the DS (Wald=7.66, *p*=.01, HR=.20, 95% CI=.06-.62) and OFC (Wald=4.28, *p*=.04, HR=.29, 95% CI=.09-.94) during reward anticipation predicted shorter time to occurrence of SUDs. These findings suggest that when people have reduced positive affect, they seek exogenously what they lack endogenously, putting them at risk for SU. This study has important implications for informing our understanding of the pre-existent risk factors and pathophysiology of SUDs, and can inform prevention efforts through psychosocial and pharmacological interventions for SUDs.

Disclosures: C.P. Bart: None. **R. Nusslock:** None. **T.H. Ng:** None. **M.K. Titone:** None. **A.L. Carroll:** None. **K.S.F. Damme:** None. **C.B. Young:** None. **C. Armstrong:** None. **J.M. Chein:** None. **L.B. Alloy:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.05/W39

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant # R01 AA025718

Title: Characterization of glycine receptor subtypes and ethanol sensitivity in the mesolimbic pathway

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Abstract: Introduction
Glycine receptors (GlyRs) are primarily expressed in spinal cord and brain stem neurons. However, recent studies in mesolimbic regions, such as the ventral tegmental area (VTA) and nucleus accumbens (nAc), have reported the presence of GlyRs of an undetermined subunit composition. It is possible to identify GlyRs based on their sensitivity to glycine (Gly), picrotoxin (PTX), general anesthetics, GTP-γ-S and ethanol (EtOH), among others. It is important to understand the properties of GlyRs in the limbic network because their presence might contribute to the rewarding effects of EtOH. The aim of this study was to characterize GlyRs properties in two regions of the reward circuit: prefrontal cortex (PFC) and VTA using Wild Type (WT), Knock In alpha 1 (KIα1) and Knock Out alpha 2 (KOα2) mice.
Results
Using electrophysiological techniques and examining concentration-response curves (CRC) in WT mice, we detected that the EC_{50} of Gly was 71±7 μM in PFC and 34±2 μM in VTA. As a comparison, the EC_{50} in nAc was 66±10 μM. Interestingly, most neurons recorded in the KOα2 mice displayed a very small current even with 1000 μM glycine preventing a complete analysis. VTA neurons, on the other hand, responded reasonably well to glycine. The values for the current density were 5±2 pA/pF in PFC neurons, 0.7 ± 0.2 pA/pF in the nAc, and 21±5 pA/pF in VTA neurons. In presence of intracellular GTP-γ-S, the current was potentiated about 80% of control in VTA, but not in PFC neurons suggesting the presence of α3 subunits. Studying the sensitivity of the glycine activated current in neurons from these regions showed that the PTX resistant current was 49±5% in PFC, 58±5% in nAc, and 73±4% in VTA. These values suggest that the GlyR complexes are predominantly of an αβx conformation.
Conclusion
Based on these data, we postulate that GlyRs present in PFC are composed mainly of alpha 2 and alpha 3 subunits that are not potentiated by EtOH or GTP-γ-S. In VTA neurons, on the other hand, heteropentameric alpha 1 GlyRs that are highly sensitive to EtOH seem to predominate. These findings provide important data to identify GlyRs in the mesolimbic system and their possible role in alcohol behavior.

Disclosures: A.I. Araya Martínez: None. **S.S. Gallegos:** None. **R. Viveros:** None. **L. San Martín:** None. **L.G. Aguayo:** None. **R.J. Harvey:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.06/W40

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA AA022651

Title: Adolescent alcohol exposure produces sex differences in BNST plasticity following adult stress

Authors: *T. A. WILLS, C. KASTEN, E. HOLMGREN, K. CARZOLI;
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Abstract: Adolescent alcohol is a known risk factor for the future development of alcohol use disorders (AUDs), yet the mechanism that contribute to this increased vulnerability are unknown. Adolescent alcohol exposure may increase this risk by producing adaptations in circuitry involved in stress and negative affect, as these are the primary contributors to relapse. The bed nucleus of the stria terminalis (BNST) is a highly sexually dimorphic brain region that is critical in these behaviors. Our lab finds that withdrawal from chronic intermittent ethanol vapor during adolescence (AIE) in male and female mice increases anxiety-like behavior and glutamatergic transmission in the BNST (through increased glutamate release). AIE also alters BNST plasticity in a sex dependent manner by enhancing NMDAR-plasticity in males and blunting $mGluR_{1/5}$-mediated LTD in females. In the current work, we set out to test the long-term consequences of AIE treatment on BNST plasticity and the effect of an adult stress challenge. Male and female C57BL/6J mice were exposed to two, 4-day cycles of alcohol vapor exposure (16 hr/day) with a 3 day period of rest in between the cycles from PND30-41. In adults (P70+) electrophysiology was performed in the BNST to measure temporal summation of NMDAR-EPSCs, DHPG ($mGluR_{1/5}$ agonist)-induced LTD, and sEPSCs in the absence or one hour following restraint stress (1hr). We found that AIE-induced changes in NMDAR-plasticity and $mGluR_{1/5}$-mediated LTD seen in adolescents were not present in adults under basal conditions (30 days after last alcohol exposure). However, following a stress challenge there was an enhancement of sEPSC frequency and no change in amplitude in mice with an AIE history indicating enhanced glutamate release in the BNST. Further, adult stress plus AIE history produced an enhancement of NMDAR-plasticity in males and an enhancement of $mGluR_{1/5}$-mediated LTD in females. Future work will explore if these sex differences in BNST plasticity are mediated by different cell types, input, and/or projections. The ultimate goal of this work is to identify specific pharmacological targets that could be manipulated to reduce the risk of developing AUDs.

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Poster

414. Neural Circuits Underlying Alcohol Dependence

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Topic: G.08. Drugs of Abuse and Addiction

Support: AA026537
AA027436

Title: Eplerenone antagonism of central amygdala mineralocorticoid receptors reduces alcohol self-administration in female rats

Authors: *V. MAKHIJANI[1,2], J. BESHEER[1,3];
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Abstract: Cortisol/corticosterone and the hypothalamic-pituitary-adrenal (HPA) axis are key regulators of alcohol drinking behaviors. While most alcohol research has focused on the functional involvement of corticosterone and its primary target, the glucocorticoid receptor (GR). Recent studies have indicated that the related mineralocorticoid receptor (MR), which binds both corticosterone and aldosterone, may also regulate alcohol drinking. Our lab has demonstrated that systemic antagonism of the MR with spironolactone can reduce alcohol self-administration but not sucrose self-administration in male and female rats. The present study first sought to understand if these effects on alcohol self-administration were due to suppression of MR mediated corticosterone negative feedback. Female rats trained to self-administer ethanol were administered spironolactone (0, 50 mg/kg; IP) prior to a self-administration session, after the session blood was collected to examine plasma corticosterone levels. Spironolactone (50 mg/kg) significantly reduced alcohol self-administration, had no effect on locomotion, and significantly increased plasma corticosterone. There were no significant correlations between alcohol self-administration and plasma corticosterone levels in either treatment group suggesting that suppression of corticosterone negative feedback was not the primary driver of reduced alcohol consumption. Parallel studies from our lab have shown that a predator odor model of stress enhanced alcohol self-administration can increase MR expression in the dorsal hippocampus of female rats, and central amygdala of male rats. To understand if these brain regions were the locus of MR antagonist action, the selective MR antagonist eplerenone (0, 100, 1000, 5000 ng/0.5 uL/side) was bilaterally infused into the dorsal hippocampus or central amygdala of female rats prior to alcohol self-administration. Eplerenone infusion in the hippocampus had no effect on alcohol self-administration or locomotion. Eplerenone infusion in the central amygdala (1000, 5000 ng/0.5 uL/side) significantly reduced alcohol self-administration, and had no effect on locomotion. These studies further support the role of MR signaling in modulating alcohol

drinking behaviors, and highlight the role of central amygdala MR signaling in alcohol self-administration.

Disclosures: V. Makhijani: None. **J. Besheer:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

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Program #/Poster #: 414.08/W42

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH ZIA- AA000421
NIH Fi2GM117604

Title: Anxiolytic properties of alcohol are elevated in mice with decreased striatal dopamine D2 receptors

Authors: *M. E. BOCARSLY[1], M. BRAVO[1], V. A. ALVAREZ[2];
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Abstract: The literature shows comorbidity between anxiety and the development of alcohol use disorder (AUD). Alcohol has known acute anxiolytic properties. One hypothesis to explain the comorbidity of anxiety and AUD is that alcohol consumption acutely ameliorates the anxiety phenotype and by providing relief enhances the reinforcing properties of alcohol. Animal models are consistent with this clinical observation: rats bred to consume large amounts of alcohol show higher basal anxiety and higher anxiolytic effects of alcohol compared to controls. Despite these behavioral observations, the neural circuitry underlying this comorbidity is unknown. In both humans and rodents, alcohol abuse is associated with low levels of dopamine D2 receptors (D2Rs) in the striatum. Further, in collaboration, our lab has shown that a down regulation of D2Rs on striatal projection neurons (SPN) is associated with anxiety-like behaviors. From these data, we hypothesize that striatal dopamine signaling might underlie the comorbidity of these two disorders. Here, we used transgenic mice with a targeted deletion of the D2R on SPNs, to better understand the common neurobiology underlying anxiety and AUD. Mice with a knockout or knockdown of D2Rs on SPNs show increased anxiety-like behavior on the elevated zero maze, open field and light-dark box compared to littermate controls as well as elevated basal serum corticosterone levels. The D2R knockout and knockdown mice also show decreased preference for 1% sucrose compared to littermate controls, indicating anhedonia. When administered ethanol (1.2 g/kg; i.p.) these mice show enhanced anxiolytic effects of alcohol as measured on the before mentioned behavioral tasks. Interestingly, mice with low levels of D2Rs on SPNs show increased D2Rs in the central amygdala, which have known effects on anxiety-

like behaviors. To better understand the circuitry driving the anxiolytic properties of alcohol we are further examining the role of D2Rs in the amygdala on anxiety and AUD. Taken together, these data begin to describe the neural circuitry underlying and perpetuating the anxiolytic effects of alcohol and comorbidity of anxiety and AUD.

Disclosures: M.E. Bocarsly: None. **V.A. Alvarez:** None. **M. Bravo:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.09/W43

Topic: G.08. Drugs of Abuse and Addiction

Support: Kansas State University
NIH Grant P20GM113109

Title: The effect of ethanol exposure on BDNF levels in enriched and socially isolated rats

Authors: *E. C. BRASE[1], T. J. WUKITSCH[1], J. P. RACK[1], M. E. CAIN[2];
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Abstract: Brain-derived neurotrophic factor (BDNF) is a protein that helps promote synaptic plasticity and regulate addiction-related behaviors. Both ethanol exposure and rearing environment manipulation have independently altered BDNF levels. The current research project examined differentially reared rats' BDNF levels as a result of adolescent intermittent ethanol (AIE) exposure. Male Long-Evans rats arrived in the lab on postnatal (PND) 21 and were randomly assigned to either isolated (IC), enriched (EC), or standard condition (SC) environments for a 30-day rearing period. Rats then underwent AIE treatment, consisting of an injection of either ethanol (2.0 g/kg w/v; i.p.) or an equivalent volume of saline for controls. Injections occurred from PND 27-49 on a schedule of one injection every other day for approximately four weeks to model binging. To determine blood ethanol content (BEC), blood samples were collected from the saphenous vein 30 minutes after the penultimate ethanol administration. BEC was quantified and analyzed to determine differences in alcohol metabolism based on environmental condition. 24 hours after the last AIE treatment, rats underwent a 30-minute locomotor test as a measurement of novelty response and anxiety-related behavior. Preliminary findings suggest that EC rats had less overall total, margin, and center distances traveled (cm) than IC and SC rats, with no main effect of treatment and no interaction between condition and treatment. The next day at PND 51 (late adolescence), rats were euthanized, and brains were extracted and flash frozen. Hippocampal and medial prefrontal cortex tissue samples were collected and homogenized to extract protein, then equilibrated prior to performing a pooled sample ELISA to quantify each experimental condition's total hippocampal and mPFC

BDNF levels. We hypothesized that AIE would reduce BDNF levels across environmental conditions, that ethanol exposed IC rats would have the lowest hippocampal and mPFC BDNF levels, and that control EC rats would have the highest BDNF levels. The ELISA results found no differences between groups; however, hippocampal and mPFC BDNF levels differed from one another as in prior literature. These preliminary results suggest that while environmental manipulation effectively altered locomotor behavior, the environmental manipulation or AIE exposure did not alter BDNF levels in either brain area. It is possible that the length and dosage of ethanol exposure were not enough to alter BDNF expression, that changes may be occurring in other brain areas such as the striatum, or that there are changes occurring in BDNF precursors, such as CREB.

Disclosures: E.C. Brase: None. **T.J. Wukitsch:** None. **J.P. Rack:** None. **M.E. Cain:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

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Program #/Poster #: 414.10/W44

Topic: G.08. Drugs of Abuse and Addiction

Support: R01-AA026844
R01-AA013892
K08-AA023545

Title: Differentiated vulnerability and resilience factors influencing control over alcohol desire in social drinkers

Authors: *C. LARKIN, R. SINHA, D. SEO;
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Abstract: Heavy drinking is a significant public health problem resulting in maladaptive social functioning and adverse health consequences. The current study used functional magnetic resonance imaging (fMRI) and daily smartphone app to examine specific neural and behavioral factors underlying heavy drinking. Participants were sixty-nine adult healthy individuals (30 female; age M = 26.6; SD = 7.14) comprised of heavy (N=17; 13 female) and moderate (N= 52; 17 female) drinkers, with no demographic differences. Participants completed a 2-hour fMRI scan with exposure to stress, alcohol, and neutral-relaxing visual cues. Follow-up self-assessments of mood, stress, alcohol-related behaviors were collected daily for 30 days via a mobile app. Individuals' drinking patterns were categorized with the Cahalan Quantity-Frequency Scale and the Alcohol Use Disorders Identification Test. Neural imaging results indicated that heavy drinkers showed greater striatal response to alcohol cue compared to moderate drinkers ($p<0.001$, whole-brain corrected). Analysis of daily behaviors using decision

trees revealed a group difference in difficulty in controlling desire for alcohol (DCD) with greater difficulties in heavy drinkers ($F(1, 1810) = 15.76$, $p<0.001$). Among all subjects, higher stress management ability was the strongest predictor of lower daily DCD ($F(1, 1808) = 29.61$, $p<0.001$). Analysis of moderate drinkers found stress regulation was the strongest predictor of DCD ($F(3, 1283) = 16.00$, $p<0.001$) with high regulation associated with low DCD. Analysis of heavy drinkers found the strongest predictor of DCD was argumentativeness, one of characteristics associated with behavioral inhibition, with higher stress regulation associated with lower DCD ($F(1, 448) = 31.36$, $p<0.001$). These results suggest that while argumentativeness is the predominant vulnerability factor for DCD among heavy drinkers, a resilience relationship exists between high stress regulation and reduced DCD for all subjects. These exploratory results suggest specific vulnerability and resiliency factors associated with heavy drinking. Heavy drinkers may have greater vulnerability to alcohol urges, explained by behavioral disinhibition which has been linked to heightened striatal activity. Heavy drinkers may have difficulty controlling alcohol desire when they are exposed to vulnerable situations (e.g., alcohol exposure or interpersonal confrontation) but may be more resilient when they are able to exert emotional control in stressful situations. This finding may bear therapeutic implications for the role of stress management in the treatment of problematic alcohol use.

Disclosures: **C. Larkin:** None. **R. Sinha:** None. **D. Seo:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.11/X1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA026638
FWF Grant J-2942-B30
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Title: Sex differences in ethanol and CRF effects on central amygdala GABAergic signaling in alcohol-dependent and naive rats

Authors: *D. KIRSON, S. KHOM, F. P. VARODAYAN, R. R. PATEL, M. ROBERTO; Neurosci., The Scripps Res. Inst., La Jolla, CA

Abstract: Alcohol dependence, equivalent to moderate to severe alcohol use disorder (AUD), is a chronically relapsing disease characterized by a loss of control over seeking and consumption

of alcohol. AUD has been linked to altered functioning of brain stress systems, as chronic alcohol use recruits these systems leading to the negative affective states seen in withdrawal, the relief of which drives further drinking. Both AUD and stress/anxiety disorders have been found to exhibit sex-specific differences among the population. Women are more likely to have an anxiety or mood disorder than men, and men are more likely to abuse alcohol than women. However, women develop addiction more quickly, and are more likely to relapse into repetitive drug use. The central nucleus of the amygdala (CeA) functions as a neuropeptidergic hub of stress and anxiety processing, and GABAergic signaling within the CeA is involved in the regulation of alcohol consumption. We have previously shown dysregulation of GABAergic and stress related peptide systems (e.g., corticotropin releasing factor/CRF) in the CeA following alcohol dependence, but mainly in male rats. In this study, we used whole cell patch clamp electrophysiological recordings to examine basal CeA GABAergic spontaneous inhibitory postsynaptic currents (sIPSC) and the effects of alcohol and CRF in both alcohol naïve and alcohol dependent Sprague-Dawley female rats induced by chronic intermittent alcohol vapor treatment. Basal sIPSC characteristics of frequency, amplitude, and kinetics were not different between naïve males and females, indicating no differences in GABA release or postsynaptic $GABA_A$ receptor functions. However, chronic alcohol vapor treatment in females did not increase sIPSC frequency as it does in males. Additionally, acute alcohol (44mM) had no effect on sIPSC characteristics in naïve or chronic alcohol treated females, in contrast to the increase in sIPSC frequency we have routinely found in males. These results provide important insight into sex differences in CeA neuronal functions with alcohol that may contribute to the development of alcohol dependence.

Disclosures: D. Kirson: None. **S. Khom:** None. **R.R. Patel:** None. **M. Roberto:** None. **F.P. Varodayan:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.12/X2

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant 1F31AA026766-01
NIH Grant R01-DA009411-17

Title: Serotonin 2A receptor agonist treatment improves circuit and behavioral consequences of alcohol exposure

Authors: *B. A. KIMMEY, A. CROICU, N. SHADANI, R. E. WITTENBERG, A. OSTROUMOV, J. A. DANI;
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Abstract: Alcohol abuse remains one of the leading causes of preventable death. A number of risk factors including stress and a history of alcohol use can trigger the initiation or resumption of severe problem drinking making clinical management of alcohol use disorder (AUD) difficult. For this reason, novel pharmacotherapeutic strategies targeting the molecular- or circuit-level neural adaptations giving rise to AUD are needed. Emerging evidence suggests that the ventral tegmental area (VTA), a reward nucleus implicated in drug abuse, is a critical substrate of experience-dependent plasticity which drives aberrant alcohol drinking. Our work, among others, demonstrates that inverted GABA-mediated inhibition of VTA GABA neurons, as reflected by a depolarized $GABA_A$receptor reversal potential (E_{GABA}), contributes to increased alcohol (ethanol) consumption in rodents. This heightened ethanol-induced excitability of VTA GABA neurons results from chloride dyshomeostasis and is mediated by reduced function of the K^+-Cl^- cotransporter, KCC2. Targeting this molecular adaptation, therefore, represents a novel strategy to mitigate AUD in vulnerable populations. KCC2 is modulated *in vivo* by a number of intracellular effectors. Among these, activation of metabotropic 5-HT_{2A}-type receptors (5-HT_{2A}Rs), which increase PKC signaling downstream of receptor stimulation, was shown to enhance KCC2 function following spinal cord injury. This interaction extends to the VTA, as our preliminary data suggest that downregulated KCC2 is restored by 5-HT_{2A}R agonist treatment following stress exposure. Here, we reveal that KCC2 function is comparably diminished by both acute and chronic ethanol exposure in mice. Acute injection of an intoxicating dose of ethanol or protracted ethanol drinking in a two-bottle choice paradigm robustly diminished chloride transport function and depolarized E_{GABA} in VTA GABA neurons. Treating mice with the 5-HT_{2A}R agonist, TCB-2, after ethanol exposure, strengthened chloride extrusion and hyperpolarized E_{GABA} to the pre-ethanol state. In parallel, TCB-2 reduced ongoing ethanol drinking behavior. Restoration of KCC2 function following TCB-2 treatment was achieved by direct chloride transport modulation in VTA GABA neurons downstream of 5-HT_{2A}R activation. Taken together, these results suggest that KCC2-mediated chloride transport dysregulation is a potential target for limiting AUD. Moreover, we provide novel mechanistic insight into the therapeutic action of 5-HT_{2A}R agonists on alcohol-induced neural adaptations in VTA GABA neurons.

Disclosures: B.A. Kimmey: None. **A. Croicu:** None. **N. Shadani:** None. **R.E. Wittenberg:** None. **A. Ostroumov:** None. **J.A. Dani:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

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Title: Glucagon-like peptide-1 system is modulated by acute and chronic exposure to alcohol: Findings from human laboratory experiments and a brain postmortem study

Authors: *B. D. BROWNING[1], M. FAROKHNIA[1,2], H. SUN[3], P. SUCHANKOVA KARLSSON[5], L. FARINELLI[1], M. LEE[1], F. AKHLAGHI[6], L. LEGGIO[1,2,4,7];
[1]NIAAA/NIDA, [2]Ctr. on Compulsive Behaviors, [3]NIAAA, NIH, Bethesda, MD; [4]Medication Develop. Program, NIH, Baltimore, MD; [5]Univ. of Gothenburg, Gothenburg, Sweden; [6]Biomed. and Pharmaceut. Sci., Univ. of Rhode Island, South Kingstown, RI; [7]Dept. of Behavioral and Social Sci., Brown Univ., Providence, RI

Abstract: Glucagon-Like Peptide-1 (GLP-1) is a 30-amino acid peptide primarily produced by endocrine cells in the intestines. GLP-1 is an incretin and regulates glucose homeostasis, appetite, and food intake via central (e.g., hypothalamus) and peripheral (e.g., pancreas) pathways. GLP-1 also acts as a neuropeptide and both the peptide and its receptor (GLP-1R) are expressed in the brain. Previous data indicate that GLP-1 signaling may play a role in biobehavioral mechanisms underlying reward processing related to not only food but also alcohol and drugs of abuse. Indeed, rodent studies suggest that GLP-1R may represent a novel therapeutic target for alcohol use disorder (AUD). Therefore, it is also important to understand how excessive alcohol drinking may affect the endogenous GLP-1 system. To this end, we conducted a series of secondary analyses on human laboratory experiments to examine the effect of alcohol administration on peripheral blood GLP-1 concentrations in heavy-drinking individuals. Specifically, four separate alcohol administration sessions were conducted: oral self-administration (variable dose), oral fixed dose, intravenous self-administration (variable dose), and intravenous fixed dose. Repeated blood samples were obtained during each session and GLP-1 concentrations were measured via a bead-based multiplex ELISA assay. In all four experiments, acute administration of alcohol consistently resulted in significant reduction of peripheral GLP-1 concentrations (p's < 0.05). Next, we looked at the GLP-1R gene expression in postmortem brain tissue from patients with AUD and healthy controls (New South Wales Tissue Resource Centre, University of Sydney). GLP-1R mRNA was extracted from five brain regions (i.e., prefrontal cortex, ventral tegmental area, nucleus accumbens, amygdala, and hippocampus), and real-time quantitative PCR with TaqMan gene expression assay was run. Results showed that fold change in GLP-1R mRNA in the hippocampus was significantly higher in patients with AUD compared to healthy controls ($p = 0.007$). Collectively, these data elucidate how exposure to alcohol influences the GLP-1 system, both in the periphery and in the brain. Future studies should replicate the present findings and investigate whether targeting the GLP-1 system may represent an effective pharmacological approach to treat AUD.

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Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

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Program #/Poster #: 414.14/X4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA020919
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Title: Effects of TrkB activation by alcohol withdrawal-mediated secretions of BDNF following chronic alcohol exposure

Authors: *A. J. PAYNE, J. N. BRUNDAGE, A. L. STOCKARD, M. A. NUMA, S. C. STEFFENSEN;
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Abstract: Brain-derived neurotrophic factor (BDNF) is implicated in varied physiological processes. Its main receptor in the central nervous system is tyrosine receptor kinase B (TrkB), and the main ligand for TrkB in the central nervous system is BDNF. It has been shown previously that activating TrkB can cause a downregulation of the chloride-exporting potassium chloride cotransporter 2 (KCC2), presumably resulting in a decreased chloride gradient. It is hypothesized that this down-regulation, caused by an increase in BDNF levels, creates hyperexcitable GABA neurons in the ventral tegmental area (VTA) due to a reduced efficacy of inhibitory currents. Here we investigate this mechanism of adaptation in the context of chronic alcohol exposure. We demonstrate that BDNF levels are elevated in the VTA during withdrawal from chronic alcohol exposure. We also observe that blocking TrkB activity decreases alcohol seeking behavior. Further, we investigate the expression patterns of KCC2 in connection with chronic alcohol administration. Additional work is underway to validate this mechanism and further elucidate its putative role in alcohol dependence and its potential as a treatment target.

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Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.15/X5

Topic: G.08. Drugs of Abuse and Addiction

Support: R01 AA022445

Title: Mesolimbic circuit function underlying individual alcohol drinking

Authors: *S. E. MONTGOMERY[1], C. MOREL[1], B. JUAREZ[2], M. FLANIGAN[1], E. S. CALIPARI[3], S. J. RUSSO[1], E. J. NESTLER[1], M.-H. HAN[1];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Pharmacol., Univ. of Washington, Seattle, WA; [3]Pharmacol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Harmful alcohol use remains a serious public health issue, resulting in 3 million global deaths per year and contributing to more than 200 disease and injury conditions. Within the United States, the prevalence of Alcohol-Use Disorder (AUD) has increased significantly from 8.5% to 12.7% over the last 10 years and whose complex etiology has limited the number of effective therapeutics currently available. An interesting phenomenon in alcohol drinking is the variability of consumption occurring within the human population; some individuals drink casually while others drink in an uncontrolled manner, escalating their consumption and eventually developing alcohol dependence. To understand the circuit-specific functions underlying this phenomenon of individual alcohol drinking variability, we utilized isogenic C57BL/6J mice, an inbred mouse strain typically used to study alcohol-drinking behaviors. This mouse model provides the unique opportunity to investigate the neurophysiological mechanisms underlying low and high alcohol drinking behaviors, independent of genetics. Furthermore, it is known that a hallmark of the progression of AUD is the dysfunction of dopamine (DA) neurons projecting from the ventral tegmental area to the nucleus accumbens (VTA-NAc), a neural circuit critical to encoding the salience of both drug and naturalistic stimuli. Using *in vivo* fiber photometry calcium imaging and *in vivo* electrophysiological recordings, we are now able to determine the neural population response of the VTA-NAc DA circuit before and after the establishment of alcohol drinking phenotype, to illuminate the transition to low or high alcohol drinking profiles. Our preliminary data show that the magnitude of the primary reinforcing VTA-NAc DA response to rewarding and salient stimuli correlates with future establishment of alcohol preference. Further, alcohol-induced neuroadaptations differentially affect naturalistic behaviors in mice, including exploration and response to reward, and determine heightened or blunted responses to future alcohol exposure. By assessing the VTA-NAc DA neuronal profile of activity during naturalistic mammalian behaviors prior to and after alcohol exposure, this project will provide novel insight into physiological and real-time predictors of future, individual alcohol drinking phenotypes and how alcohol actively and reciprocally attenuates or exacerbates VTA-NAc DA circuit function, leading to subsequent maladaptive behaviors.

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Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.16/X6

Topic: G.08. Drugs of Abuse and Addiction

Support: UMBC CNMS Pre-Professoriate Fellowship

Title: Uncovering the genetic basis of courtship and naive odor preference in *Drosophila*

Authors: R. OLIVER[1], N. REGER[2], *F. J. VONHOFF[1];
[1]Univ. of Maryland Baltimore County, Baltimore, MD; [2]UMBC, Baltimore, MD

Abstract: We are testing how the relationship between genetics and sociability in the fruit fly *Drosophila* melanogaster triggers an addiction-like state preceding their first exposure to alcohol. Previous research demonstrated that sexual deprivation in *Drosophila* males caused them to consume alcohol at a higher rate. In addition, flies show a certain degree of naïve ethanol odor preference (NEOP) because they are innately lured to fermented fruit. It has been previously suggested that flies with high NEOP levels may be predisposed to consume more alcohol through adulthood. We tested virgin and mated flies of different ages in T-maze traps containing apple juice supplemented with ethanol in one arm. Our data indicate that courtship status was relevant to NEOP in males, but not females. Three-day-old virgin males showed a significantly higher NEOP to 23% ethanol than those that just mated. By contrast, females of all ages consistently avoided the side containing the 23% ethanol, independent of their mating status. Moving forward, we plan to uncover some of the genetic and cellular mechanisms that contribute to this dynamic phenomenon, as it is a pivotal step to understand the neurobiology of addiction.

Disclosures: R. Oliver: None. **N. Reger:** None. **F.J. Vonhoff:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA024571
NIH Grant AA025481

Title: Prefrontal and orbitofrontal cortex neurons are differentially activated during passive and active alcohol acquisition: Influences of individual preference and chronic exposure

Authors: *B. KAMINSKA, M. L. BERKOWITZ-CERASANO, D. E. MOORMAN;
Univ. of Massachusetts, Amherst, MA

Abstract: Chronic and compulsive alcohol (ethanol, EtOH) use results in impoverished health and well-being, but the neural roots of this disease are still poorly understood. The goals of our studies are to understand how neuronal ensembles encode EtOH preference and motivation, and how this encoding changes in compulsive EtOH use and addiction. These studies focus on the rodent medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC) - areas with strong ties to reward preference, motivation, and addiction. Our studies test the hypotheses that specific patterns of mPFC and OFC neuronal activity encode individual differences in EtOH use and that these patterns are altered after chronic EtOH exposure.
To address these hypotheses, 6 female and 6 male Wistar rats were conditioned to drink 20% EtOH using homecage intermittent access for 1 month (acute exposure) or 3 months (chronic exposure). All rats were then implanted with intraoral catheters and drivable tetrode arrays. We recorded from mPFC and OFC neurons during 1) a passive classical conditioning task in which tone cues predict outcome (EtOH, sucrose, or quinine) delivery directly into the mouth and 2) an active classical conditioning task in which tone cues predict outcome availability at a reward port. Preference for EtOH was characterized by final homecage consumption, resistance to quinine adulteration, taking intensity during active task, and oromotor responses to intraoral EtOH in the passive task. We have thus far recorded 721 neurons from 5 rats (3 female, 2 male) in the 1 month exposure group:102 neurons in prelimbic mPFC, 185 in infralimbic mPFC and 434 in OFC. Across these populations, ~70% of mPFC and ~75% of OFC neurons responded significantly to one or more cues and/or outcomes. Neurons responded to most combinations between active/passive task and sucrose, EtOH, and quinine cues/outcomes, with an overall bias towards sucrose and EtOH associated cues and outcomes. Neurons primarily responded to a single cue (e.g., passive EtOH) or to outcome- or action-specific pairs of cues (e.g., passive and active sucrose cues). Additional recordings and analyses are underway to identify contributions of individual preference and exposure history to neural firing. The current results indicate that mPFC and OFC neurons are strongly influenced by EtOH-associated cues, outcomes, and behaviors and that we can identify the neural correlates of EtOH value relative to outcomes with more stable valence (e.g., sucrose or quinine). Based on our previous work and preliminary analysis, we also expect, that neural signals vary in relation to individual preference and motivation to seek/consume alcohol.

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Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

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Program #/Poster #: 414.18/X8

Topic: G.08. Drugs of Abuse and Addiction

Support: R37-AA014983
P60-AA011605

Title: Transmembrane AMPA regulatory protein-γ8 (TARP) knockout male mice show blunted operant responding for and reinstatement to alcohol

Authors: *S. P. FACCIDOMO[1], J. L. HOFFMAN[1], S. TAYLOR[1], M. KIM[1], C. W. HODGE[2]; [1]Alcohol Studies Ctr., [2]Psychiatry, UNC Chapel Hill, Chapel Hill, NC

Abstract: Alcohol has long been shown to disrupt glutamatergic function and related behaviors that can lead to the escalation of drinking and long-term alcohol abuse. The AMPA receptor (AMPAR) specifically has profound effects on synaptic and behavioral plasticity which are critical to the development of alcohol seeking behaviors and addiction. Transmembrane AMPA regulatory proteins (TARPs) are required for AMPAR trafficking and tethering of the receptor to excitatory synapses. The TARPs can modify AMPAR channel gating, regulate synaptic plasticity, and thereby alter post-synaptic expression and regulate output. The TARP subtype, TARP-γ8, is highly restricted to areas of the brain critical to conditioned alcohol consumption such as the frontal cortex, hippocampus, and basolateral amygdala making it a likely mechanism that mediates behavioral pathologies associated with alcohol addiction. We used two complementary strategies, a TARP-γ8 knockout mouse and pharmacological manipulation, to test the hypothesis that TARP-γ8 activity modulates the positive reinforcing effects of alcohol. TARP-γ8 KO mouse were physically and behaviorally similar to wild-type (WT) control mice. Importantly, there were no differences between the TARP-γ8 KO mice and WT controls in voluntary home-cage alcohol drinking or the sedative-hypnotic properties of alcohol. However, TARP-γ8 KO mice showed significantly reduced alcohol-reinforced lever pressing in operant conditioning chambers as compared to WT control mice. In addition, TARP-γ8 KO mice showed a blunted increase in operant alcohol self-administration following a concentration-induced escalation procedure, faster extinction of alcohol-reinforced operant responding and an absence of alcohol-seeking during a cue-induced reinstatement test. In the pharmacological study, systemic administration of JNJ-55511118, a selective negative modulator of AMPARs bound to TARP-γ8, decreased operant alcohol self-administration in WT mice, but had no effect in TARP-γ8 KO mice. Together, these data strongly suggest that TARP-γ8 regulation of AMPAR activity is required for the positive reinforcing effects of alcohol, which are crucial in the acquisition, maintenance and relapse of alcohol addiction. This work moves the field forward in

understanding the molecular mechanisms by which alcohol hijacks reward processes and has potential for the development of new pharmacotherapeutic strategies.

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Poster

414. Neural Circuits Underlying Alcohol Dependence

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA R01AA016852
NIAAA T32AA007565
NINDS R01NS105005
DOD/TSCRP TS130081
NIAAA P50 AA025117

Title: Molecular and physiological dysregulation of Kv1.1 contribute to alcohol withdrawal induced hyperexcitability within the hippocampus

Authors: *D. L. DOBBINS[1], H. X. EGIDO-BETANCOURT[1], A. T. GOLDSTEIN[1], T. SMITH[1], K. F. RAAB-GRAHAM[2], D. W. GODWIN[1];
[1]Neurobio. and Anat., [2]Physiol. and Pharmacol., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: Chronic use of alcohol perpetuates inhibition within the brain causing compensatory changes which are long lasting and lead to the symptoms associated with alcohol withdrawal (WD) syndrome. As individuals relapse and undergo repeated WD cycles kindling-like processes occur and lead to neuronal hyperexcitability. Thus, with each WD episode the severity and risk for potentially lethal seizures increase. To date little is known about the underlying molecular mechanisms responsible for shifting a neurons intrinsic properties toward a hyperexcitable state. Previous studies indicate hyperexcitability during WD may be mediated in part by specific ion channels that are differentially regulated by signaling pathways controlling protein synthesis, specifically mTOR. Increased mTOR activity has been observed during WD, is associated with many forms of epilepsy, and can repress the expression of the voltage-gated ion channel Kv1.1. Importantly, Kv1.1 contributes to the action potential frequency and helps set the resting membrane potential. Thus, we hypothesize WD mediated increase in mTOR activity reduce Kv1.1 expression and activity, leading to increased excitability. To test this, we subjected mice to a chronic intermittent exposure paradigm to assess the state of mTOR signaling and expression of Kv1.1 with repeated WD. Expression of these targets and local field potentials were examined in the hippocampus. Our results demonstrate repetitive WD cycles increase

mTOR activity and transcriptionally downregulate Kv1.1. This decrease in transcript suggests less Kv1.1 could be locally synthesized to prevent a further shift in excitability with additional WD episodes. We administered the chemoconvulsant pentylenetetrazol (PTZ) during WD to examine this possibility and found alterations to Kv1.1 and mTOR increased seizure susceptibility and correlate with seizure severity. Physiological dysregulation of these targets during WD were also observed by examining alterations to population spike activity with the administration of the mTOR inhibitor, Rapamycin, and 4-Aminopyridine (4AP). This study demonstrates mTOR and Kv1.1 dysregulation contribute to WD-induced hyperexcitability as observed through their aberrant molecular and physiological regulation within the hippocampus. These findings inform mechanisms of seizure activity during alcohol WD and present potential therapeutic targets to improve treatment for these events.

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Poster

414. Neural Circuits Underlying Alcohol Dependence

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIMD 2G12MD007592
NIAAA R15AA020996.

Title: Dopamine D2 receptor in ethanol induce behavioral sensitization

Authors: *N. M. DELGADO, C. M. SIERRA, P. R. SABANDAL, K. A. HAN;
Univ. of Texas At El Paso, El Paso, TX

Abstract: Males rarely court other males in *Drosophila melanogaster*. However, ethanol experience causes disinhibited inter-male courtship that is augmented with repeated ethanol exposures. Our previous work uncovered the requirement of dopamine signaling and the D1-like Dopamine/Ecdysone receptor, but not D1 or D5 receptors, for this type of ethanol-induced behavioral sensitization. The role of the D2-like DA D2 receptor (dD2R), however, remains uncharacterized and this study addresses this knowledge gap. To do this, we exposed the wild-type *Canton-S* (*CS*) and D2 receptor mutant (*d2r*) males to ethanol till sedation every day and monitored the behavioral responses including initial sensitivity, tolerance development, locomotor response and disinhibited courtship. Compared to *CS*, *d2r* flies exhibited normal initial sensitivity to the sedative effect of ethanol but displayed reduced tolerance, blunted locomotor response as well as substantially dampened behavioral sensitization to the disinhibition effect of ethanol. These findings reveal multiple roles of dD2R in ethanol-induced

behaviors. We found that re-expression of either of the three major D2 receptor isoforms differing in the third intracellular loop in either all or mushroom body neurons fully rescued the *d2r*‘s sensitization phenotype, indicating that the functional site of dD2R for sensitization is the mushroom body neurons. We are in the process of mapping the neural sites for the dD2R role in locomotor response. Our research may provide novel insights into the mechanisms that dD2R mediates multiple effects of ethanol.

Disclosures: N.M. Delgado: None. **C.M. Sierra:** None. **P.R. Sabandal:** None. **K.A. Han:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

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Topic: G.08. Drugs of Abuse and Addiction

Support: AA020919
DA035958

Title: Voluntary exercise protects against ethanol induced sensitization of kappa opioid receptors in the nucleus accumbens

Authors: *J. BRUNDAGE, K. BILLS, J. SANDRES, B. RICHMOND, S. STEFFENSEN, J. YORGASON;
Brigham Young Univ., Provo, UT

Abstract: Despite many advances in treatment options, the relapse rate for alcohol use disorders (AUD) remains around 50%. It is vital for recovering addicts that we better understand the mechanism of addiction in order to prevent relapse and speed the healing process. Exercise has long been prescribed as part of alcohol abuse disorder treatment despite the fact that the exact way it affects the reward system is largely uncharacterized. Recent work in our lab has shown that a regiment of aerobic exercise decreases the efficacy of K-Opioid Receptors (KOR), a protein upregulated by endogenous opioids and heavily involved in alcohol seeking behavior and alcohol abuse disorder.KORs are part of the endogenous opioid system, which is strongly associated with positive and negative reinforcement of alcohol abuse. In the addicted brain, they are known to modulate seeking behavior by attenuating dopamine (DA) release in the nucleus accumbens (NAc). Dopamine release in the NAc is highly related to seeking behavior in addiction. As stated above, previous data has shown that KORs are upregulated in alcohol addicted mice. In different studies, upregulated KORs were also linked to increased seeking behavior in mice. In this study, we found that a regiment of aerobic exercise was protective against ethanol induced sensitization in the KORs compared to non-exercised controls. This was

determined by changes in DA release as measured by *ex vivo* cyclic voltammetry. Similarly, aerobic exercise decreased seeking behavior compared to non-exercised controls in drinking in the dark models.

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Poster

414. Neural Circuits Underlying Alcohol Dependence

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Program #/Poster #: 414.22/X12

Topic: G.08. Drugs of Abuse and Addiction

Title: Role of extended amygdala in ethanol reward seeking altered by developmental adversities

Authors: *M. BJORNI, G. ETEM, N. ROVERO, A. QUAN, H. HENDERSON, M. BELNAP, L. HALLADAY;
Psychology, Santa Clara Univ., Santa Clara, CA

Abstract: Millions of adults and over half a million youth in the United States suffer from Alcohol Use Disorder (AUD), a chronic brain disease that leads to continued use of alcohol despite its negative health, social, and occupational effects. Yet we lack an exact understanding of the physiological mechanisms that underlie alcohol reward seeking behavior and how they might be affected by developmental adversities. To address this, we investigated the neural mechanisms underlying the ability of early life stress and/or exposure to alcohol in adolescence to alter adulthood alcohol seeking behavior. To model early life trauma, mice underwent maternal separation procedures in the first two weeks of life, which has been shown to lead to later behavioral deficits. Mice were separated from their mothers for 4 hours (PD2-5) or 8 hours (PD6-16) a day before being weaned early, at PD17. Control mice were left undisturbed in their home cage for the full preweanling period (< PD23). To model adolescent alcohol exposure, mice were given free access for 4 consecutive nights a week to either a 20% ethanol solution or water during adolescence (weeks 5-8 of life). We examined the contribution of specific neural mechanisms to natural versus ethanol reward preference by manipulating neural activity in the bed nucleus of the stria terminalis (BNST) or the central amygdala (CeA) using chemogenetics, as BNST and CeA have been implicated in reward seeking and addiction. Mice received surgical microinjections of inhibitory or excitatory DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) into BNST or CeA after reaching adulthood (i.e., week 8). Mice were then trained in an operant task to discriminate between a natural (10% sucrose) and ethanol reward (10% sucrose + 10% ethanol). After discrimination training, mice were presented with both levers simultaneously for the first time in a preference session. Data collected on the first preference test day provided insight to whether stress and/or adolescent alcohol exposure

affected the preference for responding to sucrose- versus sucrose + ethanol-rewarded levers. Through DREADDs activation, we manipulated neural activity on a second preference test day, using the first preference day for baseline comparison. Our preliminary data indicate that developmental adversities alter reward seeking in adulthood and that manipulating neural transmission in the BNST and CeA affects performance on our preference task. These findings contribute to our greater understanding of the neural mechanisms that influence alcohol seeking behavior, and thus help the scientific community on its goal towards developing better, targeted AUD treatments.

Disclosures: **M. Bjorni:** None. **G. Etem:** None. **N. Rovero:** None. **A. Quan:** None. **H. Henderson:** None. **M. Belnap:** None. **L. Halladay:** None.

Poster

414. Neural Circuits Underlying Alcohol Dependence

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 414.23/X13

Topic: G.08. Drugs of Abuse and Addiction

Title: Pharmacokinetics of ethanol in male and female Japanese quail

Authors: *S. E. EATON, J. E. JAGIELO-MILLER, M. A. PRENDERGAST, C. K. AKINS; Dept Psychol, Univ. Kentucky, Lexington, KY

Abstract: In humans, females tend to absorb and metabolize ethanol at different rates than males. Specifically, at similar doses, females achieve higher blood ethanol concentrations (BECs) and have faster elimination than males. Japanese quail may be an ideal model for studying the effects of ethanol consumption and sex differences. Birds are more sexually dimorphic than mammals, and hormones can easily be manipulated without surgery. Because relatively little research has examined ethanol effects in quail, there is not much information on metabolism and elimination differences between males and females. Understanding the absorption and metabolism rates of ethanol is critical to developing a working model of alcohol use disorder. Therefore, we aimed to document the BEC profile in male and female quail. Male (n=12) and female quail (n=6) were gavaged with 0.75 g/kg ethanol daily for 7 days or received 2-3 g/kg. Blood samples were taken 30, 60, 120, and 240 minutes after administration on day 1 and 7. Samples were analyzed for ethanol content. The findings indicate that the 3 g/kg dose produced higher BECs than the 0.75 g/kg dose [$F(1,3)=263.41$, $p<.05$]. The area under the curve (AUC) was similar between both males and females treated with 3 g/kg, p= n.s. Additionally, the rate of absorption and elimination for both males and females treated with 3 g/kg was similar, p=n.s. However, female quail treated with 0.75 g/kg appeared to eliminate ethanol quicker than their male counterparts [$F(2,10)=15.81$, $p<.05$]. Specifically, females had lower BECs than males at 240 min following gavage [$F(1,5)=20.47$, $p<.05$]. Both males and females treated with 0.75

g/kg had similar AUC, p= n.s, but the slope from peak to 240 min differed significantly, [$F(1,2)=24.97$, $p<.05$]. The elimination difference between sexes may be due to gastric or hepatic metabolic and alcohol dehydrogenase differences between the sexes. Additionally, both males and females had higher BECs on day 7 than on day 1 [$F(1,9)=19.04$, $p<.05$]. This increase across days may be due to remaining unmetabolized ethanol in the bloodstream from the previous day, suggesting quail metabolize alcohol slowly.

Disclosures: **J.E. Jagielo-Miller:** None. **M.A. Prendergast:** None. **C.K. Akins:** None. **S.E. Eaton:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.01/X14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA-IRP, NIH/DHHS Z1A DA000389
NIDA-IRP, NIH/DHHS Z1A DA000611

Title: Reinforcing effects of cocaine are modulated by dopamine-independent actions of modafinil: Possible involvement of gap junctions

Authors: M. MEREU, T. HIRANITA, C. J. JORDAN, L. E. CHUN, J. P. LOPEZ, M. A. COGGIANO, J. C. QUARTERMAN, G.-H. BI, J. D. KEIGHRON, Z.-X. XI, A. H. NEWMAN, J. L. KATZ, ***G. TANDA**;
NIDA-IRP, Baltimore, MD

Abstract: Modafinil has been shown to block the dopamine (DA) transporter, a mechanism shared by cocaine, methylphenidate, and other psychostimulants. Modafinil is clinically available for the treatment of narcolepsy and other sleep disorders. Recently, its non-medical use as a “smart drug” is raising concerns regarding its potential for abuse by populations that might also use illicit substances. In this study, we compared the potential for abuse of modafinil with that of methylphenidate, another prescribed medication, and their interactions with the reinforcing effects of cocaine in rats trained to self-administer cocaine. We also assessed changes in DA levels in the nucleus accumbens shell, a brain area related to reinforcing effects of drugs, using microdialysis procedures in rats. Methylphenidate (0.03-1.0 mg/kg) maintained intravenous self-administration behavior at comparable levels as active doses of cocaine (0.03-1.0 mg/kg), whereas modafinil (0.1-10 mg/kg) failed to do so at any dose tested. Nonetheless, both modafinil (10-32 mg/kg, i.p.) and methylphenidate (1.0-10 mg/kg, i.p.) pretreatments potentiated cocaine self-administration. Cocaine, at self-administered doses, produced dose-related stimulation of DA concentrations in the nucleus accumbens shell. Methylphenidate (1.0-

10 mg/kg, i.p.), but not modafinil, (10-32 mg/kg, i.p.), enhanced cocaine-induced stimulation of DA levels, indicating that the effects of methylphenidate on cocaine actions are DA-dependent, while those of modafinil are not. Modafinil is known to facilitate electrotonic coupling between cells by actions on gap junctions. Carbenoxolone, a gap junction inhibitor, reduced the potentiation of cocaine self-administration produced by modafinil but not by methylphenidate. In conclusion, modafinil shares with cocaine and methylphenidate an important action at the DA transporter, but the present data indicate a unique pharmacological stimulant profile, lacking abuse potential and facilitating electrotonic coupling. These results, together with clinical studies, further suggest a potential therapeutic use of modafinil in patients with cocaine use disorder.

Disclosures: **M. Mereu:** None. **T. Hiranita:** None. **C.J. Jordan:** None. **L.E. Chun:** None. **J.P. Lopez:** None. **M.A. Coggiano:** None. **J.C. Quarterman:** None. **G. Bi:** None. **J.D. Keighron:** None. **Z. Xi:** None. **A.H. Newman:** None. **J.L. Katz:** None. **G. Tanda:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.02/X15

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH / NIDA

Title: The role of dietary and synaptic zinc on cocaine abuse vulnerability

Authors: *J. L. GOMEZ, K. WRIGHT, M. MICHAELIDES;
Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Zinc (Zn^{2+}) is an essential life element, and dysregulation or deficiency is associated with various disorders. Zn^{2+} is highly concentrated in the brain where it is thought to exert effects on synaptic plasticity, neurotransmission, protein/enzyme function, and transcriptional regulation. Zinc can be found in two forms, bound (structural) and labile (free). This unbound free zinc can be co-packaged into vesicles and co-released with glutamate acting as an allosteric modulator. Thus, free zinc is tightly regulated by a family of 24 transporters that move zinc around the cell. Our lab has focused on ZnT3 (Slc30a3), the transporter that shuttles zinc into glutamate vesicles. Previous research by our lab has demonstrated that artificially manipulating striatal zinc, via chelator, causes a reduced level of cocaine sensitization with long lasting effects. Additionally, dietary or genetic manipulation alters reward and sensitivity to cocaine without effecting normal mouse behavior. This project explores the manipulation of dietary zinc and ZnT3 as a factor of cocaine abuse vulnerability.

Disclosures: **J.L. Gomez:** None. **K. Wright:** None. **M. Michaelides:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.03/X16

Topic: G.08. Drugs of Abuse and Addiction

Support: FONCyT. Prestamo BID PICT 2015 1622
FONCyT. Prestamo BID PICT 2012 1867
CONICET
SECyT

Title: Minocycline prevents chronic restraint stress-induced cocaine sensitization and morphological changes of microglia within nucleus accumbens core

Authors: ***M. P. AVALOS**, E. A. GOROSTIZA, M. SANCHEZ, A. S. GUZMAN, D. RIGONI, F. A. BOLLATI, L. M. CANCELA;
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Abstract: It is well known that individuals suffering from stress disorders are vulnerable to developing drug abuse. In animal models, studies from our lab showed that exposure to restraint stress engenders long-lasting neuroadaptations on glutamatergic system within Nucleus Accumbens (NA) which enables sensitized response to cocaine (Esparza et al., 2012, Garcia-Keller et al., 2013) and facilitation of cocaine self-administration (Garcia-Keller et al., 2016). On the other hand, our previous findings evidenced a role of glutamate in enduring psychostimulant-induced sensitization at the immune level in a parallel way to that occurring in the limbic system (Assis et al., 2009, 2011). However, there is no description so far of which is the role played by microglia in stress-induced cocaine sensitization. The aim of this study was to evaluate the effect of minocycline, a potent inhibitor of microglia activation, in chronic restraint stress-induced cocaine sensitization and morphological changes of microglia within NA core. Thus, Sprague Dawley rats were exposed to restraint stress 2 h daily for a week. From day 16 to day 21 after the first stress session, all animals were treated with minocycline (30 mg/Kg/12 h) or vehicle (DMSO 5%/12 h). On day 21, locomotor activity was measured following saline or cocaine challenge (15 mg/Kg). Then, in order to examine fluorescence intensity and the morphology of microglia, immunofluorescence by labeling Iba-1 was performed. Our results pointed out that minocycline was able to prevent chronic stress-induced cocaine sensitization suggesting that microglia play a key role in this phenomenon. Interestingly, stress induced hyper-ramification of microglia and enhancement of Iba-1 fluorescence intensity within NA core. Likewise, minocycline prevented those morphological changes of microglia and restored the expression of

Iba-1. Accordingly, we propose that microglia could contribute to the development of pathological neuroadaptations within NA core following chronic restraint stress to trigger cocaine sensitization. These results strongly prompt to think that minocycline might be considered as a promising therapeutic agent in preventing the comorbidity between stress and drug abuse. Next findings will attempt to deepen the study of these chronic restraint stress-induced changes to promote addiction-related disorders.

Disclosures: M.P. Avalos: None. **E.A. Gorostiza:** None. **M. Sanchez:** None. **A.S. Guzman:** None. **D. Rigoni:** None. **F.A. Bollati:** None. **L.M. Cancela:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.04/X17

Topic: G.08. Drugs of Abuse and Addiction

Title: Enhanced reward motivation and sensitized fear in adult rats exposed to a single trauma during early development

Authors: *M. M. COBB[1], A. N. HOFFMAN[1], A. GOLD[1], M. S. FANSELOW[1], P. J. KENNEDY[2];
[1]Psychology, UCLA, Los Angeles, CA; [2]Psychology, Univ. of California Los Angeles, Los Angeles, CA

Abstract: The prevalence of comorbidity between post-traumatic stress disorder (PTSD) and substance use disorders and obesity, suggests altered reward processing in individuals suffering from PTSD. Early life stress (ELS) such as repeated maternal separation increases both the risk for development of PTSD related symptoms and addiction-like behaviors in adulthood. Less is known about the long-term effects of a single, painful traumatic experience during early development. Here we exposed male juvenile rats (PND 19) to 15 unpredictable footshocks (1mA, 1s) over a 90 minute session and tested for sensitized fear learning and reward-seeking behavior in adulthood. Our findings replicate previous data showing that adult rats exposed to this paradigm of ELS demonstrate sensitized fear learning, a component of PTSD (Poulos et al., 2014). We next trained control and ELS rats to self-administer sugar pellets on an FR1 schedule of reinforcement followed by progressive ratio (PR) testing. We found that control and ELS rats did not differ in their acquisition of sugar self-administration however, ELS rats had a significantly higher breakpoint during the PR test. These data indicate that a single trauma during early development may have lasting effects on reward processing and valuation. Ongoing behavioral studies are investigating changes in motivation for drugs of abuse in combination with both longitudinal microPET and qRT-PCR analyses in striatal circuits to identify

developmental changes in dopamine receptor function and expression following early life trauma.

Disclosures: M.M. Cobb: None. **A.N. Hoffman:** None. **A. Gold:** None. **M.S. Fanselow:** None. **P.J. Kennedy:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.05/X18

Topic: G.08. Drugs of Abuse and Addiction

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Capes

Title: Conditioned place preference to cocaine in leptin receptor deficient diabetic (db/db) mice

Authors: *D. C. AGUIAR[1], J. A. S. L. GOMES[2], P. H. GOBIRA[3];
[1]Pharmacol., Univ. of Minas Gerais- UFMG, Belo Horizonte, Brazil; [2]Inst. of Biotech., Federal Univ. of Uberlandia, Belo Horizonte, Brazil; [3]Univ. of São Paulo, Ribeirao Preto, Brazil

Abstract: Introduction: The neurobiological mechanisms that regulate the response to addictive drugs are complex, and several evidence have been shown the link between neural control of appetite regulation and substance abuse. Leptin, an important adipose hormone, it´s circulating levels reflect the body's energy stores in adipose tissue. It regulates energy balance and appetite control via leptin receptors expressed in central nervous system. Previous data indicate that leptin signaling is critically involved in cocaine-conditioned reward. The infusion of the leptin receptor antagonist during cocaine conditioning increased the cocaine-conditioned place preference and the infusion of the leptin in nucleus accumbens core disrupts acute cocaine effects. Complementing these findings in animals, the leptin serum levels is inversely correlated with the severity of crack use in humans. Although data indicate that leptin signaling is inversely correlated with cocaine effects, the role of leptin signaling in regulating the intensity of the learned response to cocaine after this drug is no longer present remains unclear. Therefore, our objective was evaluate the reinforcing effects of cocaine and the persistence of memory of the conditioned reward response to cocaine in leptin receptor deficient diabetic (db/db) mice using a conditioned place preference (CPP) procedure. Methods: The CPP test consisted of four phases, namely pre-conditioning (day 1), conditioning (days 2-7), testing (day 8), and extinction (days 9-14). For the procedure, male db/db mice and their wild-type (WT) littermates (16-20 weeks old) were given intraperitoneal injection of cocaine (15 mg/kg, days 3, 5 and 7) or vehicle (days 2, 4 and 6) immediately before they are placed in their respective drug-paired chamber of CPP apparatus. During the test and extinction phase, no drug was delivered and the time spent in each compartment was registered. The CPP index was calculated according to the time spent in the

drug-paired side subtracted by the time spent in the vehicle-paired side. Results: In the test session, both groups showed significant preference for the cocaine-paired chamber ($p<0.05$ different from pre-test session, Two-way ANOVA, followed by Bonferroni). However, during the extinction phase the db/db mice presented an impairment of the extinction response in the CPP test compared to WT mice ($p<0.05$, Two-way ANOVA). Conclusion: Our data demonstrated that db/db mice displayed a more persistent memory of the cocaine-conditioned reward and suggest that the leptin signaling is also able to interfere in cocaine-CPP extinction phase.

Disclosures: D.C. Aguiar: None. **J.A.S.L. Gomes:** None. **P.H. Gobira:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.06/X19

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA DA033373

Title: Cocaine vs food choice: Neurobiological findings under a CRR

Authors: *J. J. CHOW, J. S. BECKMANN;
Univ. of Kentucky, Lexington, KY

Abstract: Choice procedures have become increasingly more common in attempts to determine the neurobehavioral mechanisms governing substance use disorders. However, in most choice procedures the relative rate of reinforcement across the drug and non-drug option vary depending on the choices that individual subjects make. Moreover, the relative rate of reinforcement is a known mediator of preference and previous research has demonstrated that differential experience with drugs of abuse, such as cocaine, can lead to differential neural adaptations. Thus, under choice procedures with this issue, the neurobiological findings regarding drug vs non-drug choice can become difficult to interpret. Herein, we utilized a controlled reinforcer ratio (CRR) schedule to investigate the role of the orbitofrontal cortex (OFC) and nucleus accumbens (NAc) on cocaine choice. Adult male Sprague Dawley rats were first trained under a series of establishing procedures to acquire food and cocaine self-administration. Rats were then placed on a CRR schedule. The CRR consisted of 5 blocks, with 6 trials/block, where the dose of cocaine increased as a function of block (0, 0.032, 0.10, 0.32, and 1.0 mg/kg/infusion) on one option, while the other option consistently offered a single 45-mg palatable-food pellet across all blocks. Under the CRR, both levers would extend on trial start, however only one reinforcer was randomly made available. Through the randomization of reinforcer availability, preference was measured via the time spent on a given alternative. Following stability of choice training under

the CRR, rats underwent two independent-test sessions where they were placed in a condition for either food preference or cocaine preference; with the second test session being the opposite. After the test sessions rats were perfused and brains were collected. Brains slices for the OFC and NAc were collected and underwent fluorescent *in situ* hybridization and fluorescent immunohistochemistry for cFos expression for food and cocaine preference, dependent on the order of presentation during the test sessions. Results revealed that behavioral measures, via the matching law, were not correlated with cFos expression when the relative rate of reinforcement was equal in either the OFC or NAc. However, when the relative rate of reinforcement was in favor for cocaine, an increased number of cFos positive cells for cocaine was observed in the OFC. Overall, these results revealed that neuronal activity for cocaine is related to cocaine intake, and not preference. In all, these findings provide impetus for careful considerations regarding the procedures used in investigating drug versus non-drug choice.

Disclosures: **J.J. Chow:** None. **J.S. Beckmann:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.07/X20

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA011064

Title: 5-HT$_{1B}$ receptor agonist attenuation of cocaine self-administration persists after a period of relapse

Authors: *R. GARCIA[1], S. SCOTT[1], T. LE[1], S. DOYLE[1], A. ESQUER[1], J. VALENZUELA[1], K. M. BLATTNER[2], B. E. BLASS[2], J. L. NEISEWANDER[1];
[1]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [2]Moulder Ctr. for Drug Discovery Res., Temple Univ. Sch. of Pharm., Philadelphia, PA

Abstract: The selective 5-HT$_{1B}$ receptor (5-HT$_{1B}$R) agonist, CP94253, enhances cocaine self-administration (SA) during maintenance of drug-taking but attenuates SA after three weeks of forced abstinence in male rats. Here we examined if the effects of CP94253 produced a similar abstinence-dependent attenuation on SA in female rats. Additionally, we examined if these attenuating effects persist or revert back to enhancing SA during a period of relapse (i.e., resumption of SA). Male and free-cycling female rats were trained to lever press for cocaine (0.75 mg/kg, IV), for a minimum of two weeks, on a fixed ratio 5 schedule of reinforcement. Rats underwent ~21 days of forced abstinence following stable performance for cocaine SA. After, rats were tested for the effects of acute treatment with either vehicle or CP94253 (5.6 mg/kg, SC) on both the training dose and a lower dose of cocaine (0.075 mg/kg, IV) for the first

and second hour of the test, respectively. Rats then resumed cocaine SA on the training dose for equal number of days as the initial training phase. Rats were then tested again with either vehicle or CP94253 done previously. We found that similar to male rats, CP94253 attenuated cocaine SA in female rats following abstinence. During resumption of SA, all rats demonstrated similar self-administration similar to the initial training phase. Interestingly, CP94253 attenuated SA in male and female rats when tested after resumption of cocaine SA. These results demonstrate that the abstinence-dependent attenuation effects of CP94253 on cocaine self-administration remain even after resumption of cocaine SA without abstinence. The findings suggest that CP94253 and other selective 5-HT_{1B}R agonists may have clinical efficacy as anti-relapse treatments for cocaine use disorders.

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.08/X21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA011064

Title: The effects of a selective 5-HT_{1B} receptor agonist on cocaine demand before and after abstinence from self-administration in freely cycling female rats

Authors: *S. N. SCOTT[1], G. L. POWELL[1], R. GARCIA[1], A. M. GARCIA[1], O. I. VILLARREAL[1], K. M. BLATTNER[2], B. E. BLASS[2], J. L. NEISEWANDER[1];
[1]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [2]Sch. of Pharm., Temple Univ., Philadelphia, PA

Abstract: In male rats, the selective 5-HT_{1B} receptor (5-HT_{1B}R) agonist, CP94253, enhances the reinforcing value of cocaine during daily drug-taking, but attenuates the reinforcing value of cocaine after 21 days of abstinence. We recently found that in female rats, CP94253 decreases cocaine self-administration during daily drug-taking at intermediate doses of cocaine regardless of estrous cycle phase; however, it is unclear whether the CP94253 effects are due to enhancement or attenuation of cocaine reinforcement. In this study, we used a behavioral economics analysis to address this question. This approach evaluates the consumption of a specific drug across a range of prices, where price is controlled using either the response requirement necessary to receive drug or by the concentration of drug received. The analysis yields two important parameters: demand intensity (Q_0) and elasticity of demand (α). Demand intensity measures consumption at an unconstrained price, while elasticity measures sensitivity

in consumption due to changes in price. The current study applied behavioral economic analysis to investigate whether CP94253 influences the demand for cocaine during the active drug-taking phase and after 21 days of abstinence in female rats. Female Sprague-Dawley rats were trained to self-administer 0.75 mg/kg, i.v. cocaine initially on a fixed ratio 1 (FR1) schedule of reinforcement for 2 hours/day and then on a within-session dose-reduction procedure where 9 doses (1 mg/kg to 0.01 mg/kg i.v.) were available for 5 min each in descending order. Once rats had less than 25 percent variability in elasticity across 3 consecutive days, they were tested for the effects of CP94253 on cocaine demand. Rats were pretreated with 5.6 mg/kg s.c. CP94253 or vehicle 15 minutes prior to testing using the same dose-reduction procedure as above. Preliminary results suggests CP94253 increased cocaine demand elasticity but had no effect on demand intensity. This suggests that CP94253 pretreatment decreases cocaine consumption as the effort necessary to acquire drug increases. This study is ongoing and if the results are reliable, then our findings are consistent with CP94253 attenuates the reinforcing properties of cocaine as a function of effort and have important implications for developing treatments for cocaine dependence.
Supported by: NIDA DA011064

Disclosures: S.N. Scott: None. **G.L. Powell:** None. **A.M. Garcia:** None. **J.L. Neisewander:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.09/X22

Topic: G.08. Drugs of Abuse and Addiction

Support: Feil Family Brain and Mind Research Institute

Title: Alpha synuclein critically modulates motivation for rewarding stimuli and shows dynamic changes in subcellular localization within ventral tegmental area axon terminals after systemic cocaine administration

Authors: O. TRUBETCKAIA[1], A. E. LANE[1], L. QIAN[1], P. ZHOU[2], ***D. A. LANE**[1];
[1]Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY; [2]Brain and Mind Res. Inst., Weill Cornell Med., New York, NY

Abstract: Addiction is difficult to overcome because drugs of abuse produce relatively permanent changes in the function of dopamine (DA) neurons involved in reward and motivated behaviors. The activity of ventral tegmental area (VTA) DA neurons is a balance of excitatory and inhibitory inputs coming from multiple brain regions, which is disrupted with repeated drug use causing a shift to increased glutamate activation of these cells. However, it is still unknown

what causes the functional shift in DA activation originally incoming from striatal inputs, which mediate the initial rewarding/hedonic effects of drugs, to cortical inputs that drive drug craving and compulsive drug intake. A potential candidate for modulating afferent information to VTA DA neurons is alpha-synuclein (α-syn), an abundant neuroprotein found predominantly in axon terminals. A-syn is elevated in blood and brain tissue samples obtained from human addicts and in brain regions undergoing developmental glutamatergic plasticity. However, very little is known about the normal function of α-syn or its contribution to addictive behaviors. As such, we examined the subcellular localization of α-syn and changes in drug-related behaviors of normal C57BL/6J and α-syn knockout (*C57BL/6N-Snca^{tm1Mjff}/J*) mice before and after systemic cocaine administration. Electron microscopic characterization of α-syn shows that cocaine-mediated presynaptic α-syn upregulation is highly dynamic and targets specific axon terminals depending upon the duration of cocaine exposure and whether cocaine was systemically present during evaluation. For instance, we show glutamatergic axon terminals with α-syn labeling adjacent to non-labeled glutamate terminals both making synaptic contacts onto the same DA dendrite. The highly precise presynaptic distribution of α-syn makes it strategically positioned to collectively fine-tune the complex afferent input to VTA dopamine neurons and regulate dopamine output. Repeated cocaine administration also upregulates postsynaptic α-syn where it is seen around endocytic vesicles and multivesicular bodies (MVBs) where it facilitates exosome release and is necessary for normal MVB formation. Our behavioral studies compliment the changes seen in the subcellular α-syn distributions and demonstrate that α-syn is necessary for preference to rewarding stimuli and for the cognitive flexibility needed for changing previously learned behavioral strategies, two critical factors influencing addiction.

Disclosures: O. Trubetckaia: None. **A.E. Lane:** None. **L. Qian:** None. **P. Zhou:** None. **D.A. Lane:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

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Program #/Poster #: 415.10/X23

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01-DA044489

Title: Lipid profiles of brain extracellular vesicles of cocaine-treated mice

Authors: *M. SAITO[1,2], R. LINN[1], A. HASHIM[1], H. SERSHEN[1,2], B. BARRETO[1], M. SAITO[1,2], E. LEVY[1,2];
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Abstract: Repeated use of cocaine induces addiction and other adverse effects through changes in the expression and functions of specific proteins, lipids, and RNAs, including molecules relevant to the rewarding pathway. Exosomes, endosome-derived small extracellular vesicles (EVs), are secreted from many cell types, eliminate cellular toxic materials, and carry various molecules that affect physiological and pathological functions of recipient cells. Cocaine's effects may also be partially mediated by exosomes uniquely modified by cocaine. In this study, the effects of cocaine on lipid profiles of brain EVs were examined, because some of the exosomal lipids are considered signaling molecules. 3-month-old mice (both sexes) were injected with saline (control) or cocaine (10 mg/kg) daily for two weeks. Under this condition, mice acquired locomotor sensitization and CPP to cocaine. 30 min after the final injection, small brain EVs released into the extracellular space were isolated [Perez-Gonzalez et al. J Biol Chem 2012, 287:43108] using OptiPrep density gradient centrifugation as the final step, which separated three EV fractions with different densities. We found that cocaine treatment differentially affected the amount of EVs in these fractions: decreased EVs with the medium density and increased EVs with the highest density. Then, we analyzed the major lipids of these EVs by HPTLC. In both control and cocaine samples, the lipid compositions of these three EV fractions were different. Specifically, sphingolipids (e.g., ceramide and gangliosides) were highly enriched in EVs in the lowest density group compared to brain homogenates. For example, in the control animals, ceramide was 45±8 times more enriched in the lowest density EV group compared to 7±0.4 times enrichment of cholesterol. Cocaine treatment increased the amounts of cholesterol, phosphatidylcholine, phosphatidylethanolamine, and GD1a ganglioside, but no changes in phosphatidic acid and phosphatidylserine in EVs with the highest density, along with the increase in the EV protein levels of this density group. Also, despite the decrease in the protein level of EVs with the medium-density by cocaine treatment, gangliosides, including GD1a, increased in this fraction. Thus, cocaine treatment uniquely altered distribution of EVs with different densities and also altered EV's lipid profiles. Especially cocaine affected the content and distribution of gangliosides profoundly. This may be related to previous studies suggesting that gangliosides enhance the rewarding properties of cocaine.

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.11/X24

Topic: G.08. Drugs of Abuse and Addiction

Support: Rutgers Brain Health Institute
Busch Biomedical Grant Program

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Title: Behavioral and electrophysiological evidence for "high risk" cocaine addiction identified in a subset of outbred Long Evans rats

Authors: *N. J. BEACHER[1], A. DAO[4], M. KARNAM[2], S. J. LEICHNER[3], J. KULIK[5], A. P. PAWLAK[5], M. O. WEST[3];
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Abstract: A troubling component of drug addiction is subsequent drug relapse after a period of abstinence. A substantial portion of the addicted population is susceptible to relapse triggered by drug-associated cues. During abstinence, 'drug-craving' can be triggered by drug-cues which re-invigorate drug-associations in motivational brain regions such as the nucleus accumbens (Nac). As such, identifying differences in cue reactivity is important for the long term prevention of drug-relapse. Cue reactivity differences have been studied in humans and animals using an autoshaping test, which identifies two distinctive behavioral phenotypes; Sign-trackers (ST) and Goal-trackers (GT). This phenomenon is thought to involve incentive salience, where the "self-rewarding" predictive-cue becomes associated with future reward. Historically, GT have been largely ignored in drug addiction modeling and have been overshadowed by a common focus on their ST counterparts despite recent literature suggesting group similarities. Our study asked whether individual differences in STGT phenotype would predict differences in drug seeking behavior and Nac firing patterns across a 14-day cocaine self-administration (SA) paradigm. Following autoshaping, adult male long evans rats received 14 days' training (6 hours/day) in a tone discrimination paradigm where cocaine availability was contingent on a nose poke response during a 30 sec tone S^D. Single unit Nac responses to the tone S^D were tracked for the same neuron during different behavioral events the same day (Hits vs. Misses) and compared between High vs. Low Intake and GT vs. Non-GT groups. Despite overall behavioral and electrophysiological group similarities, we identified Nac FR patterns unique to High Intake GT: "silent" neurons on Misses were "active" during Hits within the same session. Behaviorally, the same subset of High Intake GT animals demonstrated high uncued drug seeking (evidence of drug craving) and significantly different drug level on Hits vs. Misses (evidence of an inability to titrate). Results suggest a novel model for studying individual differences in drug abuse vulnerability and advance our understanding of Nac firing patterns that contribute to relapse.

Disclosures: **N.J. Beacher:** None. **A. Dao:** None. **M. Karnam:** None. **S.J. Leichner:** None. **J. Kulik:** None. **A.P. Pawlak:** None. **M.O. West:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

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Program #/Poster #: 415.12/X25

Topic: G.08. Drugs of Abuse and Addiction

Support: Foundation for Polish Science Homing/2016-1/10

Title: Plastic changes in central nuclei of the amygdala driven by natural reward and addictive learning

Authors: *L. D. BIJOCH, M. PEKALA, J. KLOS, S. MITRA, L. KACZMAREK, A. BEROUN;
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Abstract: Since few years, drug addiction is considered to be a disease based on plastic changes on synapses in different brain regions. Similar changes can be observed also as results of natural forms of memory formation and it is believed that they encode information - engrams. Thus, in this study we were trying to determine how much similarities shares the initial exposure to addictive substances with natural reward learning. Both addictive and positive experiences are known to have strong emotional context, so we focused our experiments on two specific brain pathways of the limbic system: connections between posterior Basolateral Amygdala (pBLA) and medial or lateral part of Central Amygdala (mCeA or lCeA).
In our studies we used adolescent mice of both sexes. Our model of "addictive" experience was cocaine intraperitoneal injections, while sucrose self-administration represented the natural reward one. To ensure pathway specificity, viruses carrying information about channelorodopsin 2 were injected into pBLA. In such mice, after natural or addictive experience, plastic changes were studied by whole-cell patch clamp recordings with use of optogenetic stimulation.
Collected data indicate that both cocaine injection and sugar administration lead to changes in pBLA-to-mCeA pathway. In both paradigms, we observed similar generation of silent synapses - immature synaptic contacts. Silent synapses contain mostly NMDA receptors (and not AMPAR) and may function as indicator of plastic changes. Results indicate that these synapses increase their effectiveness (we observed LTP-like changes) after both sugar and cocaine treatment. However, specifically after cocaine injections we also observed indicators of plastic changes in lCeA and these changes were accompanied with decreased activity of neurons in that nucleus (LTD-like changes).
Thus, drug exposure affects the pathway processing positive memories (pBLA-to-mCeA) similarly as natural learning do. However, at the same time it also decreases the activity of lCeA, which is known for inhibiting mCeA. Thus, such reduction leads to disinhibition of mCeA and even stronger activation of mCeA. Our results shed light on controversies around considering

addiction as a form of simple, appetitive learning. We are also describing principles of cocaine experience, which is related with both LTD and LTP processes in the amygdala at the same time.

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

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Program #/Poster #: 415.13/X26

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA P01DA047233
NIDA R01DA007359
Boehringer Ingelheim Fonds

Title: Retinoic x receptor alpha signaling in the nucleus accumbens contributes to cocaine- and opioid-induced transcriptional and behavioral alterations

Authors: *A. GODINO, M. SALERY, R. FUTAMURA, C. J. BROWNE, F. J. MARTINEZ-RIVERA, P. J. HAMILTON, D. M. WALKER, E. J. NESTLER;
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Abstract: Addictive behaviors are thought to depend largely on long-lasting drug-induced transcriptional adaptations in key regions of the brain's reward circuitry. It is therefore critical to characterize the precise molecular mechanisms through which such transcriptional changes are first established following drug exposure to sustain drug-related associative learning. While some key players in this process have been identified, the extremely complex nature of the transcriptional programs triggered by drug exposure suggests more subtle and fine-tuned mechanisms of regulation. To that end, we predicted upstream regulators of genes whose expression levels were altered after prolonged withdrawal from cocaine self-administration and also positively associated with addiction-like behavioral features, and identified Retinoic X Receptor alpha (RXRa) as a putative key transcriptional mediator in the nucleus accumbens (NAc). We further investigated the contribution of RXRa, a transcription factor and nuclear receptor for retinoids also known as NR2B1, to the transcriptional and behavioral adaptations induced by drug exposure. First, we used RNA-sequencing to assess the transcriptional consequences of viral-mediated RXRa overexpression in NAc neurons, in order to confirm its role in driving addiction-relevant gene networks. Second, bidirectional manipulation of RXRa expression levels in the NAc using overexpression and knockdown strategies modulated conditioned place preference behavior for both cocaine and morphine in both male and female mice. However, pharmacological inhibition of RXRa canonical function seemingly failed to

elicit similar behavioral effects, suggesting a more complex mechanistic role for RXRa in drug-associated reward learning. Consequently, we are now examining the role of non-canonical mechanisms for RXRa function, such as a hypothetical interaction with CREB - a well-characterized plasticity-related transcription factor implicated in addiction mechanisms. Together, this study demonstrates a novel role for RXRa in the formation of drug-related memories, and paves the way for future studies of retinoid signaling in the context of drug abuse.

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

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Program #/Poster #: 415.14/X27

Topic: G.08. Drugs of Abuse and Addiction

Support: K99-DA045795; PJH
R37-DA0007359; EJN

Title: CREB-mediated activation of Zfp189 in nucleus accumbens drives behavioral responses to psychostimulants, but not opiates

Authors: C. D. TEAGUE[1], C. J. BROWNE[1], R. FUTAMURA[1], F. J. MARTINEZ[1], P. MEWS[1], A. MINIER-TORIBIO[1], A. GODINO[1], Z. S. LORSCH[1], R. L. NEVE[2], ***P. J. HAMILTON**[3], E. J. NESTLER[1];
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Abstract: Repeated administration of psychostimulants or opiates elevates cyclic AMP-responsive element binding protein (CREB)-mediated transcription in the nucleus accumbens (NAc), a major brain reward region. Over-expression of CREB in NAc decreases the rewarding effects of both psychostimulants and opiates, indicating that CREB activity in this region regulates the addictive properties of these drugs. However, manipulations of this kind cause transcriptional changes at hundreds or thousands of gene loci, limiting mechanistic insight. To clarify this complexity, we generated a novel fusion construct consisting of the nuclease-dead, RNA-guided, DNA-binding protein Cas9 tethered to the active form of CREB (S133D) (dCas9-CREB). When combined with viral delivery methods, this enables us to target CREB to specific gene loci within mouse brain. Here, in mouse NAc, we targeted CREB to the *Zfp189* gene, which is implicated in the regulation of excitatory synaptic transmission and is activated by CREB in

animal models of psychostimulant abuse. We assessed the causal contribution of the CREB-*Zfp189* interaction to psychostimulant- versus opiate-induced behaviors. Our experiments were carried out in male and female mice on a C57 background between 8-10 weeks of age and sample sizes were calculated to yield sufficient statistical power. We observed that co-delivery of herpes simplex virus (HSV)-dCas9-CREB and HSV-*Zfp189*-sgRNA to the NAc elevates *Zfp189* gene expression (mRNA) relative to non-targeting controls. CREB-mediated activation of *Zfp189* in both male and female mice reduces conditioned preference behaviors for cocaine, but does not affect morphine preference. Further, inducing CREB-*Zfp189* interactions potentiates locomotor responses to cocaine, yet does not alter locomotor responses to morphine. We are currently performing drug self-administration, brain slice electrophysiology, and spine morphological analysis to explore these divergent drug responses. These experiments indicate that CREB's action at *Zfp189* causally contributes to CREB's ability to reduce the rewarding effect of psychostimulants. However, CREB-*Zfp189* interactions in the NAc did not affect behavioral responses to morphine, suggesting that CREB's capacity to reduce the rewarding properties of opiates is not mediated by CREB's action at *Zfp189*. This work is evidence that *in vivo* neuroepigenetic editing allows us to model a single drug-induced molecular interaction and identify its causal contribution to the broader syndrome, and that a potential psychostimulant-specific transcriptional mechanism may reveal therapeutic targets for psychostimulant addiction.

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

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Support: P50MH096890 (EJN)
R01MH051399 (EJN)
P01DA047233 (EJN)
K99DA042100 (DMW)

Title: Adolescent stress results in sex-specific reprogramming of the reward circuitry transcriptome in adulthood

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Abstract: Adolescence, a time of heightened sensitivity to rewarding stimuli, is associated with vulnerability to psychiatric disorders. Male rodents that experience adolescent social isolation stress (SI) form stronger preferences for drugs of abuse in adulthood. However, little is known about how females respond to SI. Our preliminary data suggest that SI reverses sex differences in adult reward-associated behaviors and permanently reduces baseline sex differences in anxiety-related behaviors. Given these behavioral alterations, we tested the hypothesis that SI alters the transcriptome in a persistent and sex-specific manner in the nucleus accumbens (NAc), ventral tegmental area (VTA), and prefrontal cortex (PFC). Mice were isolated or group housed (GH) from P22 - P42, then GH until ~P90. Transcriptome-wide changes in NAc, VTA, and PFC were investigated by RNA-seq after acute or chronic cocaine or saline administration. SI reduces sexually dimorphic gene expression across all three brain regions. Further analysis revealed that SI results in expression profiles in males that more closely resembles GH females, suggesting that SI "feminizes" the male transcriptome. Importantly, when SI females are exposed to the first dose of cocaine, their transcriptional profiles resembled GH males in the NAc and PFC but not VTA, suggesting that SI "masculinizes" the female transcriptional response to acute cocaine. This effect is lost after chronic exposure to cocaine in the PFC. Together, these data suggest that SI has region-specific effects on sex-specific transcriptional responses to cocaine. Currently, we are utilizing gene co-expression network analysis to identify reward-circuitry conserved key drivers of the sex-specific transcriptional responses to cocaine which are reversed by SI. We predict that these key driver genes will have enormous sex-specific therapeutic potential given their conservation across multiple brain regions. Together, these data show that SI disrupts sex-specific adolescent development of transcription throughout the reward circuitry.

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.16/X29

Topic: G.08. Drugs of Abuse and Addiction

Support: R01DA014133

Title: Cell type-specific morphological and transcriptional adaptations to cocaine in the nucleus accumbens

Authors: *M. SALERY[1], A. GODINO[1], M. K. FAUSTIN[1], R. FUTAMURA[1], C. J. BROWNE[1], J. F. FULLARD[2], P. ROUSSOS[2], E. J. NESTLER[1];
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Abstract: Pathological motivation for drug-seeking and consumption, along with the high risk of relapse observed after long periods of withdrawal, are thought to result from persistent adaptations in brain circuits involved in reward learning. In the nucleus accumbens (NAc), drug exposure affects neuronal physiology differently among distinct cell populations, with D1- and D2-expressing medium spiny neurons (MSNs) exhibiting divergent cellular responses. While remodeling of NAc connectivity is known to depend on stable changes in the synaptic architecture of MSNs, the relative contribution of each MSN subtype in this process remains unclear. To tackle this issue, we performed a cell type-specific analysis of dendritic morphology following long-term withdrawal from chronic cocaine exposure. A Cre-dependent virus expressing channelrhodopsin-2 (ChR2) fused to EYFP was injected into the NAc of D1-Cre and D2-Cre transgenic mice. Due to its plasma membrane targeting, EYFP-coupled ChR2 allowed for clear resolution of dendritic spine morphology within D1 or D2 MSNs, demonstrating divergent adaptations in synaptic organization within the two NAc neuronal subpopulations in response to cocaine. Beyond the D1- vs D2-MSN dichotomy, the activation level of individual NAc neurons can be used to further segregate neuronal ensembles that are recruited during drug exposure. The identification and molecular characterization of these specific subsets of neurons is critical to deepen our understanding of how drugs of abuse reshape neuronal networks. The immediate early gene *Arc* is a highly reliable marker of activity used to define neuronal populations recruited during learning processes. In the NAc, Arc is induced by cocaine and stands as a pertinent tool to identify drug-activated MSNs. We use the tamoxifen-inducible ArcCreER^{T2} mice, which allow for the stable expression of fluorescent reporters in Arc-positive cells, to permanently label neuronal populations activated by cocaine. Then, we isolate these tagged cells or their nuclei with Fluorescence Activated Cell Sorting (FACS) and perform single-cell RNA sequencing to phenotype the cell types recruited during distinct phases of cocaine exposure, as well as to characterize the transcriptional signature specific to activated cells. Together this study employs new methods to advance our understanding of the neuronal processes engaged in subsets of neurons that are specifically recruited during and after exposure to cocaine. Supported by R01DA014133

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R37DA007359
P01DA047233

Title: Cell type-specific transcriptional patterns in nucleus accumbens differentiating cocaine versus morphine exposure: Actions of ΔFosB

Authors: *C. K. LARDNER[1], P. J. HAMILTON[2], A. RAMAKRISHNAN[1], H. G. KRONMAN[1], D. M. WALKER[1], L. SHEN[1], E. J. NESTLER[1];
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Abstract: The onset and persistence of drug addiction is, in part, effected via cell-type specific mechanisms of transcription in the brain. However, between classes of abused drugs, little is known about the overlap of transcriptional mechanisms and how these converge and diverge to effect an addictive phenotype. Within nucleus accumbens (NAc), ΔFosB, a Fos family transcription factor, accumulates in D1 medium spiny neurons (MSNs) following repeated exposure to cocaine, but in both D1 and D2 MSNs after chronic morphine. This phenomenon makes ΔFosB and its downstream effectors promising therapeutic targets but it is unknown how ΔFosB coordinates the unique effects of stimulants and opiates. To determine the D1- vs D2-MSN specificity of ΔFosB targets, we fused dCas9 to the transcription factor CREB to selectively induce ΔFosB in a MSN-specific manner. We then performed RNAseq in male and female whole NAc after ΔFosB induction as well as evaluated the effects of ΔFosB activation in D1- or D2-MSNs on cocaine and morphine place conditioning. We found both MSN-subtype and drug dose-dependent effects of ΔFosB induction on cocaine and morphine reward. Our RNAseq data reveal that ΔFosB induction in D1- or D2-MSNs, or both, each account for distinct gene expression changes. Understanding the cell type-specific roles of ΔFosB in mediating cocaine and morphine action clarifies their divergent and convergent mechanisms and reveals new avenues for therapeutic development. Moreover, our method for studying the genomic patterns coordinated by a single transcription factor more broadly serves as a novel mode of investigation into understanding how cell type-specific transcription and epigenetic patterns interact with environmental input to guide behavior.

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01DA014133
P01DA047233
Yale/NIDA Neuroproteomics Center Pilot Project Grant

Title: Proteomic profiling of nucleus accumbens synaptosomes following short- and long-term withdrawal from cocaine self-administration in mice

Authors: *Y. YIM[1], C. J. BROWNE[1], J. WANG[2], R. S. WILSON[3], A. C. NAIRN[3,4], Y. DONG[2], E. J. NESTLER[1];
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Abstract: Addiction is a devastating disorder that is exceptionally difficult to treat due to the high propensity for relapse even long after terminating use. The persistence of addiction is mediated by drug-induced changes in the physiology of reward-processing regions of the brain. Dysregulated signaling within the nucleus accumbens (NAc) is thought to play a critical role in promoting drug-seeking and relapse. Determining these changes may reveal more effective targets to treat drug addiction and relapse. However, the molecular details underlying these adaptations remain incompletely understood. Here, we extend previous work focused mainly on candidate proteins of interest by examining whole-proteome changes induced by cocaine self-administration that persist through extended abstinence. Mice underwent 6 days of intravenous cocaine or saline self-administration followed by a period of 24 hours or 45 days of forced abstinence, after which mice were euthanized and the NAc was extracted. NAc synaptosomes were purified and ran on lipid chromatograph tandem (LC-MS/MS) mass spectrometry follow by label free quantification in collaboration with the Yale/NIDA neuroproteomics core. In this preliminary study, we are able to show lists of synaptic proteins with induction or repression after self-administered cocaine followed by different time points of withdrawal. Interestingly, long-term withdrawal is associated with repression, rather than induction, of protein expression. Further extension of this proteomic study will lead to the characterization of synaptic proteins in various brain reward regions and make important advances in our understanding of the molecular basis of addiction and relapse.

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: R37DA007359
P01DA047233
5T32DA007135-33

Title: E2F3a transcription factor regulates cocaine- and morphine-related behaviors in males and females in a cell-specific manner

Authors: *F. J. MARTINEZ-RIVERA[1], A. TORRES-BERRÍO[1], P. J. HAMILTON[2], A. M. MINIER-TORIBIO[1], C. J. BROWNE[1], Y. YIM[1], R. FUTAMURA[1], R. L. NEVE[3], E. J. NESTLER[1];
[1]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Neurosci., Virginia Commonwealth Univ. Hlth. Syst., Richmond, VA; [3]Neurol., Massachusetts Gen. Hosp., Cambridge, MA

Abstract: The development of drug addiction is characterized by epigenetic changes in the brain reward circuit, leading to the transition from recreational drug use to drug dependence. Most of these epigenetic processes, such as histone modifications, chromatin remodeling and alterations in the expression of transcription factors, have been well characterized in the nucleus accumbens (NAc). Our group recently identified the transcription factor E2F3 as a novel upstream regulator of cocaine-induced gene expression in the NAc. Consistent with this, we also demonstrated that viral manipulations of the E2F3a isoform in NAc, but not of E2F3b, enhances cocaine-induced locomotor and conditioned place preference (CPP) behaviors in adult male mice. Interestingly, further cell-specific analyses revealed that E2F3a acts as a key upstream regulator of *Fosb*and induces expression of the canonical addiction-mediating transcription factor, ΔFosB, in D1 receptor-expressing medium spiny neurons (D1-MSNs) selectively. Here, we used viral-mediated gene transfer combined with CPP to assess whether E2F3adifferently regulates drug-rewarding behaviors depending on: 1) sex, 2) MSN cell type and 3) type of drug. Our preliminary results showed that selective E2F3a overexpression in D1-MSNs increased cocaine CPP in female mice, whereas E2F3a overexpression in D2-MSNs has no effect. Current experiments withmorphine CPP will reveal the influence of E2F3a in a sex and drug specific manner.Together, our results highlight E2F3a as a novel regulator of drug-elicited transcriptomic modifications in D1-MSNs.

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Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

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Support: NIH Grant DA044308
NARSAD Young Investigator Grant

Title: Microbiome depletion disrupts transcriptional homeostasis and reduces persistence of cocaine sensitization

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Abstract: Psychostimulant addiction represents a public health crisis leading to extensive morbidity and mortality. Despite this, there are currently no FDA-approved medications for treatment of psychostimulant use disorders. Increasing evidence suggests that the bacterial composition of the gut microbiome significantly affects brain and behavior in models of psychiatric disease. Our lab has previously demonstrated that depletion of the gut microbiome significantly affects the formation of cocaine conditioned place preference and locomotor sensitization. However, no currently published studies have examined the role of the gut microbiome in modulating the persistence of cocaine-induced changes in neuronal or behavioral plasticity. Given that prevention of relapse following abstinence is the most clinically challenging issue in treating patients with substance use disorders, studies examining the effects of microbiome manipulations in relapse relevant models are critical for the development of translational strategies in this space. For these studies mice had their microbiome depleted with non-absorbable antibiotics in their drinking water and were compared to untreated controls. Locomotor sensitization was induced with five daily injections of cocaine, and after 28 days of withdrawal a cocaine challenge was given to assess the persistence of locomotor sensitization. Despite normal development of sensitization, antibiotic-treated mice exhibited significantly reduced persistence of locomotor sensitization after prolonged withdrawal. To identify molecular signatures underlying these behavioral changes, animals were sacrificed 60 minutes following drug administration, and the nucleus accumbens was dissected out for RNA-sequencing analysis.

While cocaine-challenged control animals showed changes in approximately 200 genes, antibiotic-treated mice had significant changes in 3,610 genes. To further interrogate gut-derived factors that might be playing a role in these changes seen in microbiome-deficient mice, key bacterial metabolites, the short-chain fatty acids (SCFA), were replenished via the drinking water in a series of experiments. When levels of these metabolites were restored to normal in microbiome-deficient mice, the persistence of locomotor sensitization returned to normal. Taken together, these findings suggest that the microbiome may serve as a key homeostatic regulator of persistence of behavioral response and regulation gene expression in the brain, and suggest that the microbiome has potential as a translational research target.

Disclosures: K.R. Meckel: None. **R.S. Hofford:** None. **J.B. Hanks:** None. **N.L. Mervosh:** None. **P. Roussos:** None. **D.D. Kiraly:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.21/X34

Topic: G.08. Drugs of Abuse and Addiction

Support: NARSAD Young Investigator Award
NIH grant DA044308
Yale/NIDA Neuroproteomics Center

Title: Granulocyte-colony stimulating factor during abstinence from cocaine self-administration alters expression of glutamatergic synaptic proteins in nucleus accumbens

Authors: *R. S. HOFFORD[1], T. EUSTON[1], R. WILSON[3], T. T. LAM[3], E. S. CALIPARI[4], D. D. KIRALY[2];
[1]Psychiatry, [2]Psychiatry / Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Yale/NIDA Neuroproteomics Ctr., New Haven, CT; [4]Pharmacol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Recent work from our laboratory implicates granulocyte-colony stimulating factor (G-CSF) as a neuroimmune modulator of cocaine reward. Levels of G-CSF are increased in blood and brain following cocaine administration and levels of G-CSF correlate with cocaine intake. Injections of G-CSF enhance low-dose cocaine self-administration in a threshold task, and inhibition of G-CSF in the nucleus accumbens (NAc) prevents formation of cocaine conditioned place preference. However, treatment with G-CSF during extinction of cocaine self-administration accelerates extinction, and administration during abstinence attenuates cue-induced cocaine-seeking. Given the importance of associative and instrumental learning mechanisms in tests of cocaine reward, we hypothesize that G-CSF is producing these varied

effects by enhancing the learning mechanisms that mediate these behaviors. Thus, animals experience increased cue sensitization during active intake, but also faster extinction learning during extinction and reinstatement. Since the molecular actions of G-CSF in brain are unclear, the current set of experiments utilized data-independent acquisition proteomics to compare protein expression changes in NAc from rats given daily injections of G-CSF or saline during abstinence from cocaine self-administration. Due to the variation in cue-induced reinstatement occurring within the G-CSF treated group, additional comparisons were conducted between G-CSF "responders" (i.e. those that had the lowest cue-induced reinstatement scores) and G-CSF "non-responders" (i.e. those that had the highest cue-induced reinstatement scores). Proteomic analysis identified 125 proteins differentially regulated between responders and non-responders. Pathway analysis indicated that many of these proteins localize to glutamatergic synapses (top gene ontology terms included glutamateric synapse, cell membrane, postsynapse, and regulation of chemical synaptic transmission). When protein expression was graphed against lever presses during cue-induced reinstatement, levels of the glutamatergic synaptic scaffold protein Shank3 significantly correlated with cue-induced reinstatement in all rats, but levels of GluA1 correlated with lever presses in G-CSF treated rats only. While much work needs to be done, these analyses suggest that G-CSF might alter cocaine-seeking by affecting the composition of glutamatergic synapses in the NAc.

Disclosures: R.S. Hofford: None. **T. Euston:** None. **R. Wilson:** None. **T.T. Lam:** None. **E.S. Calipari:** None. **D.D. Kiraly:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.22/X35

Topic: G.08. Drugs of Abuse and Addiction

Support: Yale/NIDA Neuroproteomics Center
Brain Behavior Research Foundation

Title: Investigating the role of granulocyte colony stimulating factor as a regulator of the striatal proteome

Authors: *T. J. EUSTON[1], R. S. HOFFORD[1], R. WILSON[2], T. LAM[2], D. D. KIRALY[3]; [1]Psychiatry, Icahn Sch. of Med., New York, NY; [2]Yale Univ., New Haven, CT; [3]Psychiatry / Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Pathologic use of illicit drugs represents a major public health concern and creates significant economic and social costs. Addiction to cocaine and other psychostimulants remains a major cause of this morbidity. The pathophysiological mechanisms that lead to persistent and

dysregulated drug use remain incompletely understood, and there are currently no FDA-approved pharmacotherapies for treatment of psychostimulant use disorders. There is growing evidence that dysregulation of the immune system plays a role in the pathophysiology of multiple psychiatric disorders including major depressive disorder and schizophrenia. While cocaine is known to have immunomodulatory effects, the link between these immune interactions and pathological use behaviors has only recently been investigated. We recently identified granulocyte-colony stimulating factor (G-CSF) as a cytokine that is increased in serum and brain by chronic cocaine. Peripheral administration of G-CSF enhances cocaine place preference and cocaine intake in a self-administration model, and also facilitates cocaine-induced neuronal activation in the nucleus accumbens and prefrontal cortex. While G-CSF has clear effects on synaptic and behavioral plasticity, the molecular mechanisms underlying these effects remains unclear. To interrogate changes induced by G-CSF in the setting of active cocaine treatment a 2 x 2 design was utilized with animals injected with Saline or Cocaine (7.5mg/kg) and PBS or G-CSF once daily for seven days. This was followed by discovery proteomics analysis of the nucleus accumbens (NAc) using data-independent acquisition mass spectrometry analysis. As expected, there were many proteins that were significantly altered by one week of cocaine treatment. However, treatment with G-CSF alone resulted in regulation of an even larger number of proteins, and co-treatment with the two resulted in the largest number of significantly regulated proteins - suggesting a significant interaction between the two. Gene ontology analysis of samples from G-CSF + Cocaine treated animals showed that there was a strong upregulation of proteins associated with the synaptic compartment, with a number of both pre and postsynaptic proteins demonstrating significant alterations. Confirmatory Western blot analysis demonstrate similar increases in levels of PSD-95 and GluR1 by G-CSF + cocaine in a separate cohort of animals. These findings complement our previous work and suggest that in the presence of G-CSF the ability of cocaine to cause synaptic remodeling is enhanced.

Disclosures: **T.J. Euston:** None. **R.S. Hofford:** None. **R. Wilson:** None. **T. Lam:** None. **D.D. Kiraly:** None.

Poster

415. Neural and Behavioral Mechanisms of Addiction: Cocaine

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 415.23/X36

Topic: B.08. Intrinsic Membrane Properties

Support: NIH R01AA025784
Behavior Research Foundation grant (#24989).

Title: High risk of cocaine addiction in adolescents: Differential contribution of diverse cortical areas

Authors: D. HUANG[1], S. IM[2], J. M. CIALDELLA[2], A. V. SALINAS[2], *Y. Y. MA[1]; [1]Pharmacol. and Toxicology, Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Binghamton Univ., Binghamton, NY

Abstract: The cerebral cortex, particularly the anterior cingulate cortex (ACC), the prefrontal cortex (PrL) and the infralimbic cortex (IL), play an important role in relapse of drug taking. However, only a few studies have examined the contribution of the primary (M1) and secondary (M2) motor cortices in the persistence of addiction as a habituated behavior. In order to closely mimic the chronic drug history, most addiction researchers have attempted to model drug exposure with extended daily sessions (≥ 2-6 hrs) during a prolonged period of time (≥10 days). However, during adolescence, even brief exposure to the drug can lead to devastating behavioral, emotional, and cognitive consequences, predisposing the young brains to drug addiction and other mental disturbances. Inspired by the fact that the adolescent stage is usually when the drug taking behaviors are initiated, we predicted that this age group is exquisitely sensitive to the abusive potential of drugs. This vulnerability of adolescents may be fully masked by extended drug administration. Thus, limited drug exposure, i.e., intravenous self-administration (IVSA) of cocaine, 1 hour per day for 5 days, was used in the current experiments in order to explore the neuronal substrates of high addiction risk among adolescent Sprague-Dawley rats using whole-cell patch clamp recordings of pyramidal neurons in the IL, PrL, ACC, M1 and M2. Relative to 1 day after adolescent cocaine IVSA (i.e., 1 day withdrawal, abbreviated as WD 1) and WD45 after adolescent saline IVSA, our data showed a significantly incubated cocaine seeking, and increased excitability (indicated by an increased number of spikes induced by current injections of increasing intensities) in the M2, but not in the IL, PrL, ACC and M1, on WD 45 in rats with a cocaine IVSA history during their adolescent stage. Although, relative to adult saline IVSA, cocaine IVSA during the adult stage lead to increased excitability in the M2 on both WD1 and WD45, no difference of M2 excitability was observed in rats with a history of cocaine IVSA between WD1. vs. WD45. Further analysis showed increased membrane input resistance of pyramidal neurons in the M2 from adolescent cocaine IVSA-treated rats on WD45, but not WD1; however, no membrane resistance change was detected in adult cocaine IVSA-treated rats on WD1 or WD45. Our conclusions are that (1) limited drug exposure can be used as an addiction model to explore the vulnerability of drug addiction among adolescents, and (2) membrane input resistance-induced excitability changes in the M2 may be a novel neurobiological target for preventing relapse of addiction.

Disclosures: D. Huang: None. **S. Im:** None. **J.M. Cialdella:** None. **A.V. Salinas:** None. **Y.Y. Ma:** None.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.01/X37

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH-NIGMS #2R25GM082406
Ponce Research Institute
NIM-HD #007579
NIH-NIGMS #2R25GM096955

Title: Sex differences in effects of voluntary exercise during extinction of cocaine-seeking behavior

Authors: *R. J. MORALES SILVA[1], U. GELPI-DOMÍNGUEZ[2], D. NIEVEZ-TORRES[4], A. ECHEVARRIA- RIVERA[2], M. T. SEPULVEDA-ORENGO[3];
[1]Basic Sci., [3]Dept. Basic Sci., [2]Ponce Hlth. Sci. Univ., Ponce, Puerto Rico; [4]Univ. of Puerto Rico, Ponce, Puerto Rico

Abstract: One of the main problems in cocaine addiction is the high incidence of relapse. Several studies have shown that physical activity, such as exercise, can prolong drug abstinence and alleviate drug-seeking symptoms in humans. Although recent clinical trials have successfully incorporated exercise as part of the treatment for drug addiction, it is still unknown how exercise affects cocaine-induced molecular processes, thus leading to reduced drug-seeking behavior. Studies in hippocampus have shown exercise increases levels of brain-derived neurotrophic factor (BDNF). Moreover, studies showed that infusion of BDNF into the nucleus accumbens core (NAc) and/or medial prefrontal cortex (mPFC) decreases cocaine seeking behavior. We hypothesized that aerobic exercise would increase of BDNF levels in the NAc and PL, leading to reduced cocaine-seeking behavior in male and female rats. To test our hypothesis, we used an addiction model of cocaine self-administration. Rats were exposed to 12 sessions of short-access cocaine self-administration, followed by 16 sessions of extinction. After each extinction session, rats were placed in boxes equipped with a running wheel for a period of 6 hrs. Once extinction was achieved, they were subjected to a cue-primed reinstatement session to access cocaine-seeking behavior. After the behavioral protocol, levels of the proteins tropomyosin receptor kinase B (TrkB), BDNF receptor, and BDNF from NAc and mPFC from male and female rats were measured by western blots. As in previous studies, our preliminary data suggest that concurrent access to a wheel (6 hrs) during extinction from cocaine self-administration reduces reinstatement of cocaine-seeking in male rats. However, the effects of exercise in female rats seems to be estrus cycle dependent. Preliminary data from female rats suggests that exercise increases TrkB receptor levels in the mPFC, but not in the NAc from female rats, which may correlate with reduced cue-primed reinstatement in estrus female rats. We are currently examining TrkB receptor and BDNF protein levels in male and female rats for comparison. Sex-specific differences in the effects of voluntary exercise on cocaine-seeking behavior might be related to differences in hormonal levels between males and females.

Disclosures: R.J. Morales Silva: None. **U. Gelpi-Domínguez:** None. **D. Nievez-Torres:** None. **A. Echevarria- Rivera:** A. Employment/Salary (full or part-time):; Ponce Research Institute. **M.T. Sepulveda-Orengo:** None.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.02/X38

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA033372

Title: Functional sex differences in glutamate trafficking and their potential role in cocaine addiction-like behavior

Authors: *M. C. KNOUSE[1], A. U. DEUTSCHMANN[2], L. A. BRIAND[1];
[1]Psychology, [2]Temple Univ., Philadelphia, PA

Abstract: Despite the fact that more men are diagnosed with substance use disorder, women exhibit a faster transition from use to abuse, increased craving during withdrawal, and poorer treatment outcomes. Furthermore, as our cultural expectations of men and women have changed, there has been an increase in drug and alcohol use in women and this increase is likely to persist. Preclinically, female rodents show stronger behavioral responses to drugs of abuse during initiation, escalation, and reinstatement of drug seeking. These behavioral differences are accompanied by alterations in structural plasticity within the mesocorticolimbic reward system. However, very little work has been done to determine what functional sex differences exist in glutamate transmission in these circuits. The goal of these experiments was to determine functional sex differences in reward circuitry that may underlie behavioral sex differences in addiction. We found that female mice exhibit alterations in short-term plasticity within the nucleus accumbens core, indicative of differences in presynaptic glutamate transmission. To determine which inputs might be responsible for these presynaptic changes, we performed *ex vivo* optogenetics to specifically interrogate medial prefrontal (mPFC), ventral hippocampal (vHIPP), and basolateral amygdalar (BLA) projections to the nucleus accumbens core. We found that the sex differences seem to be primarily driven by alterations in mPFC and vHIPP inputs. Examination of whole cell recordings in the mPFC demonstrate that female mice may have increased glutamate transmission within this NAc projection region, as indicated by an increase in inward rectification. Work is ongoing to further elucidate functional sex differences in ventral hippocampal projection neurons. Taken together these findings suggest there are functional sex differences at many levels without the mesocorticolimbic reward system and gaining a better understanding of these differences could provide insight into sex-specific treatments for addiction.

Disclosures: M.C. Knouse: None. **A.U. Deutschmann:** None. **L.A. Briand:** None.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.03/X39

Topic: G.08. Drugs of Abuse and Addiction

Support: R00 DA033372
T32 DA007273

Title: A sex-specific role for PICK1 in the prefrontal cortex during cocaine seeking

Authors: *M. M. WICKENS, J. M. KIRKLAND, L. A. BRIAND;
Psychology, Temple Univ., Philadelphia, PA

Abstract: Protein interacting with C-Kinase 1 (PICK1) is a postsynaptic scaffolding protein that regulates glutamate receptor trafficking. PICK1 binds to GluA2 AMPA subunits leading to the subsequent internalization of AMPA receptors. Global knockout of PICK1 and local infusion of PICK1 inhibitors into the nucleus accumbens dampen cocaine taking and seeking. However, nothing is known about the role of PICK1 in the prefrontal cortex. As glutamate receptor trafficking within the prefrontal cortex (PFC) is altered by cocaine administration, examining the role of proteins involved in trafficking could shed light on the mechanisms underlying addictive phenotypes. The current study examined how site-specific knockout of PICK1 in the PFC affects food and cocaine taking and seeking in both male and female mice. Prefrontal knockout of PICK1 did not affect food self-administration or the acquisition of an operant response in either male or female mice. Additionally, no differences in cocaine intake or responding were seen following PFC knockout of PICK1 during the self-administration phase. However, when we examine cocaine seeking during a cue-induced reinstatement session, we find that male mice with a PFC-specific PICK1 knockout exhibit a decrease in cocaine seeking compared to GFP-injected control male mice. In contrast, female mice exhibit an increase in cue-induced cocaine seeking following prefrontal knockout of PICK1. These results suggest that PICK1 in the prefrontal cortex is playing opposing roles in cocaine seeking in male and female mice. This suggests that the synaptic mechanisms underlying substance use disorder could be distinct in males and females and sex-specific treatments may be needed.

Disclosures: M.M. Wickens: None. **J.M. Kirkland:** None. **L.A. Briand:** None.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.04/X40

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R00DA038110

Title: Investigating neuroadaptations underlying sex differences in cue-induced cocaine craving

Authors: C. M. CORBETT, Y. SHIFMAN, *J. A. LOWETH;
Cell Biol. and Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ

Abstract: Although clinical studies indicate sex differences in both cocaine addiction and relapse vulnerability, the majority of preclinical studies in this area have been conducted with male rats. Our recent work has focused on investigating differences in cue-induced relapse vulnerability between adult male and female rats and assessing how this changes across the estrous cycle. Interestingly, we and others have found that females show enhanced cue-induced seeking behavior compared to males at certain withdrawal time points and that the estrous cycle has a significant impact on the time-dependent intensification ("incubation") of cue-induced cocaine craving that occurs during withdrawal from extended-access self-administration. More specifically, female rats in estrus show enhanced incubation of cue-induced cocaine craving compared to females in non-estrus. To investigate neuroadaptations that are driving these sex differences, we conducted western blots in males and females following both early and late withdrawal from extended-access cocaine self-administration and assessed changes in glutamatergic signaling pathways in corticolimbic regions involved in drug-seeking behavior. We also assessed whether these neuroadaptations were affected by changes in estrous cycle. These studies will help identify mechanisms that could be driving sex differences in cue-induced relapse vulnerability.

Disclosures: C.M. Corbett: None. **Y. Shifman:** None. **J.A. Loweth:** None.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.05/X41

Topic: G.08. Drugs of Abuse and Addiction

Title: GluA1 expression in differentially reared rats following cocaine self-administration

Authors: D. KUIPERS, A. BURTON, A. M. DICLEMENTI, F. PIGNONE, A. SCHWARZ, A. ZIMBELMAN, L. WITTENKELLER, M. T. STEFANIK, ***M. J. GILL**;
North Central Col., Naperville, IL

Abstract: Early environmental experience impacts susceptibility to drug abuse later in life. In particular, rearing rats in enriched (EC), impoverished (IC), or social (SC) conditions results in rearing-induced neurobiological, neurochemical, and behavioral changes (Bowling, Rowlett, & Bardo, 1993). When EC rats are provided the opportunity to self-administer drug during adolescence and early adulthood, they self-administer less drug, and reinstate at lower rates compared to IC or SC rats. This suggests that enrichment during childhood protects rats, making them less susceptible to addiction during adolescence and adulthood. We believe that it may be the glutamatergic system in particular, that is responsible for this protective effect based on work implicating accumbal GluA1 in cocaine self-administration (Pierce & Wolf, 2013), and reinstatement (Anderson et al., 2008). The current study sought to determine if glutamatergic pathways altered during the rearing period, produce the protective effect observed in EC rats. We hypothesize that glutamatergic signaling is altered in EC compared to IC and SC rats during rearing, resulting in greater GluA1 expression of the nucleus accumbens (NA), prelimbic cortex (PL), and infralimbic cortex (IL) of EC rats compared to IC and SC rats following reinstatement of cocaine seeking behavior. Female Sprague-Dawley rats arrived in the lab at 21 days of age, and were assigned to either EC, IC, or SC contexts, where they were reared for 30 days. Following the rearing period rats underwent standard 2-hr cocaine (0.1 mg/50 μL bolos) or saline self-administration, extinction, and reinstatement. Following cue-induced reinstatement brains were extracted and processed for immunoblotting. Immunoblotting was used to quantify GluA1 protein levels in the NA, PL and IL of differentially reared rats. During cue-induced reinstatement EC rats displayed attenuated cocaine-seeking behavior compared to SC rats. Following cue-induced reinstatement, GluA1 protein levels are reduced in the NA, IL and PL of rats that underwent cocaine self-administration, compared to saline self-administration, independent of rearing group. These results contrast previous research showing an upregulation of GluA1 in the accumbens following cocaine-primed reinstatement.

Disclosures: D. Kuipers: None. **A. Burton:** None. **A.M. Diclementi:** None. **F. Pignone:** None. **A. Schwarz:** None. **A. Zimbelman:** None. **L. Wittenkeller:** None. **M.T. Stefanik:** None. **M.J. Gill:** None.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.06/X42

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH-N01DA-13-8908
Basic Research Seed Grant-UNTHSC

Title: Age-related alterations in morphine and cocaine seeking behavior as measured by conditioned place preference

Authors: *R. A. SHETTY[1], R. CHAUBEY[1], R. BUI[2], M. J. FORSTER[1];
[1]Univ. Of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [2]Emory Univ., Atlanta, GA

Abstract: The latest reports published by agencies such as Substance Abuse and Mental Health Services Administration and the National Survey of Drug Use and Health have indicated a steady rise of substance use between the ages of 50-65. However, the potential health care issues stemming from increased drug use in the aging population is poorly understood. We hypothesize that normal aging entails an impaired function of reward pathways that diminishes the rewarding potency of abused drugs and concurrently increases drug-seeking behavior. Therefore, to better understand the nature of the aging/substance abuse interaction, we studied the effects of morphine and cocaine in drug naïve C57BL/6 mice from age groups representing young adulthood (2-3 months) and senescence (17-18 months) in a conditioned place paradigm (CPP). Our results suggests that responsiveness to illicit drugs are markedly different in older drug-naïve age groups when compared with young adults. Furthermore, stable drug-seeking and drug-avoiding behavioral phenotypes that were previously observed in young mice were altered with advanced age. These results suggest, that neurobiological changes during aging modify drug-seeking behavior in a manner that may facilitate addiction/dependence.

Disclosures: R.A. Shetty: None. **R. Chaubey:** None. **R. Bui:** None. **M.J. Forster:** A. Employment/Salary (full or part-time):; University of North Texas Health Science Center.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.07/X43

Topic: G.08. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse (NIDA) Grant R00 DA033372 (L.A.B.)

Title: Adolescent social isolation stress alters synaptic plasticity in the nucleus accumbens: Role of neuroimmune signaling

Authors: *A. MCGRATH[1], L. A. BRIAND[2];
[2]Psychology, [1]Temple Univ., Philadelphia, PA

Abstract: People who experience traumatic events early in life are at increased risk to develop substance use disorders (SUDs) later in life. This phenomenon has also been replicated in animal models. For instance, previous data from our lab has shown that adolescent social isolation leads to increased motivation for cocaine and greater vulnerability to cue-induced relapse in adulthood. However, little is known about how adolescent social isolation alters the brain to make an individual more vulnerable to addiction. Cocaine exposure leads to many neuroadapations in the nucleus accumbens, including increased dendritic spine density. These changes in dendritic spines can be long-lasting and have been theorized to play an important role in the maintenance of cocaine craving even after long periods of abstinence. The current studies utilize the adolescent isolation stress model that elicits increased addiction-like behavior in mice. We found that adolescent social isolation stress leads to a persistent increase in spine density in both the core and shell regions of the nucleus accumbens in adulthood. These increases in spine density were region specific as we did not see changes in spine density in the prefrontal cortex of adult mice exposed to adolescent social isolation. Both stress and cocaine have been shown to activate microglia and our isolation stress model also alters cytokine expression. Experiments are underway to determine how disrupting microglial activation alters the effects of adolescent social isolation. The findings of the current studies will provide insight into potential interventions to decrease vulnerability to SUDs in individuals who have experienced stress early in life.

Disclosures: A. McGrath: None. **L.A. Briand:** None.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA15758 to John Mantsch
NIDA Grant DA038663 to John Mantsch and Cecilia Hillard
NIDA Grant K01DA045295 to Jayme McReynolds

Title: Role of endocannabinoid signaling in cocaine-taking and cocaine-seeking behavior following chronic electric footshock stress-induced escalation of cocaine self-administration in rats

Authors: *J. R. MCREYNOLDS[1], J. C. MATHY[1], R. SCHAPS[1], K. A. SHANNON[1], C. P. WOLF[1], D. M. STARCK[1], C. J. HILLARD[3], J. R. MANTSCH[2];
[1]Biomed. Sci., [2]Marquette Univ., Milwaukee, WI; [3]Med. Col. Wisconsin, Milwaukee, WI

Abstract: Stress is an important contributing factor to addiction and is problematic as stress is unavoidable in daily life. Addiction can be characterized by a loss of control over drug intake

that is modeled by escalating patterns of drug self-administration (SA). We have shown that a stressor, electric footshock stress, administered daily at the time of SA induces an escalation of cocaine intake in rats under short-access conditions (2-h/day). Stress-induced escalation of SA is likely the consequence of long-lasting neuroplastic changes that involve neurobiological mediators that connect stress-responsive and reward systems in the brain, such as the endocannabinoid system (eCB), likely occur in regions implicated in both stress and reward, such as the nucleus accumbens (NAc) shell and ventral tegmental area (VTA). We hypothesize that repeated stress at the time of SA induces a persistent increase in eCB signaling, particularly in the NAc shell and VTA, which results in escalation of cocaine use and increased susceptibility to later reinstatement. Male SD rats were trained to SA cocaine (0.5 mg/kg/inf) on a FR 4 schedule in 4 X 30 min SA sessions separated by 5-min drug-free periods. Some rats received shock in the SA chamber during the 5 min drug-free period over 14 days. Systemic administration of the CB1R antagonist AM251 (1 mg/kg), intra-NAc Shell or intra-VTA infusions of AM251 (1, 3 µg) prior to the SA session attenuated cocaine intake in stress-escalated rats. Interestingly, cocaine SA increases CB1R binding and repeated stress at the time of SA further increases CB1R binding in the VTA while neither self-administration condition changes CB1R binding in the NAc. Studies examining changes in VTA DA neuronal activity and NAc shell DA signaling, via fiber photometry imaging of GCaMP6f or dLight, are currently ongoing. Separate groups of rats were tested for reinstatement of drug-seeking behavior to a priming injection of cocaine, footshock stress, or the pharmacological stressor yohimbine. Rats who received shock during SA demonstrated augmented reinstatement to cocaine, footshock stress, and yohimbine. Furthermore, as with SA, the CB1R antagonist AM251 given prior to injection of cocaine (10 mg/kg, ip) significantly attenuated cocaine-primed reinstatement only in stress-escalated rats. Studies examining the contribution of eCB signaling to stress-induced reinstatement are ongoing. These data suggest that stress-induced neuroplastic changes occur, likely in the eCB system, in regions of the brain that influence expression of escalated cocaine intake and augmented cocaine-primed reinstatement and these changes may be glucocorticoid-dependent.

Disclosures: **J.R. McReynolds:** None. **J.C. Mathy:** None. **R. Schaps:** None. **K.A. Shannon:** None. **C.P. Wolf:** None. **D.M. Starck:** None. **C.J. Hillard:** None. **J.R. Mantsch:** None.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.09/X45

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01 DA046457 (RJS)

Title: Goal-directed and habitual cocaine seeking: Further assessment of noncontingent cocaine as a method to cause satiety and outcome devaluation

Authors: *B. JONES, H. SPENCER, T. H. KIM, R. J. SMITH;
Dept Psychological and Brain Sci., Texas A&M Univ., College Station, TX

Abstract: Addiction has been hypothesized to stem from a failure to exert goal-directed control over habits. To evaluate the role of habits in addiction, our lab recently developed a novel outcome devaluation procedure to assess goal-directed and habitual responding for intravenous (IV) cocaine in rats. We hypothesized that noncontingent administration of IV cocaine would mimic preferred blood levels, resulting in temporary satiety and outcome devaluation. Previously, our lab found that pre-training NMDA lesions of dorsomedial striatum (DMS) caused rats to be insensitive to outcome devaluation, whereas lesions of the dorsolateral striatum (DLS) caused rats to be sensitive to outcome devaluation. Supporting the validity of this procedure, these findings are consistent with established roles of DMS and DLS in goal-directed and habitual responding, respectively. We found that non-lesioned rats show a great degree of variability in their sensitivity to outcome devaluation, regardless of schedule of reinforcement. This may reflect individual differences in response strategies for cocaine self-administration, or instead may be explained by differences in preferred cocaine levels, which might affect sensitivity to non-contingent cocaine. Here, we sought to evaluate individual differences in preferred levels of cocaine using pharmacokinetic modeling to estimate cocaine levels during free access self-administration sessions. Male Sprague Dawley rats were trained on a seeking-taking chained schedule of cocaine self-administration (0.5 mg/kg/infusion). Outcome devaluation was carried out via noncontingent administration of IV cocaine followed by evaluation of responding on the seeking lever for 10 minutes under extinction conditions. Similar to our previous results, we found variability among animals in their sensitivity to outcome devaluation. However, there was no correlation between sensitivity to outcome devaluation and preferred blood levels of cocaine. In addition, there was no difference in preferred blood levels of cocaine when comparing animals with DMS or DLS lesions, despite clear differences in sensitivity to outcome devaluation. Finally, pharmacokinetic modeling revealed that noncontingent IV cocaine (1 mg/kg) resulted in blood levels that are greater than the preferred levels for all animals, indicating that this dose might result in temporary satiety across animals. These findings support the premise that noncontingent IV cocaine induces temporary satiety, and that individual differences in subsequent responding are related to differences in response strategy (goal-directed vs. habitual), rather than differences in satiety.

Disclosures: B. Jones: None. **H. Spencer:** None. **T.H. Kim:** None. **R.J. Smith:** None.

Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.10/X46

Topic: G.08. Drugs of Abuse and Addiction

Support: Commonwealth of Pennsylvania CURE Addiction Center of Excellence: Brain Mechanisms of Relapse and Recovery (Childress)
NIDA U54 DA039002 Cocaine Cooperative Medication Development Center (Kampman, Center PI; Childress, PI Imaging Project)
NIDA R01DA039215 (Childress, PI)

Title: Can agony fuel ecstasy? Cocaine and opioid patients carrying a genetic variant linked to over-response of the stress (cortisol) system demonstrate heightened limbic brain response to appetitive drug cues

Authors: *A. R. CHILDRESS, K. JAGANNATHAN, R. CRIST, III, P. REGIER, G. ARAUCO-SHAPIRO, S. DARNLEY, M. TAYLOR, J. J. SUH, Z. MONGE, T. FRANKLIN, R. WETHERILL, K. YOUNG, M. GAWRYSIAK, R. SZUCS-REED, K. KAMPMAN, C. P. OBRIEN, D. D. LANGLEBEN;
Psychiatry, Univ. PENN Perelman Sch. Med., Philadelphia, PA

Abstract: AIM: Preclinical research highlights intimate, "hot-wired" inter-connections between the brain's stress and reward systems -- such that (behavioral or pharmacologic) activation of the stress circuitry (“agony”) can boost the taking (“ecstasy”), and cue-triggered seeking, of drug rewards. We wondered whether genetics known to increase stress (cortisol) reactivity might also be reflected in stronger cue-triggered limbic brain responses, a relapse-relevant phenotype in addiction. A small pilot study in cocaine patients (CPDD, June 2019) provided support for this hypothesis. To confirm these findings in an expanded, diverse cohort, we compared drug cue-triggered limbic activation in cocaine and opioid patients with, and without, a genetic vulnerability linked to higher response of the cortisol stress system (i.e., the minor allele of rs3800373, for the FKBP5 gene).
METHODS: Using event-related BOLD fMRI, we scanned detoxified, stabilized, treatment-seeking cocaine and opioid inpatients (n=56 total) during quasi-random presentations of 500 msec evocative (cocaine or opioid, aversive, sexual) and comparator (neutral) cues, 48 presentations per cue category. We analyzed (SPM 8) pre-planned contrasts, comparing carriers of the minor vulnerability allele (TG, GG; n=31) for rs3800373 of FKBP5, vs. TT homozygotes (n=25) carrying the major allele. Our anatomical focus was nodes of the mesolimbic dopamine system, a critical substrate for drug reward and drug cues.
RESULTS: Consistent with our hypothesis, the cohort of cocaine and opioid patients with a

genetic variant (TG,GG) linked to greater stress (cortisol) responding demonstrated a robust brain response to drug cues in multiple nodes of the mesolimbic system, including the ventromedial prefrontal (orbitofrontal) cortex, amygdala, striatum, pallidum, insula and midbrain (parametric T maps thresholded from 2 to 5, for display), as well as the posterior cingulate and visual association cortex. The activation pattern in TT homozygotes was circumscribed (limited to portions of the striatum, inferior frontal gyrus, and visual cortex).
CONCLUSION: These findings in a diverse cohort highlight both the important "cross-talk" between the brain's aversive and appetitive motivational systems, and the genetic contributions to this interaction by FKBP5, a gene with proposed roles in anxiety/PTSD, depression, chronic pain, and addiction. Refining the genetic links between "agony" (stress) and "ecstasy" (drug-related phenomena) could offer future gene-level targets for prevention of relapse, and potentially, for addiction itself.

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Poster

416. Factors Influencing Cocaine Use

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 416.11/Y1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA042029

Title: Rats using habitual, dorsolateral striatum-dopamine-dependent response strategies to self-administer cocaine are resistant to cue extinction compared to goal-directed rats

Authors: *B. N. BENDER, M. M. TORREGROSSA;
Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Cue exposure therapy is a potential treatment for substance use disorders that involves repeated, unreinforced exposure to drug-associated cues. Although cue exposure has shown promising results in preclinical models, its effects are more modest in clinical settings. Habitual components of human drug seeking that are often not captured by animal models could contribute to this lack of translation. Cue extinction had been shown to reduce reinstatement of cocaine seeking in animals likely using non-habitual, goal-directed strategies that rely on dopamine in the dorsomedial striatum (DMS). However, it is not known how cue extinction affects habitual cocaine-seeking behavior, which relies on dopamine in the dorsolateral striatum

(DLS). Therefore, in the present study, we examined if our model of cue exposure has differential efficacy in rats trained under two schedules of reinforcement that facilitate either goal-directed (fixed ratio; FR) or habitual (second-order, SO) behavior, respectively. Adult, male, Sprague Dawley rats were trained to self-administer cocaine intravenously for 20 days under different schedules of reinforcement. One group was implanted with DLS guide cannula. To examine whether behavior was dependent on DLS dopamine at different timepoints during training, rats were given drug-seeking tests following bilateral intracranial microinfusion of the non-specific dopamine antagonist cis-flupenthixol (10 μg) or vehicle. After FR training, dopamine antagonism in the DLS did not affect drug-seeking behavior, suggesting that behavior was not DLS-dopamine-dependent, and was therefore goal-directed. However, after SO training, dopamine antagonism in the DLS significantly reduced drug-seeking behavior, suggesting habit formation. A separate group of rats underwent cue extinction (120 or 240 cues) or a control procedure (0 cues) following training, and then underwent cue-induced reinstatement. In rats trained to self-administer cocaine on FR schedules to facilitate goal-directed responding, 120 and 240 cues reduced reinstatement, but only 240 cues reduced reinstatement in rats trained on SO schedules exhibiting habitual behavior. Additionally, western blot analysis revealed increased expression of proteins involved in synaptic plasticity in the dorsolateral striatum of rats habitually seeking cocaine. Overall, the present study suggests that different reinforcement schedules can be used to facilitate goal-directed and habitual cocaine seeking, and rats using more habitual response strategies are resistant to the effects of cue extinction compared to rats using goal-directed response strategies.

Disclosures: B.N. Bender: None. **M.M. Torregrossa:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.01/Y2

Topic: G.08. Drugs of Abuse and Addiction

Support: National Basic Research Program Grants 2015CB553501
National Natural Science Foundation of China 31871111
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Key Laboratory of Mental Health, Institute of Psychology, CAS

Title: Distinctive roles of hippocampal ten-eleven translocation methylcytosine dioxygenase 1 (TET1) and TET3 in the acquisition of morphine self-administration in rats

Authors: *J.-J. ZHANG, F.-Z. JIANG, W. ZHENG, N. SUI;
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Abstract: DNA methylation represents an appealing mechanism for long-lasting changes in brain functions responsible for learning, memory, and various neuropsychiatric disorders including substance use disorder. Conversely, the DNA demethylation, induced by ten-eleven translocation (Tet) family of methylcytosine dioxygenases, may represents a novel approach to ameliorate various behavioral problems associated with drug addiction. In this study, we determined the necessary role of TET1 and TET3 in the acquisition of morphine self-administration (SA) in rats. First, we showed that the expression of TET3 in the hippocampal CA1 region but not in the nucleus accumbens (NAc) shell was significantly up-regulated after one day but not seven days of morphine SA (0.3 mg/kg/infusion) training or after the yoked morphine injection. Similarly, the expression of TET1 in the CA1 but not in the NAc shell was significantly down-regulated after seven days of morphine SA training but not after one day or the yoked morphine injection. Saccharin SA training did not affect the expression of TET1 and TET3. More importantly, virally knocking down TET3 expression in the CA1 enhanced the acquisition of morphine SA, whereas overexpression the catalytic domain of TET1 by AAV-TET1(CD)-OE in the CA1 significantly attenuated it. Together, these findings reveal a previously unknown epigenetic mechanism of morphine-reinforced excessive operant responding and provide a new insight into how TET1 and TET3 differentially respond for the DNA methylation in neuropsychiatric disorders *in vivo*.

Disclosures: J. Zhang: None. **F. Jiang:** None. **W. Zheng:** None. **N. Sui:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.02/Y3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant P50 DA05312

Title: Escalation and reinstatement of fentanyl self-administration in male and female rats

Authors: *S. MALONE, L. HAMMERSLAG, M. BARDO;
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Abstract: Purpose: Opioid use disorder is characterized by increased intake over time and a high likelihood of relapse. This study determined if escalation of fentanyl self-administration between male and female rats over extended (6-hr) sessions enhanced craving as measured by fentanyl- and yohimbine-induced drug seeking following a period of extinction.
Methods: Adult Sprague-Dawley rats (12 male, 12 female) were trained to self-administer (SA) i.v. fentanyl (2.5 ug/kg/infusion) across seven 1-hr sessions, followed by 21 additional SA sessions of either 1- or 6-hr duration. Both groups were then extinguished on 14 consecutive 1-hr

sessions and reinstatement was assessed following pretreatment with either fentanyl (10 or 30 ug/kg, s.c.) or yohimbine (1 or 2 mg/kg, i.p.)
Results: During the initial 1-hr acquisition sessions, a lever x session interaction showed that responding increased across sessions on the active lever only, and a lever x sex interaction indicated that females responded on the active lever more than males. After rats were split into 1- and 6-hr SA groups, a linear regression analysis indicated that the change in intake across sessions depended on group, with an increase in the 6-hr group only. Escalation in the 6-hr SA group also occurred when only the first hour was considered. Overall fentanyl SA was greater in females in the 1-hr SA group, but not the 6-hr SA group. Across extinction sessions, 1- and 6-hr SA groups decreased active lever responding similarly; however, females responded more than males in the 1-hr SA group. Fentanyl induced a dose-dependent reinstatement of fentanyl seeking, with no difference between 1- and 6-hr SA groups. Yohimbine also induced a dose-dependent reinstatement of fentanyl seeking; however, there was a significant interaction between yohimbine dose and group. Only the 1-hr SA group showed increased fentanyl seeking with 1 mg/kg yohimbine, whereas only the 6-hr SA group showed increased fentanyl seeking with the 2 mg/kg yohimbine. With both fentanyl- and yohimbine-induced reinstatement, females showed overall greater responding in the 1-hr SA group, but not the 6-hr SA group.
Conclusion: These results demonstrate that fentanyl self-administration escalates with extended access, and that sex differences in intake are no longer evident after escalation. Females trained with 1-hr SA sessions responded more overall than males during acquisition, extinction, and both reinstatement conditions. Regardless of sex, extended access potentiated yohimbine-induced drug-seeking, but not fentanyl-induced drug-seeking, indicating that escalation of opioid intake increases vulnerability to stress-induced relapse.

Disclosures: S. Malone: None. **L. Hammerslag:** None. **M. Bardo:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.03/Y4

Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC Discovery Grant

Title: The effect of an acute food-deprivation stress on heroin seeking after punishment-imposed abstinence in rats

Authors: *C. BORGES, A. CHISHOLM, D. RIZZO, N. QUTEISHAT, T. CAPOLICCHIO, A. ROMANO, U. SHALEV;
Psychology Dept., Concordia Univ., Montreal, QC, Canada

Abstract: The punishment-imposed abstinence model has been used to better mimic the self-imposed abstinence that humans initiate due to the adverse consequences associated with drug taking. The voluntary abstinence model has been used in experiments using different types of substances of abuse such as methamphetamine, cocaine and alcohol, however, it has never been done with heroin. Furthermore, it is known that acute stress plays a key role triggering relapse in humans and animal models. We have previously demonstrated a robust acute food-deprivation-induced reinstatement of extinguished cocaine and heroin seeking, which can be readily translated to humans. In order to study the effect of acute food deprivation on heroin seeking after a punishment-imposed abstinence, 10 male Long Evans rats were trained to self-administered heroin (0.1 mg/kg/infusion) under a seek-take procedure with the 'seek' lever under RI30 and the 'take' lever under FR1. Heroin infusions were also associated with a presentation of a 20 s light-tone cue. After the establishment of stable responses on the seek-take chain, a mild foot shock was delivered on 30% of the completed seek links instead of the extension of the take lever. Foot shock intensity was increased by 0.1 mA per daily session until the rats stopped taking the drug. Once the voluntary abstinence was achieved, heroin seeking was tested following 21 h food deprivation and under sated condition, in a counterbalanced within-subject design. Tests were conducted under extinction conditions or with heroin available.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.04/Y5

Topic: G.08. Drugs of Abuse and Addiction

Support: Miami University College of Arts and Sciences
Miami University Department of Psychology
Miami University Office for the Advancement of Research and Scholarship

Title: Development of a mouse model of compulsive-like oral fentanyl self-administration

Authors: *S. MONROE, K. SCHUH, A. K. RADKE;
Miami Univ., Oxford, OH

Abstract: Fentanyl is commonly prescribed for postoperative pain and is regarded by the Drug Enforcement Agency as one of the most significant drug-related threats to the U.S. Much illicit heroin is contaminated with fentanyl, and as many as 4 out of 5 heroin users report that their opioid use began with prescription opioids, suggesting that fentanyl's relationship to the opioid epidemic is profound. Despite continuing efforts, opioid addiction is poorly treated and relapse

rates remain high. Integral to this cycle is the emergence of compulsive drug use, or continuing to use despite the associated negative consequences. As such, the development of preclinical research investigating the neurobiology and behavioral mechanisms that mediate aversion-resistant opioid use is vital.
With this goal in mind, 35 C57BL/6J mice (n=18 female, 17 male) self-administered oral fentanyl (10μg/ml) for 2 h, 5 d/week in operant chambers outfitted with a cup for drug delivery. Rewards were earned on a fixed ratio (FR1) schedule of reinforcement in which a correct response delivered 50 μl of fentanyl solution. Self-administration was monitored for 15 sessions. To investigate aversion-resistant drug-seeking, we utilized two measures of compulsive-like seeking in two separate cohorts. In cohort 1, the bitter tastant quinine was added to the fentanyl solution over a series of days in increasing concentrations after the initial 15 sessions (0, 100, 250, and 500 μM). Both male and female mice robustly self-administered fentanyl under these conditions, and quinine did not reduce fentanyl consumption at any concentration in either sex. A two-bottle choice task was next done in the home cage to confirm mice find the selected concentrations of quinine aversive. This experiment showed that mice significantly reduce their consumption of water when it is mixed with quinine and exhibit a strong preference for plain water. In cohort 2, a conditioned suppression paradigm was used to elicit a fear response to a cue that predicts a footshock (0mA, 0.25mA, or 0.55mA). Animals were then returned to self-administration and presented with the cue to determine whether they continue to respond for fentanyl in the presence of the cue. Results suggest that mice demonstrate resistance to punishment under this paradigm.
Together, our results suggest that these methods may be useful in studying aversion-resistant opioid seeking in mice, including the underlying neurobiological mechanisms that contribute to opioid dependence.

Disclosures: **S. Monroe:** None. **K. Schuh:** None. **A.K. Radke:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.05/Y6

Topic: G.08. Drugs of Abuse and Addiction

Support: DA042499

Title: Dissecting the role of dorsal hippocampus in reinstatement of drug seeking behavior

Authors: *T. MARKOVIC, N. MASSALY, J. GARCIA, J. YI, J. MORON-CONCEPCION; Anesthesiol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Opioid misuse and addiction represents an alarming national health issue with 40 to 60 percent of patients relapsing after periods of abstinence. Long term maintenance of associations between the reinforcing effects of the drug and the environment in which they are administered are a leading cause of relapse. Indeed, exposure to the "drug-environment" can lead to drug cravings and drug seeking behavior. Numerous studies indicate that glutamatergic transmission in the dorsal hippocampus (dHPC) is crucial for the formation of the learned associations between the rewarding effects of opioids and the context in which they are given. Additionally, dHPC projects to nucleus accumbens, a key area for processing of cue-predicted reward related behaviors. Thus we hypothesized that dHPC is necessary for relapse in drug seeking behaviors induced by drug-associated contextual cues. In this study, I demonstrate the necessity of dHPC in contextual cue-induced reinstatement of opioid self-administration. To assess the role of dHPC in reinstatement of drug seeking I used a chemogenetic (DREADD) approach to selectively silence dHPC excitatory neurons. My findings demonstrate that silencing excitatory dHPC neurons during re-exposure to drug-context significantly attenuates drug-seeking reinstatement. Importantly, control groups injected with control virus or DREADD ligand alone showed robust reinstatement. In addition, silencing dHPC did not alter either short term or long term memory as measured using an object location task. This result demonstrates that the observed attenuation of reinstatement is not due to memory retrieval impairment but rather to a selective disruption of drug-reward association. While these findings uncover the necessity of dHPC in cue induced reinstatement, the exact changes in activity of dHCP driving relapse are yet to be uncovered. To this end I am utilizing fiber photometry to investigate the dynamics of dHPC activity during both, the extinction and reinstatement of drug-seeking behavior . Such experiments will lead to a better understanding of the temporal resolution of hippocampal networks activity underlying context induced relapse in opioid-seeking behavior.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.06/Y7

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R41 DA045398

Title: Rat behavioral changes due to implanted striatal magnetic particles activated with externally-applied magnetic fields

Authors: *D. N. TECENO[1], P. WALIA[1], M. BATISTA-LINHARES[1], L. O. MAIR[2], S. JAFARI[2], P. STEPANOV[2], O. HALE[2], C. ROPP[2], A. HEVAGANINGE[2], I. N. WEINBERG[2], F.

M. VASSOLER[1];
[1]Dept. of Biomed. Sci., Tufts Univ., North Grafton, MA; [2]Weinberg Med. Physics, Rockville, MD

Abstract: While many techniques are available to manipulate deep brain structures (optogenetics, deep brain stimulation (DBS), ultrasound, transcranial magnetic stimulation, etc.) to treat psychiatric and neurologic disorders, there are critical issues that decrease clinical utility. For example, DBS is invasive and only able to directly impact a small portion of the brain at a time. The goal of the current study is to determine if mechanical stimulation of neurons could serve as an alternative mechanism for neurological manipulations to treat various disorders. Recent data has shown that mechanical stimulation of neurons depolarizes cells and increases their firing rate. Here we developed a technique to engineer magnetic nanorods that can be implanted into neural tissue. We then designed a magnetic coil to provide external magnetic stimulation to the implanted nanorods. Particle injected animals (n=15) were stereotaxically implanted with magnetic nanorods, while control animals (n=13) experienced sham surgeries. One week following surgery, animals were placed in a small open field within the magnetic coil. Animals were acclimated to the field for 1-minute, followed by 4 minutes of low-magnitude (5mT, 20 Hz) external magnetic stimulation. The magnet was then turned off and the animal remained in the field for an additional 2 minutes. Total locomotion, rotations, and vacuous chewing behaviors for the duration of the experiment (7 minutes) were scored by a blind observer. Results showed no difference in overall locomotion or number of rotations. However, particle implanted animals showed significantly increased levels of chewing behavior compared to sham animals. An additional group of animals (particle implanted n=7, sham n=7) were euthanized eight weeks post-operation and 1-hour post-magnetic stimulation. Brains were sliced (25nm) and appropriately stained to observe neuronal activation, immune responses, and particle spread surrounding the injection site. Results showed no difference in immune response between groups. Additionally, increased neuronal activation was observed in the piriform cortex and cingulate gyrus of animals implanted with nanorods. These data demonstrate the ability of mechanical stimulation of striatal neurons to modify rat behavior. Behavioral tests examining the efficacy of magnetic particle stimulation for attenuation of oxycodone reinstatement are currently being investigated. Potential future applications of the technology include the use of wearable generators of low magnetic fields applied to intra-nasally administered magnetic nanoparticles.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.07/Y8

Topic: G.08. Drugs of Abuse and Addiction

Support: The Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Effects of Nalfurafine on Oxycodone Self-Administration in Adolescent Male and Female Mice

Authors: *Y. ZHANG[1], M. KREEK[2];
[1]Rockefeller Univ., New York, NY; [2]The Rockefeller Univ., New York, NY

Abstract: Prescription opioid oxycodone abuse has affected millions of adolescents and young adults in the United States. Kappa opioid agonists can counterbalance the euphoria effects of mu opioid agonists, such as oxycodone. Nalfurafine is a clinically used kappa agonist in Japan for the treatment of pruritus; its effects on oxycodone self-administration (SA) in adolescents have not been studied. **Objective:** The current study examines the effects of nalfurafine on oxycodone SA in adolescent male and female mice. **Methods:** Adolescent mice (5 week old) first received surgery during which a catheter was implanted into their jugular vein. After recovering from the surgery, mice were then placed into the self-administration chambers and allowed to self-administer oxycodone, two hour per day for 14 days. Following 14-day oxycodone SA, each mouse was then injected with a single dose of nalfurafine (0, 50, 20, 10 ug/kg, s.c.) 10 min before oxycodone self-administration for four consecutive days, respectively. **Results:** The high dose of nalfurafine (50 ug/kg) significantly reduced oxycodone SA; lower dose of nalfurafine (20 ug/kg) did not significantly affect oxycodone SA. The lowest dose of nalfurafine (10 ug/kg) may have increased oxycodone SA in the same mice. **Conclusion:** This is the first study to examine the effects of nalfurafine on oxycodone SA in adolescent mice. The effect of nalfurafine on the rewarding property of oxycodone will be further studied.

Disclosures: Y. Zhang: None. **M. Kreek:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.08/Y9

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH NIDA DA034886

Title: Decreased cocaine but increased opioid reward in offspring of males exposed to morphine during adolescence

Authors: *P. WALIA, A. M. TOORIE, D. N. TECENO, T. D. PATTON, E. M. BYRNES, F. M. VASSOLER;
Dept. of Biomed. Sci., Tufts Univ., North Grafton, MA

Abstract: Since the late 1990's, the United States has been suffering through an opioid epidemic. A growing body of evidence indicates possible transgenerational effects that extend the impact of opioid exposure to future generations. The goal of these experiments is to examine the effect of adolescent male opioid exposure on the next (F1) generation's drug self-administration behavior and developmental neuronal gene expression as well as potential mechanisms that may contribute to the transmission of these effects using a rat model (Sprague Dawley). Males were administrated increasing doses of morphine (5-25mg/kg, s.c.) for 10 days during adolescence (P30-39). Age-matched control males received saline injections at equivalent volumes. Animals were left drug-free until adulthood (P70-80) when they were mated with drug-naïve females (n = 24). Brains from F1 offspring were collected on P1, P7, P28, and P70. Male and female offspring were also examined for drug (morphine, oxycodone, or cocaine) self-administration in adulthood (P60-80). After mating, testes and spermatozoa from the cauda epididymis were collected from F0 males and acetylated H3 was examined. Results show that F1 animals have increased levels of opioid self-administration yet decreased cocaine self-administration. Male F0 seminiferous tubules displayed increased levels of acetylated histone H3 indicating developmental differences in spermatogenesis and genome packaging. Experiments examining developmental neural gene expression differences are ongoing. The results demonstrate that opioid exposure in the F0 generation has lasting effects, making future generations vulnerable to the harmful impact.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.09/Y10

Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC

Title: Effect of chemogenetic activation of thalamo-accumbens projections on the augmentation of heroin seeking induced by chronic food restriction

Authors: *A. CHISHOLM, D. RIZZO, É. FORTIN, V. MOMAN, N. QUTEISHAT, J.-P. MANOLIADIS, T. CAPOLICCHIO, A. ROMANO, P. PARSA, J. GASPARRI, C. BORGES, U. SHALEV;
Dept. of Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Drug addiction is a chronic disorder that is characterized by compulsive drug seeking and involves switching between periods of compulsive drug use, abstinence, and relapse. In both human addicts and animal models of addiction chronic food restriction has been shown to increase rates of relapse. Previously, our laboratory has demonstrated a robust increase in drug seeking following a period of withdrawal in chronically food-restricted rats compared to sated rats. To date, the neural mechanisms that mediate the effect of chronic food restriction on drug seeking have not been elucidated. Evidence from our laboratory indicates that chemogenetic activation of the paraventricular nucleus of the thalamus (PVT) reduces heroin seeking in chronically food-restricted rats. The PVT sends glutamatergic projections to the Nucleus Accumbens (NAc). Thus, the objective of the current study was to study the effect of chemogenetic activation of the PVT- NAc Shell and Core neuronal pathways on heroin seeking under food restriction conditions.
Male Long Evans rats were injected with a viral vector carrying an excitatory Designer Receptor Exclusively Activated by Designer Drug (DREADD) into the PVT, and implanted with a guide cannula aimed at the NAc Shell or Core. Next, rats were trained to self-administer heroin over 10 days (0.1 mg/kg/infusion; i.v.). Following training, rats were removed from the operant conditioning chambers and placed into drug withdrawal for 15 days. Over the withdrawal period, rats were exposed to a mild food restriction (90% of baseline body weight) or were given unrestricted access to food. On the 15th day of the withdrawal period, a drug-seeking test was conducted in which rats were intracranially injected with CNO (1.0 mM) into the NAc Shell or Core, to activate the PVT-NAc pathway, or vehicle. Injectors' placement was verified using immunohistochemistry.
All rats reliability learned to self-administer heroin. As expected, food-restricted rats demonstrated an augmented heroin seeking during the heroin-seeking test in comparison to sated rats. Preliminary data indicate that activation of the PVT-NAc Shell pathway attenuates heroin seeking in chronically food-restricted rats.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.10/Y11

Topic: G.08. Drugs of Abuse and Addiction

Title: Role of the ghrelin system in oxycodone self-administration and brain stimulation reward

Authors: *Z.-B. YOU[1], E. L. GARDNER[1], A. MOORE[1], T. BUCK[1], C. J. JORDAN[1], B. HUMBURG[1], G.-H. BI[1], Z. XI[1], L. LEGGIO[2];
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Abstract: Prescription opioid abuse is currently a serious worldwide public health problem. Identifying novel mechanisms underlying opioid addiction and developing innovative medications for treatment of opioid use disorders are high priorities in addiction research. Ghrelin is an orexigenic hormone secreted primarily from stomach and acts via the growth hormone secretagogue-receptor 1a (GHS-R 1a, a.k.a. ghrelin receptor) in regulating food intake and reward. Ghrelin has also been found to modulate mesolimbic dopamine transmission and regulate the rewarding effects of alcohol and psychostimulants. Much less is known about the role of the ghrelin system in contexts associated with prescription opioid misuse and abuse. In this study, we assessed responses of ghrelin in bloodstream and brain ghrelin receptors to oxycodone self-administration and self-administration-related behaviors, and the effects of ghrelin receptor antagonism on such behaviors using the selective ghrelin receptor antagonist JMV2959. We found that acquisition of oxycodone self-administration (0.1 mg/kg/infusion) in rats is associated with significant elevations in plasma ghrelin concentrations. Acquisition of oxycodone self-administration significantly altered mRNA expression of ghrelin receptors in the ventral tegmental area/substantia nigra (VTA/SN), a brain region critical for drug reward and motivation. Pretreatment of oxycodone trained rats with JMV2959 (0-5 mg/kg, i.p.) dose-dependently reduced oxycodone self-administration tested under either 0.05 or 0.0125 mg/kg/infusion of oxycodone. JMV2959 pretreatment also dose-dependently decreased the breakpoint for oxycodone self-administration tested under progressive ratio reinforcement. JMV2959 pretreatment (5 mg/kg, i.p.) in mice significantly inhibited intracranial self-stimulation maintained by optogenetic activation of VTA dopamine neurons. Our findings indicate that oxycodone and oxycodone experience is associated with development of hyperactivity of endogenous ghrelin in rats and suggest a contributory role of ghrelin signaling in the maintenance of oxycodone self-administration and in motivation for oxycodone-taking behavior. Thus, manipulations of ghrelin systems may represent a feasible approach for treatment of prescription opioid addiction. Supported by funds from NIDA-IRP

Disclosures: **Z. You:** None. **E.L. Gardner:** None. **A. Moore:** None. **T. Buck:** None. **C.J. Jordan:** None. **B. Humburg:** None. **G. Bi:** None. **Z. Xi:** None. **L. Leggio:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA045771
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Title: CRF-5-HT interactions and motivation for stress-induced opioid relapse

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Abstract: Previous studies have shown that stressors can inhibit 5-HT neuronal activity and release by stimulating release of the stress neurohormone corticotropin-releasing factor (CRF) within the serotonergic dorsal raphe nucleus (DRN). CRF effects on 5-HT DRN neurons are indirect, mediated by CRF-R1 receptors located on GABAergic afferents. Our laboratory is pursuing the potential role of these neurochemical interactions in stress-related psychiatric disorders including substance abuse. More recently, we have demonstrated a unique sensitization of 5-HT DRN neurons to GABAergic inhibition that is correlated with relapse to multiple drugs of abuse including opioids and in multiple models of stress-induced relapse. Furthermore, stimulation of GABA afferents to 5-HT DRN neurons is both necessary and sufficient for stress-induced relapse of previously extinguished morphine conditioned place preference (CPP). The current study demonstrates that stimulation of CRF-R1 within the DRN with the CRF-R1-preferring agonist ovine CRF reinstates morphine CPP in the absence of a stressor. Furthermore, intraDRN pretreatment with the CRF-R1 antagonist NBI 35965 blocks swim stress-induced reinstatement, indicating that this circuit is both necessary and sufficient for stress-induced opioid relapse. We also examined the role of this circuitry in stress-induced negative affect with ultrasonic vocalizations (USVs). USVs are naturally emitted by rats in response to environmental challenges: 50 kHz calls indicate positive affective responses to stimuli such as social interaction whereas 22 kHz calls indicate negative affective responses to stimuli such as pain, stress and drug withdrawal. We found that a footshock stressor, commonly used in models of stress-induced reinstatement of drug self-administration, elicits strong 22 kHz distress calls in rats. However, if animals are pretreated with intra-DRN NBI 35965, these 22 kHz distress calls are

significantly blunted. These data collectively support the hypothesis that stressors can elicit negative affective responses via their impact on CRF-5-HT circuits that motivate opioid relapse.

Disclosures: **N. McCloskey:** None. **C. Li:** None. **L.G. Kirby:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

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Program #/Poster #: 417.12/Y13

Topic: G.08. Drugs of Abuse and Addiction

Support: 1SC3GM130430-01

Title: Environmental enrichment reduces cue-induced heroin-seeking in male and female rats

Authors: L. LOUGHLIN[1], S. EWING[2], *R. RANALDI[1,2];
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Abstract: Previous studies have shown that environmental enrichment (EE) implemented after the development of a heroin self-administration habit significantly reduced cue-induced reinstatement of heroin-seeking behavior in male rats. Although EE seems to be a beneficial behavioral intervention for male rats, the potential for similar benefits in females has yet to be explored. The aim of the current study is to test whether or not EE reduces cue-induced reinstatement of heroin seeking in females as it has been shown to in males. In the current study rats were trained to self-administer heroin with contingent cues (stimulus light above the lever and pump sound) present. Afterwards, they were randomly assigned to either EE (novel stimulus objects and running wheel) or standard housing (non-EE). This was followed by extinction, which consisted of fifteen sessions in which lever pressing did not result in heroin nor heroin-associated cues. On the sixteenth day, a cue-induced reinstatement session occurred in which the drug cues were presented non-contingently twice, two minutes apart, at the start of the session and contingently upon active lever pressing for the remainder of the session. Thus far, results show that exposure to EE significantly reduces responding in reinstatement for males and we are finding similar patterns in the females. The findings demonstrate that EE can reduce cue-induced reinstatement of heroin seeking in both male and female rats. As such, these findings suggest that environmental enrichment may be a useful behavioral therapeutic strategy for the treatment of heroin use disorder in both sexes.

Disclosures: **L. Loughlin:** None. **S. Ewing:** None. **R. Ranaldi:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

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Program #/Poster #: 417.13/Y14

Topic: G.08. Drugs of Abuse and Addiction

Title: Does the A118G mu-opioid receptor polymorphism predict sensitivity to reward downshifts?

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Abstract: Understanding the emotional reaction to loss, or psychological pain, is a critical problem for the field of mental health. Animal models of loss have pointed to the opioid system as a nexus of psychological pain, physical pain, and substance abuse. However, few attempts have been made to connect the results of animal models of loss to human behavior. Allelic differences in the human mu opioid receptor gene, notably the A118G single nucleotide polymorphism, have been linked to individual differences in social rejection, substance abuse, and PTSD symptoms. The present study explored the relationship between the A118G SNP and behavior in a reward downshift task in humans. Participants were trained to touch a target on a screen for points for 10 trials, and subsequently received 8 trials with an 8:1 reduction in points. The relative change in the number of button presses following the downshift was the primary variable of interest. Then, participants were tested in the cold pressor test to assess pain sensitivity. Finally, participants provided a sample of DNA which was screened for the A118G mutation in the mu opioid receptor. DNA was extracted from the saliva sample and amplified by polymerase chain reaction. Amplification was confirmed by agarose gel electrophoresis and amplified DNA was purified. Sequencing was outsourced and sequencing traces analyzed. All behavioral data was collected prior to genotyping to ensure that the experimenter was blind to genotype during training. We found that A-heterozygotes were more sensitive to the downshift, exhibiting a transient decrease in button pressing during the downshift. G-carriers were insensitive to the reward downshift. In the cold pressor task, there was insufficient evidence to link genotype with pain sensitivity. The insensitivity to reward downshifts we observed are counterintuitive in the context of previous studies in which G-carriers exhibited increased sensitivity to physical pain. However, based on evidence that the mu opioid system plays a critical role in comparing hedonic values, we propose a new hypothesis about individual differences in reward downshifts. The present results can be understood by considering that individuals that have a normally functioning mu receptor are capable of making comparisons across a wide range of experience. However, for G-carriers in which the mu receptor function is reduced, hedonic values may be rendered all-or-nothing, such that all rewards are more equally rewarding and all pain is more equally painful.

Disclosures: **A.M. Daniel:** None. **B.K. Rushing:** None. **K. Tapia:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.14/Y15

Topic: G.08. Drugs of Abuse and Addiction

Title: Fentanyl vapor self-administration in mice: A novel non-invasive preclinical opioid addiction model

Authors: ***M. ORTIZ**[1], K. MOUSSAWI[2], B. J. TUNSTALL[2], R. C. MARCHETTE[2], G. F. KOOB[3], A. BONCI[2], L. F. VENDRUSCOLO[2];
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Abstract: Opioid addiction is a growing public health concern in the United States with fentanyl usage accounting for more than half of opioid overdose fatalities. Intravenous drug self-administration in mice has been frequently used to study the reinforcing effects of drugs. However, intravenous self-administration studies require surgery and are complicated by limitations of catheter failures, the likelihood of which increases with duration of self-administration training. Here, we developed a non-invasive fentanyl vapor self-administration model of opioid addiction in mice, which circumvents the pitfalls of intravenous self-administration. First, we conducted a dose-response function of the analgesic and locomotor effects of vaporized fentanyl to determine an effective fentanyl concentration. Mice were then trained to self-administer fentanyl vapor on a fixed ratio 1 (FR1) schedule of reinforcement in an air-tight operant chamber equipped with both active and inactive nosepoke holes. Poking the active nosepoke operandum resulted in vaporized fentanyl delivery to the operant chamber for approximately one minute. A light cue was paired to the drug delivery. Mice readily learned to self-administer fentanyl in 1-hour sessions. Fentanyl levels in the blood were proportional to the dose of vaporized fentanyl. Following self-administration, mice went through extinction training where they were placed into the operant chambers in the absence of light cues and fentanyl vapor. After extinction, mice exposed to the light cue reinstated drug seeking. Further, mice allowed long access (LgA; 12 h/session) to fentanyl escalated drug intake over the course of 14 sessions, whereas mice allowed short access (ShA; 1-hour sessions) to fentanyl exhibited stable fentanyl self-administration over time. The LgA mice showed more naloxone-precipitated signs of somatic withdrawal when compared to ShA mice. In summary, we developed and validated a non-invasive fentanyl vapor self-administration model that recapitulates the main features of intravenous models. Advantages of this model include: 1) utilizing a vast repertoire of behaviorally selected and genetically modified mouse lines, 2) conducting prolonged experimental protocols that require extensive training (i.e., LgA sessions) or longitudinal studies

without the risk of catheter failure, and 3) facilitating *in vivo* calcium imaging and electrophysiology experiments that require a tether in freely moving mice.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA040414
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Title: GluA1 protein levels in the nucleus accumbens core and shell after the incubation of oxycodone craving

Authors: A. WHEATLEY[1], M. E. WOLF[2], ***M. T. STEFANIK**[1];
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Abstract: The recurring desire to take drugs, even after years of abstinence, is among the most insidious features of addiction. It is often triggered by cues previously associated with drug use. Cue-induced craving progressively intensifies (‘incubates’) over weeks of forced abstinence or withdrawal. This ‘incubation of craving’ has been reliably observed during withdrawal from different classes of drugs of abuse in rodents and humans. Recently, prescription opioid abuse and dependence has increased to epidemic levels. A prominent contributor to this trend is oxycodone (Oxy), a semisynthetic opioid, and the most widely prescribed opioid painkiller. Currently, however, little is known about the incubation of Oxy craving.
Incubation for psychostimulants depends on time-dependent strengthening of AMPA receptor (AMPAR) transmission onto medium spiny neurons in the nucleus accumbens (NA) through the accumulation of high-conductance, GluA2-lacking, Ca^{2+}-permeable AMPARs (CP-AMPARs). Our studies assess the incubation of *Oxy* craving and underlying neuroadaptations in the NA. It is hypothesized that expression of the incubation of Oxy craving also depends on strengthening of NA synapses via CP-AMPAR inclusion.
Male Sprague Dawley rats underwent 10 days of extended-access Oxy self-administration (6hr/day, 0.15mg/kg/infusion). Rats returned to the operant chamber for a 30-min test on withdrawal day 1 (WD1), and either WD15 or WD30, measuring the time course of cue-induced seeking. Results show that incubation of oxycodone seeking occurs at maximal levels around WD15. Next, animals were killed at each time point, and tissue from the NA (both core and shell

sub-regions) was biotinylated to test whether Oxy incubation is accompanied by elevated cell-surface levels of homomeric GluA1 receptors, the type of CP-AMPAR that is increased after incubation of cocaine craving. Preliminary results indicate that increased GluA1 levels parallel drug-seeking behavior in the NA core. Current studies are underway examining AMPAR receptor composition in the NA shell. By understanding incubation's cellular basis, we gain insight into mechanisms underlying the persistent vulnerability to relapse.

Disclosures: A. Wheatley: None. **M.E. Wolf:** None. **M.T. Stefanik:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant 1U01DA045300
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Title: Individual variation in the vulnerability and resilience to opioid dependence

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Abstract: There has been a significant rise in opioid use disorder (OUD) in the United States over the past decade, making the understanding of the genetic and behavioral components that contribute to OUD necessary to explore. In the current study, rats underwent various testing procedures to assess the behavioral determinants associated with vulnerability versus resilience to opioid dependence. A heterogeneous stock rat line was used in order to better capture genetic and behavioral diversity within a rodent model. Stress and anxiety-related behaviors were assessed via the elevated-plus maze and an open field test, while analgesic thresholds were determined using a tail-flick test. Next, rats underwent 3 weeks of long-access (LgA, 12-hr sessions) heroin self-administration training, immediately followed by a progressive ratio test to determine the motivation to work for the drug. Following 4 more sessions of LgA, rats underwent a within-session extinction training and heroin-induced reinstatement (0.25 mg/kg, s.c.) test and then 6 days of extinction training. Immediately following the last day of extinction training, a test for cue-induced reinstatement was performed. The behavioral measures previously mentioned to assess stress, anxiety and analgesic threshold were repeated. Data thus far demonstrates that both male and female rats learn to acquire heroin self-administration at the same rate, and do not differ in their motivation to work for heroin, or in reinstatement of drug-seeking behavior. However, a correlation was present between total distance travelled during the open field test and cue-induced drug-seeking behavior. In fact, if rats are characterized based on

a high level of locomotor activity (high-responders, HRs) versus a lower level of locomotor activity (low-responders, LRs) in the open field test, HRs show greater rates of heroin-induced and cue-induced drug-seeking behavior compared to yoked-saline control rats. The HR/LR model is commonly used to examine individual variation in the acquisition of drug-taking behavior, particularly psychostimulants, but here we show that this model may also be relevant for predicting individual variation in drug-seeking behavior following heroin self-administration. These data highlight the value of focusing on individual variation in drug-seeking behavior and progress our understanding of behavioral differences that may be associated with vulnerability versus resilience to OUD. Further testing will focus on the genetic, epigenetic, and gut microbiome factors that contribute to individual variation in heroin dependence.

Disclosures: B.N. Kuhn: None. **A.T. Roberts:** None. **P.W. Kalivas:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA046143
NIH Grant DA003906

Title: Cellular specificity of matrix metalloproteinase activation on accumbens medium spiny neurons during heroin relapse

Authors: *V. CHIOMA[1], A.-C. BOBADILLA[2], P. W. KALIVAS[3];
[2]Dept. of Neurosci., [1]Med. Univ. of South Carolina, Charleston, SC; [3]Neurosci. Res., Med. Univ. S Carolina, Charleston, SC

Abstract: Heroin abuse is a leading cause of drug overdose-related deaths in the United States, highlighting a need for further research elucidating effects of maladaptive neuroadaptations following prolonged heroin use. Activation of the tetrapartite synapse in nucleus accumbens core (NAcore), which comprises of pre- and postsynapse, astrocytic processes, and surrounding extracellular matrix (ECM), has been linked to increased relapse vulnerability. Specifically, degradation of the ECM by activated matrix metalloproteinases (MMPs) is involved in both constitutive and transient extracellular synaptic remodeling. Following chronic heroin self-administration and extinction training, transient increases in MMP-9 activity in NAcore were elicited after 15 mins of cued heroin seeking compared to heroin-extinguished and saline control rats. Although increases in MMP-2,9 fluorescence can be localized to the soma and dendritic processes of medium spiny neurons (MSNs) in accumbens, it is unknown which specific cell types harbor changes in MMP activity under heroin-extinguished and cued reinstatement

conditions. We hypothesized that D1-receptor expressing MSNs express increased pericellular localization with MMPs during transient cued heroin seeking, while D2-receptor expressing MSNs express increased localization following extinction. We used an AAV cre-dependent mCherry virus to transfect accumbens MSNs in D1 and D2 cre-dependent rats and measured the localization of activated MMP-2,9 after FITC-gelatin microinjection under extinguished and reinstated conditions. For D1 MSNs, we observed increased MMP-2,9 localization with dendritic surfaces in reinstated animals compared to both yoked saline controls and heroin-extinguished animals. While D2 MSNs showed increased MMP-2,9 localization only in heroin-extinguished animals, but MMP-2,9 localization after 15 min reinstatement was reduced to yoked saline levels. We also investigated whether the previously mentioned increased MMP activity selectively around D2 MSNs is mediated by extinction training. Next, we used pharmacological MMP-2,9 inhibitors to determine which were contributing to increased localization, specifically around D1 MSNs during reinstatement and D2 MSNs after extinction. Finally, we studied the involvement of tissue inhibitors of metalloproteinases (TIMPs) in NAcore during reinstatement to determine if local MMP inhibition around D2 MSNs is necessary for cued heroin seeking. These findings reveal how NAcore extracellular matrix signaling underlying constitutive and transient synaptic plasticity relies in part on specific cell-types.

Disclosures: V. Chioma: None. **A. Bobadilla:** None. **P.W. Kalivas:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.18/Y19

Topic: G.08. Drugs of Abuse and Addiction

Support: R01-DA009411-19

Title: Adolescent nicotine exposure alters ventral tegmental area inhibitory transmission and enhances morphine reward learning in adulthood

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[1]Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [2]Anesthesiol. and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Opioid use disorder is a rapidly-growing source of preventable mortality. Identifying underlying neural mechanisms that give rise to opioid abuse is critically needed. Paralleling the recent rise in opioid abuse, nicotine use in adolescence has escalated**.** The long-term consequences of prior exposure to nicotine remain poorly understood. Our lab has shown that the acute effects of adult nicotine exposure in the ventral tegmental area (VTA), and on drug reinforcement behavior, are persistent until adulthood if nicotine exposure occurs during

adolescence. Others have revealed male ICR mice given nicotine during adolescence show greater preference for a morphine-paired context during adulthood relative to mice that receive saline during adolescence. However, the effect of sex and strain on the ability of early exposure to nicotine to enhance later drug-context association is unknown. Also, the underlying neural mechanisms that drive the ability of early nicotine exposure to enhance associative learning in adulthood, and whether these mechanisms differ when nicotine is given in adolescence as compared to acutely in adulthood, are unknown. To target these knowledge gaps**,** we exposed adolescent mice to nicotine (0.5mg/kg, i.p.) daily for two weeks and probed the behavioral- and circuit-level adaptations in morphine reward when the animals were adults. We examined the influence of adolescent or adult nicotine exposure in male and female mice on two measures of associative learning in adulthood: morphine conditioned place preference (presented here) and touchscreen autoshaping (in progress). As in male ICR mice, two weeks of adolescent nicotine in male C57BL/6J mice enhanced time spent in the morphine-paired compartment relative to mice that received saline during adolescence. Likewise, a single acute injection of nicotine given 15h prior to the first morphine-context pairing in adulthood also enhanced morphine place conditioning. These behavioral changes corresponded with an alteration in VTA GABA signaling. VTA GABA neurons demonstrated a depolarizing shift in the GABA reversal potential and paradoxical heightened action potential firing in response to morphine following adolescent nicotine exposure, as compared to animals treated with saline during adolescence. Together, our data show adolescent nicotine exposure enhances morphine reward learning in adult mice in concert with increased excitation in VTA GABAergic circuitry. We are now testing the impact of these altered GABA neurons on VTA-striatal circuits as well as the role of sex-specific differences in this heightened reward sensitivity to morphine following adolescent nicotine exposure.

Disclosures: R.E. Wittenberg: None. **B.A. Kimmey:** None. **A.J. Eisch:** None. **J.A. Dani:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA045000

Title: Effects of acute oxycodone on brain stimulation reward in male and female rats using intracranial self-stimulation

Authors: N. CONSTANTINO[1], S. PAGE[2], M. MAVRIKAKI[1], T. LINTZ[1], *E. H. CHARTOFF[2];
[1]Psychiatry, [2]Harvard Med. Sch., Belmont, MA

Abstract: Oxycodone has potent analgesic effects, but also produces sedation and has a high abuse liability, making it one of the most widely misused prescription painkillers. Oxycodone can elicit rewarding and reinforcing effects, as it is readily self-administered. Despite epidemiological studies examining prevalence rates of opioid use disorder in men and women, little is known about biologically-based sex differences in the effects of oxycodone on reward processes. We recently showed that male rats will initially self-administer more oxycodone than females—an effect that normalizes after extended self-administration. This raises the possibility that oxycodone is more rewarding in males. Alternatively, oxycodone may be less rewarding in males, resulting in higher levels of intake to achieve the same reinforcing effects. To test this, we used intracranial self-stimulation (ICSS), which measures the ability of a drug to facilitate or depress brain stimulation reward. This is measured either by a decrease in the frequency of stimulation that first sustains operant responding (stimulation threshold), or by an increase in the percent maximum control rate of responding (%MCR). We administered acute injections of oxycodone (0.1, 0.3, 1.0, and 3.0 mg/kg, IP) to adult male and female Sprague Dawley rats in a within-subjects design, with each rat receiving increasing followed by decreasing doses once every 2 days. Using both analysis methods, we show that oxycodone can both facilitate and depress ICSS responding over the 90-minute ICSS session, with higher drug doses having profound motor-suppressing effects within the first 45 minutes, followed by emergence of reward facilitation. Males show a nominally greater increase in reward-related effects of oxycodone compared to females, as well as a reduced motor-suppressing effect. These data support our hypothesis that oxycodone facilitates reward function more in males than females, which is consistent with males initially self-administering more oxycodone. Consistent with other work showing development of opioid-induced reward facilitation over time, the second administration of some oxycodone doses produced a greater rewarding effect compared to the first. In conclusion, oxycodone produces broadly similar effects on reward- and motor-related processes measured with ICSS in males and females, although there are significant sex differences that may ultimately influence opioid misuse.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA DA25267
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NIDA UG3 DA048353 01

Title: Evaluation of the opioid receptor pharmacology of the Kratom (Mitragyna speciosa) alkaloids Mitragynine and 7-Hydroxymitragynine in cell membranes and rats

Authors: *T. HIRANITA[1], S. OBENG[1,2], J. LEON OYOLA[2], A. E. PENNINGTON[1], M. E. REEVES[1], L. F. RESTREPO[1], J. S. FELIX[1], A. PATEL[1], N. P. HO[1], J. L. WILKERSON[1], C. R. MCCURDY[2], L. R. MCMAHON[1];
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Abstract: Of the approximately 40 alkaloids identified in the Southeast Asia medicinal and recreationally used plant *Mitragyna speciosa* (kratom), the alkaloids mitragynine and its metabolite 7-hydroxymitragynine have received much attention due to their opioid receptor pharmacology. However, details of this opioid pharmacology have not been fully established. Here, receptor binding in CHO cell membranes and drug discrimination in rats were used; the extent to which pharmacological actions vary as a function of route of administration was of particular interest. Mitragynine had 91-fold less affinity than morphine to displace [^{3}H]DAMGO binding to human μ opioid receptors (Ki: 600 and 6.57 nM, respectively). In rats discriminating mitragynine (32 mg/kg, i.p.), its ED_{50} value for producing discriminative stimulus effects was 9.7 mg/kg. The μ-opioid receptor agonists morphine (0.32-32 mg/kg) and fentanyl (0.0056-0.32 mg/kg) produced a maximum 65% and 75% mitragynine-lever responding, respectively. When administered p.o., mitragynine was 5.9-fold more potent than when it was administered i.p. In rats discriminating morphine (3.2mg/kg, i.p.), its ED_{50} value for producing discriminative stimulus effects was 1.75 mg/kg. Fentanyl (0.0032-0.32 mg/kg) and the low efficacy μ-opioid agonist nalbuphine (1.78-32 mg/kg) fully substituted for the morphine discriminative stimulus (ED_{50}: 0.018 and 8.2 mg/kg, respectively). 7-Hydroxymitragynine fully substituted for morphine and was 4.4-fold more potent (ED_{50} = 0.40 mg/kg). Mitragynine, when administered i.p. and p.o., produced up to 74% and 76% morphine-lever responding; potency p.o. was 3.8-fold less potent than i.p. (ED_{50}: 99.0 and 26.4 mg/kg, respectively). The opioid receptor antagonist naltrexone (0.032mg/kg, i.p.) shifted the dose-effect function of mitragynine rightward and downward due to the failure of naltrexone to antagonize the rate-decreasing effects of mitragynine. Naltrexone produced surmountable antagonism of the morphine discrimination dose-effect function (8.4-fold). The present results strongly suggest that 7-hydroxymitragynine is a more selective μ agonist than mitragynine. Opposing differences in the potency of mitragynine when it is administered p.o. versus i.p. in the mitragynine versus morphine discrimination procedures are consistent with pharmacokinetics yielding different metabolites that translate into different pharmacodynamic profiles.

Disclosures: T. Hiranita: None. **S. Obeng:** None. **J. Leon Oyola:** None. **A.E. Pennington:** None. **M.E. Reeves:** None. **L.F. Restrepo:** None. **J.S. Felix:** None. **A. Patel:** None. **N.P. Ho:** None. **J.L. Wilkerson:** None. **C.R. McCurdy:** None. **L.R. McMahon:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.21/Y22

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH T32-NS007413 (SJS/AJE)
NASA 80NSSC17K0060 (AJE)
CHOP Department of Anesthesiology and Critical Care Development Funds (AJE)

Title: Ultrasonic vocalizations (USVs) during intravenous oxycodone self-administration and context-elicited reinstatement in male rats

Authors: *S. J. SIMMONS[1], W. H. HAURY[1], R. E. GREENBAUM[1], J. M. SCHWARTZ[2], H. M. DEUTSCH[2], A. J. EISCH[1,3];
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Abstract: Opiates were linked to 42,000 overdoses in the United States in 2016, and prescription opioids - like oxycodone - have contributed to the current opioid epidemic. Self-reports suggest oxycodone possesses “unparalleled addictive potential”, and measuring this subjective ‘likability’ of oxycodone in animals is important to better evaluate oxycodone’s abuse potential. To meet this need, we recorded ultrasonic vocalizations (USVs) - interpreted to reflect affective states based on emission frequency - in rats trained to self-administer oxycodone intravenously. Adult male Long-Evans rats (n=7) trained (3h/d, 6d/wk, 18d; “acquisition”) to bar press for 3-s infusions of oxycodone (days 1-12: 0.10 mg/kg/inf; days 13 to 18: 0.05 mg/kg/inf). Rats then extinguished bar pressing in an alternate physical context (3h/d, 6d/wk, 12d; “extinction”). After extinction, half of the rats were returned to the oxycodone-paired context for reinstatement (ABA; n=4) while half were placed in the extinction context (ABB; n=3). USVs were recorded on days 1 and 13 of acquisition and during reinstatement. Preliminary results show rats acquired and extinguished oxycodone self-administration behavior comparably as in prior work. During acquisition, rats emitted 50-kHz USVs 242% more often prior to the first oxycodone infusion on day 13 vs. day 1 [T-=1.00, p=0.080] as has been seen in cocaine self-administering rats. During reinstatement, ABA rats pressed 268% more vs. extinction day 12, while ABB rats only pressed 48% more vs. extinction day 12 [t(5)=3.045, p=0.029]. Consistent with prior work, these data show that an oxycodone-paired context elicits drug-seeking after the operant response is extinguished. Regarding USVs during reinstatement, ABA rats emitted 319% more 50-kHz USVs compared to ABB rats [U(ABB)=6.00, p=0.057]. These novel data show that rats emit more 50-kHz USVs when placed in a context used previously for oxycodone self-administration

relative to USVs emitted when rats are returned to the extinction context. These data support the development of an anticipatory response that may reflect individual differences in 'likability' associated with oxycodone. Future studies will probe the circuitry hypothesized to underlie the contextual learning shown to imbue physical environments with salience and relapse-driving properties.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.22/Y23

Topic: G.08. Drugs of Abuse and Addiction

Support: UCI HEAL Grant

Title: Gut-brain interactions in intravenous fentanyl self-administration

Authors: *M. REN[1], S. LOTFIPOUR[2];
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Abstract: The United States is currently experiencing an opioid addiction epidemic. The number of drug-related deaths has spiked over the last five years primarily due to fentanyl. It is therefore necessary to investigate the mechanisms mediating fentanyl's motivational properties in order to assist in the development of successful treatment strategies. Our lab has developed a rat model of intravenous fentanyl self-administration to investigate factors that influence fentanyl intake, including sex, drug dose, and gut bacteria. Gut bacteria communicate with the brain, and vice versa, via the gut-brain axis to regulate brain function, mood, and behavior. Preclinical research has shown a significant impact of intestinal bacteria on regulating addiction-related behaviors. We show that male and female Sprague Dawley adult rats are able to learn response requirements via reinforced nosepokes to acquire fentanyl infusions at 1.25 or 2.5 versus a 0 μg/kg/infusion dose on an escalating schedule of reinforcement, i.e. fixed ratio schedule (FR) 1, 2, 5 and progressive ratio. Additionally, males treated with prolonged oral antibiotics vs. water have decreased discrimination of response requirements for fentanyl, suggesting a role of gut bacteria in drug-related behavior, including the motivation to attain an opioid reinforcer. Our findings provide feasibility for an intravenous fentanyl self-administration animal model and uncover potential factors mediating drug intake, which may lead to effective addiction interventions to decrease opioid-related deaths.

Disclosures: M. Ren: None. **S. Lotfipour:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.23/Y24

Topic: G.08. Drugs of Abuse and Addiction

Title: Relation between acute withdrawal from morphine and predictors of drug abuse risk in rats

Authors: W. WHITE, *I. M. WHITE;
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Abstract: In a prior study, when morphine (5 mg/kg) was periodically administered to rats, considerable individual differences were seen in activity 12 to 24 hours after administration. Some animals had a very large decrease in activity by the end of the day following administration. The reduction in activity appeared to be a sign of acute withdrawal, and these animals appeared to be acute withdrawal sensitive. On the other hand, other animals showed no decrease in activity, and they appeared to be acute withdrawal insensitive. The present study began to assess how the measure of acute withdrawal was related to other measures that predict drug abuse. Twenty male, Wistar rats were run on an assessment of acute withdrawal. Every five days rats received a dose of morphine, either 2.5, 5, or 10 mg/kg, and their activity was monitored for the next 24 hours. Results produced by morphine were compared to those produced by saline. In addition, several other measures thought to be predictive of drug vulnerability were collected. These included time spent in closed and open arms of an elevated maze (a measure of anxiety); distance moved in a novel open field (a measure of sensation seeking); and distance moved in response to a moderate dose of amphetamine (0.5 mg/kg) before and after repeated morphine treatment (a measure of cross sensitization). All measures produced considerable individual differences. Higher anxiety, lower sensation seeking, and lower cross sensitization were modestly related to intensity of acute withdrawal produced by morphine (5 mg/kg). Relating measures may provide clues about common mechanisms and suggest how measures might be combined to produce better predictors of drug vulnerability.

Disclosures: W. White: None. **I.M. White:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.24/Y25

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA043676

Title: A smartphone-based tool for neuroeconomic prediction of treatment outcomes in opioid use disorder treatment

Authors: *A. MELLIS[1], A. B. KONOVA[2], S. LOPEZ-GUZMAN[3], T. BURLEIGH[4], H. BAYER[5], P. W. GLIMCHER[6];
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Abstract: Since the turn of the century, the annual rate of opioid overdose deaths has nearly quadrupled. However, even participants who receive the current front-line treatment for opioid use disorder, medication assisted therapy (MAT), are likely to relapse and drop out of treatment. However, given that most treatment facilities are operating near full capacity, determining how to deploy limited resources to those at highest risk of relapse and dropout is critical. Mobile health applications provide unique opportunity for monitoring using ecological momentary assessments, improving the resolution of predictions. Our past work in an opioid MAT program (86% male, 50% white, average age = 48.1) has demonstrated that a combination of neuroeconomic tasks and clinical assessments can provide accurate posterior probabilities of opioid use events, above the predictive power of clinical assessments alone.
To address this need we developed a gamified neuroeconomic tool for deploying these assessments on a daily basis to subjects in MAT programs. We specifically developed incentive-compatible gamified versions of the Levy risk task (Levy et al., 2010) and a standard intertemporal choice task (Kable & Glimcher, 2007). Our neuroeconomic tasks use restricted choice sets (20-35 trials, rather than 120-150), calibrated to determine task performance in under 3 minutes and can provide accurate estimates of risk attitude, ambiguity attitude and discount rate. We embedded these tasks in a commercial gamified environment and in a non-OUD cohort found high levels of compliance across subjects. We combined these tasks with other instruments that measure daily self-reports of treatment compliance, relapse to illicit opioid use, craving, and exposure to drugs, to produce a suite appropriate for use in a MAT cohort. In a pilot project, participants recruited from a MAT program are scheduled to complete 21 days of mobile

assessment to demonstrate the feasibility and acceptability of a mobile health app in an outpatient MAT setting.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.25/Y26

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant DA009815

Title: The long-acting glucagon-like peptide-1 receptor agonist, Liraglutide, reduces heroin self-administration and relapse in rats

Authors: *J. E. DOUTON, S. BALLARD, P. S. GRIGSON;
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Abstract: Drug addiction is commonly understood as the usurpation of reward-related learning processes that alters the normal functioning of the brain reward system and directs the individual to exclusively seek and take drug. However, the motivated state of an individual when craving drug is similar to the state observed in animals that crave food when hungry, water when thirsty, or salt when sodium deficient - where there is only one goal and no substitute. In this context, drugs of abuse appear to have an effect on the 'need' system, affecting the overall metabolic state of the individual. Recently, much research has focused on the effect of feeding-related hormones, such as glucagon-like peptide-1 (GLP-1), on non-feeding motivated behaviors involving drugs of abuse. Here, we assessed the effects of the long acting GLP-1 receptor agonist, liraglutide (Lir), on heroin use and addiction. Rats had the opportunity to self administer heroin for six hours for a total of 10 days. After drug-taking behavior was acquired, rats were treated for 12 days with vehicle or 1mg/kg of Lir daily, one hour before being placed in the experimental chamber. Thereafter, rats began a 15-day abstinence period in which daily treatment with vehicle or Lir continued. Following abstinence, a test was performed to assess cue-induced reinstatement and drug-induced relapse. Here, we show that chronic treatment with Lir significantly reduced two key components of heroin addiction: heroin self-administration and drug-induced reinstatement of heroin seeking behavior (i.e.relapse). One hour pretreatment with Lir, however, was not effective in reducing cue-induced reinstatement. Importantly, while

effective in reducing both self-administration and 'relapse', prolonged treatment with this dose of Lir did not affect blood glucose or body weight. Taken together, these findings show that the long acting GLP1-R agonist, Liraglutide, is a candidate for the treatment of relapse in individuals with an opioid use disorder and even for the reduction of heroin taking for individuals motivated to begin the process of quitting.

Disclosures: **J.E. Douton:** None. **S. Ballard:** None. **P.S. Grigson:** None.

Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

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Office of Naval Research (ONR) 00014-19-1-2149
The Hope for Depression Research Foundation
The Pritzker Neuropsychiatric Research Consortium

Title: Differences in behavioral response to morphine in a genetic model of temperament

Authors: ***M. A. EMERY**[1], C. AYDIN[1], E. K. HEBDA-BAUER[1], P. M. MARAS[1], A. PARSEGIAN[1], A. V. STEFANOV[1], A. TANG[1], S. J. WATSON, Jr.[2], H. AKIL[2];
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Abstract: Our lab has developed a genetic model of temperament using a selective breeding strategy based on degree of locomotor response in a novel environment. Using this strategy, we have generated and maintain two lines of rats with distinct behavioral and neurochemical phenotypes, termed selectively-bred high-responders (bHRs) and low-responders (bLRs). Our prior work using these lines have demonstrated that their temperamental differences affect their propensity to seek and take psychostimulant drugs. Specifically, bHRs demonstrate a higher basal propensity to take drugs, due to a broadly sensation-seeking phenotype; whereas bLRs appear less susceptible at baseline but will seek and take drugs in response to psychosocial stress. Further, gene expression studies have revealed broad differences in gene expression levels between these two lines which are likely to mediate the differences in temperament. Notably, gene expression differences include members of the endogenous opioid system in multiple reward-related brain areas. In addition to basal differences in the opioid system, exposure to stress during adolescence alters opioid gene expression differentially between the two lines. However, to date bHR/bLR differences in the response to morphine have not yet been

characterized. Here, we characterize behavioral responses of bHR and bLR rats to morphine in a variety of behavioral paradigms, including locomotor sensitization, conditioned place preference, and rate of analgesic tolerance development. In addition, we expand the characterization of basal differences in the expression patterns of opioid system components between these lines. Results reveal strain differences in the behavioral response to morphine. Further, basal differences in the biology of the endogenous opioid system as well as differences in the biological response to morphine administration may, at least in part, mediate these differential behavioral responses. These findings provide a foundation to use this model to probe the genetic and neurobiological antecedents that mediate individual differences in the path to develop and maintain opioid addiction.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.27/Y28

Topic: G.08. Drugs of Abuse and Addiction

Support: Boettcher Foundation Webb-Waring Biomedical Research Award

Title: Oral prescription opioid-seeking behavior in male and female mice

Authors: *A. PHILLIPS[1], D. MCGOVERN[2], J. LEE[2], K. RO[2], D. HUYNH[2], S. ELVIG[2], K. FEGAN[2], D. H. ROOT[2];
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Abstract: A significant portion of prescription opioid users self-administer orally rather than intravenously; however, most animal opioid research has been conducted using the intravenous route. These models have demonstrated that cues associated with intravenous use are sufficient to cause relapse. Our first objective was to determine whether oral drug-associated cues are sufficient to cause relapse. It is also clinically shown that preference to self-administer orally at least partially relates to the user's sex, leading to our second objective to determine whether sex differences exist in susceptibility to relapse. Mice orally self-administered escalating doses of oxycodone under postprandial and non-postprandial conditions and exhibited robust cue-induced reinstatement of extinguished drug-seeking behavior. An additional group of mice showed that oral self-administration under non-postprandial conditions is sufficient to support cued reinstatement. Because we discovered that female mice earned significantly more mg/kg oxycodone than male mice, subsequent gonadectomy studies were conducted to evaluate the

effects of gonadal sex steroids on oral self-administration. Contrary to our initial hypothesis that these procedures would reverse the female to male imbalance of oxycodone oral self-administration, we found that ovariectomy prandial-dependently enhanced, while orchiectomy across dose and prandial-independently suppressed oxycodone self-administration. These studies establish that 1) oral drug cues are sufficient to cause reinstatement that is independent of prandial conditions, 2) earned oral oxycodone is larger in female mice compared with male mice, and 3) gonadectomy produces divergent effects on oral oxycodone self-administration between sexes. Future projects regarding these findings will focus on the neuronal role of ventral tegmental area neurons during the extinction-reinstatement phase of our paradigm, leptin receptor antagonism outcomes during postprandial conditions, and further investigation of gonadal steroid hormones with attention on their organizational effects.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

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Topic: G.08. Drugs of Abuse and Addiction

Support: VA Merit Review Award I01RX001144
VA Interprofessional Polytrauma and Traumatic Brain Injury Rehabilitation Research Fellowship
NIH Grant R01 DA045000

Title: Exploring the impact of biological sex on oxycodone place conditioning behavior in male and female rats

Authors: *J. A. BABB[1,2], N. CONSTANTINO[3], M. HAMMOND[3], G. B. KAPLAN[4,2], E. H. CHARTOFF[1,3];
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Abstract: Prescription opioid misuse can escalate to opioid dependence and opioid use disorder and continues to represent a major factor in the ongoing U.S. opioid epidemic. Women report different motivations for initiating drug use and experience different acute and chronic effects of drugs than men. Despite evidence that sex may impact addictive behavior in humans, preclinical rodent models investigating drug-seeking behavior have largely omitted females. In this study, the rewarding properties of the prescription opioid oxycodone were evaluated in adult male and

female rats using a well known rodent behavioral paradigm: place conditioning. A repeated measures ANOVA was used to analyze the effect of sex on pre- and post-conditioning preference for drug-associated contexts. In response to two days of twice daily conditioning sessions with 3mg/kg oxycodone and saline, both male and female rats acquired significant conditioned preference for the drug-paired context compared to the vehicle-paired context (main effect of conditioning; $F_{(1,14)} = 45.6$, $p<0.001$), and to a similar degree (no main effect of sex or sex by conditioning interaction). Preliminary data suggest however, that although initial context-drug associations are similar across sexes, female rats may take longer to extinguish preference for drug-paired contexts in the absence of drug. Ongoing experiments are exploring this possibility, and potential neural mechanisms underlying this phenomenon. These experiments could provide novel insight into differential opioid seeking behavior between male and female rodents which may lead to novel therapeutic compounds targeted to men and women in a sex-specific manner.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 417.29/Y30

Topic: G.08. Drugs of Abuse and Addiction

Support: DA23205
DA25267
DA48353

Title: The sigma1 receptor antagonist CM304 enhances the antinociceptive effects of cannabinoid CB1 agonists, but not mu-opioid receptor agonists, in mice

Authors: *S. OBENG[1], S. INTAGLIATA[2], M. MOTTINELLI[2], L. F. RESTREPO[1], P. AVI[1], V. A. TAYLOR[1], M. E. REEVES[1], A. E. PENNINGTON[1], C. R. MCCURDY[2], L. R. MCMAHON[1], T. HIRANITA[1];
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Abstract: There is an overdose epidemic associated with the use of illicitly manufactured as well as prescription mu-opioid agonists. Sigma1 receptor (σ_1R) antagonists in combination with other drugs may provide a viable, safe pharmacological option for treating pain. The present study compared the pharmacological effects of the σ_1R antagonist CM304 alone and in combination with mu-opioid (nalbuphine, morphine and fentanyl) or cannabinoid CB_1 [CP55,940 and (−)-*trans*-Δ^9-tetrahydrocannabinol (THC)] receptor agonists in C57BL/6J mice.

Rectal temperature, tail withdrawal latency from warm water of various temperatures (45°C, 50°C and 55°C) and counts of unhabituated locomotor activity were measured in this order. Basal latency for tail withdrawal systematically decreased from 10 seconds at 45°C to 1.3 seconds at 55°C. Morphine (cumulative 1-100 mg/kg, s.c.) and fentanyl (0.0032-0.32 mg/kg, s.c.) dose-dependently increased maximum possible effects (MPE) up to 100% at 55°C (ED_{50} values: 7.50 and 0.068 mg/kg, respectively). Nalbuphine (3.2-320 mg/kg, s.c.), CP55,940 (0.032-3.2 mg/kg, i.p.) and THC (3.2-320 mg/kg, i.p.) were less active at 55°C (Emax values: 74.0%, 56.6% and 58.2% for nalbuphine, CP55,940 and THC, respectively). CM304 (56 mg/kg) produced an upward shift in the CP55,940 dose-effect function such that 100% MPE was achieved at 3.2 mg/kg CP55,940, while CM304 (56 mg/kg) produced a 3-fold leftward shift in the dose-effect curve of THC. On the other hand, CM304 did not enhance the antinociceptive effects of morphine and fentanyl. In contrast to tail withdrawal latency, there were no consistent effects of CM304 on cannabinoid-induced decreases in rectal temperature and locomotor activity. While CM304 alone did not significantly alter tail withdrawal latency at 55°C, it did decrease activity and rectal temperature. However, CM304 produced an upward shift in the dose-effect function of hyperactivity induced by morphine.
The present results may not support the development of a σ_1R antagonist as an adjunct to opioids for treatment of acute pain. However, the results indicate that an σ_1R antagonist could be used specifically to increase the analgesic effectiveness of cannabinoid CB_1 receptor agonists.

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Poster

417. Opioids: Mechanisms Underlying Seeking Behavior

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Support: K99 DA045765
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NHMRC CJ Martin Award 1072706
F31 DA042588
K12 GM093854

Title: Higher numbers of orexin-expressing neurons are associated with increased motivation for psychostimulants, opioids and palatable food

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Abstract: ***Introduction:*** Orexin signaling is critically involved in the expression of motivated seeking of drugs of abuse and palatable food. Orexins are produced by a discrete population of neurons in caudal hypothalamus, ranging mediolaterally from dorsomedial hypothalamus (DMH) through perifornical (Pf) to lateral hypothalamus (LH). Recent evidence indicates that the number of endogenous orexin-expressing neurons, particularly those in LH, is associated with the magnitude of motivation for cocaine. Further, there is evidence of plasticity in orexin expression, such that chronic exposure to cocaine or morphine is associated with an increase in the number of orexin-expressing neurons. Here we compared these phenomena across several drugs of abuse, including psychostimulants and opioids, as well as palatable foods. ***Methods:*** To examine the relationship between endogenous orexin cell numbers and motivation for drug, male Sprague Dawley rats (8wk) were trained to self-administer cocaine (n=12) or the short-acting opioid remifentanil (n=10), before being tested on our within-session behavioral economics paradigm. Rats were perfused and the number of orexin-expressing neurons was quantified and correlated with demand elasticity, an inverse measure of drug motivation. To examine plasticity of orexin-expression, rats were trained to self-administer cocaine (n=27) or fentanyl (n=13) on an intermittent access (IntA) or short access (ShA) paradigm; we have previously shown IntA to enhance motivation for drug relative to ShA. In a separate group of female Long Evans rats (n=47), binge-like eating was induced by exposing rats to sweetened fat (vegetable shortening/10% sucrose) for 30 min, twice/wk for 4wk. A control group was given ad-libitum access to chow. All rats were perfused 30-150d after drug/binge exposure and orexin-expressing neurons were quantified. ***Results:*** Animals with high baseline demand for cocaine and remifentanil had significantly higher numbers of LH orexin-expressing neurons. High demand for remifentanil was also associated with higher DMH/Pf orexin neurons. IntA to cocaine and fentanyl, as well as a history of binge-like eating, was associated with a persistent increase in orexin cell numbers in LH. IntA to fentanyl was also associated with increased DMH/Pf orexin neurons. ***Conclusions:*** Higher numbers of LH orexin neurons are associated with enhanced 'trait' and 'state' motivation for psychostimulants, opioids and palatable food. The number of DMH/Pf neurons appears to be uniquely linked with motivation for opioids. These data point to the orexin system as a common neurobiological system underlying aberrant motivation across reinforcers.

Disclosures: S.L. O'Connor: None. **M.H. James:** None. **J.E. Fragale:** None. **C.B. Pantazis:** None. **B.S. Bentzley:** None. **A. Mohammadkhani:** None. **K.A. Porter-Stransky:** None. **G. Aston-Jones:** None.

Poster

418. Attention and Neuromodulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 418.01/Y32

Topic: H.01. Animal Cognition and Behavior

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R01EY019882
R01EY027402
R01EY008890
T32EY007135
U54HD083211
Nvidia Corporation

Title: Microcircuitry of visual attention: Laminar organization of attentional selection in area V4

Authors: *J. A. WESTERBERG, A. MAIER, J. D. SCHALL;
Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: The modulation of visual responses during attentional selection originates through interactions within and across cortical areas, such as area V4 and the frontal eye field. Anatomical studies have shown that the primary feedforward visual activation in area V4 enters in the middle, granular layers while feedback targets extragranular layers. To investigate the laminar organization of attentional selection in V4, we performed acute laminar recordings in V4 using linear multielectrode arrays in macaque monkeys performing a color singleton visual search task. Fluid reward was earned for shifting gaze to the singleton. While spiking activity across all layers was enhanced following attentional selection of the singleton, the synaptic depolarizations had unique laminar dependencies. Target selection was observed in the current sinks of the CSD almost exclusively in the supragranular layers. This preceded the changes in the population spiking activity. The laminar specificity of this effect suggests that target selection of stimuli either originates in area V4 or is fed back to V4 through its extensive connections with frontal cortex or other cortical areas. These results provide the first description of the temporal evolution of target selection along the layers of extrastriate area V4 in a visual search task, which provide new constraints on the generation of event-related potential indices of visual attention like the N2pc.

Disclosures: **J.A. Westerberg:** None. **A. Maier:** None. **J.D. Schall:** None.

Poster

418. Attention and Neuromodulation

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Topic: H.01. Animal Cognition and Behavior

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R01EY019882
R01EY027402
R01EY08890
T32EY007135
U54HD083211
Nvidia Corporation

Title: Contribution of area V4 to the N2pc event-related potential index of attention

Authors: *M. S. SCHALL, J. A. WESTERBERG, A. V. MAIER, J. D. SCHALL, G. F. WOODMAN;
Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Research into mechanisms of visual attention has relied on an event-related potential known as the N2pc, because it indicates where and when attention is allocated. The N2pc was discovered and characterized in humans, so its neuronal origins are unknown. Several investigators have conjectured that extrastriate visual area V4 can be a generator of the N2pc based on the similarity of patterns of modulation. Our group has been investigating that conjecture. We have established that the N2pc manifests in macaque monkeys performing visual search. We have established that inverse solutions of the N2pc identify a current generator in the vicinity of V4. Now, to determine most directly whether V4 contributes to the generation of the N2pc, we performed laminar recordings of area V4 concurrent with extracranial EEG in macaque monkeys performing visual search for a singleton. A target stimulus (red or green) was presented among several distractors (homogenous green or red) in an array around a central fixation point. Monkeys shifted gaze to the singleton to earn fluid reward. With field potentials recorded across all cortical layers, we calculated current source density (CSD) of net synaptic depolarizations. On a trial-by-trial basis we compared the magnitude of CSD and polarization of the N2pc. Trial-to-trial variability in the synaptic depolarizations of V4 explained a significant amount of variance in the extracranial voltage fluctuations during the N2pc. This relationship was found only when the singleton was the preferred color of the recorded location. These results demonstrate that V4 is a contributor to the extracranial index of attentional selection.

Disclosures: M.S. Schall: None. **J.A. Westerberg:** None. **A.V. Maier:** None. **J.D. Schall:** None. **G.F. Woodman:** None.

Poster

418. Attention and Neuromodulation

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R01EY027402
R01EY008890
T32EY007135
U54HD083211
Nvidia Corporation

Title: Microcircuitry of visual attention: Attentional priming in area V4

Authors: *J. D. SCHALL, J. A. WESTERBERG, A. V. MAIER;
Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Behavioral priming improves performance in psychophysical tasks. To investigate the neural mechanisms of visual priming, we recorded neural spiking and field potentials across all layers of area V4 in monkeys performing visual search under conditions of priming of popout. In this task response accuracy and time improve with repeated presentation of the same singleton in a search array (e.g., red among green), and performance is impaired when feature assignments change. Neurophysiological substrates of these behavioral changes have been discovered in the frontal eye field (FEF) (Bichot & Schall 2002 J Neurosci). We investigated whether area V4 contributes to priming of popout. Monkeys performed a color pop-out task where the sequences of trials were such that priming would occur. Acute linear electrode arrays were introduced into V4 and recorded neural activity across the layers during task performance. Both synaptic depolarizations, measured through current source density (CSD), and neural spiking were measured. As expected, priming of pop-out improved search performance. We found that priming coincided with earlier target selection time measured across all layers of V4 in neural spiking and in the supragranular layers in CSD sinks. The laminar specificity of the changes in target selection time in the CSD suggest that V4 either generates the priming effect or inherits it from another cortical area, perhaps FEF, rather than through bottom-up changes in visual processing from earlier visual areas. These results provide new information about the mechanisms of visual memory and provide new constraints on the generation of event-related potential indices of visual attention like the N2pc.

Disclosures: J.D. Schall: None. **J.A. Westerberg:** None. **A.V. Maier:** None.

Poster

418. Attention and Neuromodulation

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Robin and Richard Patton through the E. Bronson Ingram Chair in Neuroscience

Title: Speed-accuracy tradeoff of visual processing in supplementary eye field: Comparison with frontal eye field and superior colliculus

Authors: *T. R. REPPERT, R. P. HEITZ, J. D. SCHALL;
Vanderbilt Univ., Nashville, TN

Abstract: Two macaque monkeys performed visual search with speed-accuracy tradeoff (SAT) cued in short blocks of emphasis on either speed or accuracy of responding. With cued emphasis on speed, behavioral responses were quicker, and error rate, higher. Different stimulus arrays supported higher or lower efficiency search during each recording session. During less efficient search, the SAT performance curve shifted to slower responding with increased error rate. We quantified and compared effects of SAT and search efficiency on visually-responsive neurons in SEF, FEF, and SC. Previously, we showed that visual response magnitude was greater in FEF and SC when speed was emphasized (Heitz and Schall 2012; Reppert et al. 2018). We found the same to be true in SEF. In SC, we observed an interaction of search efficiency and SAT condition on response magnitude, with response magnitude elevated in the Fast condition during inefficient search but elevated in the Accurate condition during efficient search. In SC, we also observed no effect of search efficiency on response magnitude. In FEF there was no interaction of search efficiency and SAT condition on response magnitude, nor was there a main effect of search efficiency. Previously, we showed that TST was earlier in FEF and SC when speed was emphasized (Heitz and Schall 2012; Reppert et al. 2018) and that neurons in SEF do not produce TST (Purcell et al. 2012). Here, we found that ~1/3 of visually-responsive SEF neurons distinguished the target from distractors. Among those SEF neurons, we observed an interaction of SAT condition and search efficiency on TST, with TST delayed in the Accurate relative to the Fast condition during more efficient but not less efficient search. As observed previously in FEF, we observed TST delayed during less relative to more efficient search, but this was not found in the sample of SC neurons. These results complement prior observations of SAT-related effects in

SC and FEF and extend our understanding of the distributed neural circuitry that accomplishes SAT of visual search.

Disclosures: T.R. Reppert: None. **R.P. Heitz:** None. **J.D. Schall:** None.

Poster

418. Attention and Neuromodulation

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Topic: H.01. Animal Cognition and Behavior

Support: R01 EY08890P30
EY008126
U54 HD083211
E. Bronson Ingram Chair in Neuroscience

Title: Separate modifiability of stages of target selection for visual search in macaques

Authors: *K. A. LOWE[1], T. R. REPPERT[2], J. D. SCHALL[1];
[1]Psychology, [2]Vanderbilt Univ., Nashville, TN

Abstract: The computational and neural architecture supporting visual search has not been elucidated despite many years of investigation. We employed the logic of separate modifiability through multi-dimensional factorial manipulations to address these fundamental questions. We trained two male macaque monkeys to perform visual search with selection on color and response on shape of a singleton. One monkey was novice, and the other had experience with other color-shape visual search tasks. We manipulated separately *singleton identifiability* (color similarity of singleton and distractors) and stimulus-response *cue discriminability* (elongation of singleton cuing pro/anti/no saccade). Using a powerful mathematical approach known as systems factorial technology, we distinguished alternative processing architectures (series or parallel) and stopping rules (exhaustive or self-terminating). The novice monkey used a coactive strategy in which singleton identification and cue discrimination are pooled to produce responses. The experienced monkey used a parallel exhaustive strategy in which singleton identification and cue discrimination were completed separately but in tandem to produce responses. The signatures of different processing architectures offer novel hypotheses to guide analysis of the neural signals in frontal eye field (FEF) sampled during task performance.

Disclosures: K.A. Lowe: None. **T.R. Reppert:** None. **J.D. Schall:** None.

Poster

418. Attention and Neuromodulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 418.06/Y37

Topic: H.01. Animal Cognition and Behavior

Support: R01AG050518

Title: Effects of an orexin-2 receptor agonist on attention in rats following loss of cortical cholinergic projections

Authors: *S. A. BLUMENTHAL[1], E. B.-L. MANESS[2], J. R. FADEL[3], J. A. BURK[1]; [1]Psychological Sci., [2]Applied Sci., William and Mary, Williamsburg, VA; [3]Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Deterioration to the basal forebrain cholinergic system (BFCS) is linked to age-related cognitive impairment, specifically to the pathology of Alzheimer's disease (AD). Animals with BFCS damage perform poorly on learning, memory, and attention tasks, indicating cognitive deficits. The orexin neuropeptide system, comprised of two neuropeptides (orexin A and orexin B), has also been implicated in the cognitive decline associated with aging, likely due to the role of orexins in promoting attention. Two orexin receptor subtypes exist, orexin 1 (Ox1R) and orexin 2 (Ox2R). Studies have examined the effects of stimulation and blockage of both receptors together and Ox1R alone on attention; but no studies have examined the role of Ox2Rs in attention through the use of Ox2R agonists. Ox2Rs may be implicated in attentional processes and the loss of orexin neurons seen in age-related cognitive decline. In order to examine the role of Ox2Rs in attention following BFCS deterioration, the present study administered the Ox2R agonist, YNT-185, to rats given intrabasalis infusions of either saline (n = 12) or 192 IgG saporin (n =11), an immunotoxin which selectively destroys the BFCS. Animals received infusions of YNT-185 to the lateral ventricle (LV) in doses of 0, 1, 10, and 100nM across four separate sessions and performance was then assessed on a sustained attention task requiring discrimination between signal and non-signal trials through lever presses. The 100nM dose of YNT-185 improved attentional performance, as compared to the 0nM dose, for rats given the immunotoxin, but worsened performance for rats given saline lesions. YNT-185 may be efficacious in aiding attentional function in animals with vulnerable cholinergic systems but may lead to overexcitation for those with intact cholinergic function.

Disclosures: S.A. Blumenthal: None. **E.B. Maness:** None. **J.A. Burk:** None. **J.R. Fadel:** None.

Poster

418. Attention and Neuromodulation

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Program #/Poster #: 418.07/Y38

Topic: H.01. Animal Cognition and Behavior

Support: R01AG050518

Title: Dual orexin receptor antagonism attenuates dizocilpine-induced attentional impairments in a rat model of acute psychosis

Authors: *E. B.-L. MANESS[1], S. A. BLUMENTHAL[2], J. BURK[2];
[1]Applied Sci., [2]Psychological Sci., William and Mary, Williamsburg, VA

Abstract: Schizophrenia (SZ) is a psychiatric condition wherein those afflicted demonstrate a combination of positive symptoms, such as hallucinations and delusions, as well as negative symptoms, including alterations of processing that disrupt sociality, mood, and cognition. Widespread failure of corticofugal inhibition caused by reduced NMDA receptor input to GABA interneurons results in mesolimbic overstimulation, leading to sensory disturbances and psychosis. Current antipsychotic medications are known to produce unpleasant physiological side effects like sedation, Parkinsonism, and diabetes; additionally, they can bring forth or exacerbate negative and cognitive symptoms. As such, exploring alternative pharmacotherapies which alleviate the positive symptoms of SZ while boosting motivational and cognitive integrity is a worthwhile endeavor. The lateral hypothalamic orexinergic system, a widespread neuromodulatory network involved in wakefulness, appetitive drive, and incentive- and fear-linked behaviors, plays a key role in regulating excitatory neurotransmission throughout the central nervous system. The sedative-hypnotic nature of orexin receptor inhibitors is hypothesized to calm subcortical hyperexcitation while sparing cognitive function. In the present study, the dual orexin receptor antagonist filorexant (MK-6096) was intracerebroventricularly infused following acute intraperitoneal administration of dizocilpine (MK-801), an NMDA receptor antagonist, and performance was assessed in a visual sustained attention test. 0.1 mM of filorexant normalized signal detection impairments induced by dizocilpine without diminishing performance on its own. Additionally, dizocilpine-linked trial omissions were reduced following filorexant infusions. Based on these findings, drugs which dampen orexin receptor activity may restore attentional and motivational abilities for those with SZ, possibly by assuaging subcortical hyperactivity and normalizing cortical function.

Disclosures: E.B. Maness: None. **S.A. Blumenthal:** None. **J. Burk:** None.

Poster

418. Attention and Neuromodulation

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 418.08/Y39

Topic: H.01. Animal Cognition and Behavior

Support: Start up funds from Vassar College

Title: Sustained attention leads to brain region specific changes in glutamine synthetase

Authors: *J. BONANNO[1], E. KHARITONOVA[1], T. WANG[1], L. A. NEWMAN[2];
[2]Dept. of Psychological Sci., [1]Vassar Col., Poughkeepsie, NY

Abstract: Research suggests that astrocytic dysfunction contributes to neurodegenerative diseases and neuropsychiatric disorders such as Alzheimer's disease, attention-deficit/hyperactivity disorder (ADHD), and schizophrenia. We examined healthy astrocytic activity during sustained attention to better our understanding of how these processes can go awry. We focused our study on the role of astrocytes in glutamate and GABA recycling. In the glutamate-glutamine cycle, astrocytes take up neuronally secreted glutamate and GABA and convert it to glutamine to send back to neurons for continued neurotransmission. This process, facilitated by the astrocyte-specific enzyme glutamine synthetase (GS), prevents neurotoxicity and allows for phasic signalling in the synapse. In this study, we assessed changes in GS levels during a sustained attention task (SAT) in Long Evans rats. The animals were first trained on the water reinforced SAT. Those that reached criterion performance on the SAT were sacrificed 15 minutes after the completion of the task along with animals that did not reach criterion performance. An additional group of cagemate controls was also examined to assess the effects of any training on the SAT. GS levels were quantified in the prelimbic cortex (PL), hippocampus, and dorsolateral striatum using immunohistochemistry. We found a brain region specific effect of GS. While rats that reached criterion performance on the SAT had higher GS levels in the PL and the upper limb of the dentate gyrus (ULDG), rats that failed to learn the task had lower levels of GS expression in the PL and ULDG. Our results suggest that success on the sustained attention task may be dependent on sufficient GS levels in the prefrontal cortex and hippocampus. These findings shed light on the dynamic changes in glutamate and GABA recycling in astrocytes during cognition and may point to dysfunctional astrocytes as a therapeutic target in neurodegenerative diseases and neuropsychiatric disorders.

Disclosures: J. Bonanno: None. **E. Kharitonova:** None. **T. Wang:** None. **L.A. Newman:** None.

Poster

418. Attention and Neuromodulation

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Program #/Poster #: 418.09/Y40

Topic: H.01. Animal Cognition and Behavior

Support: Vassar College Startup Funds

Title: Inhibition of glutamine synthetase in the prelimbic cortex with methionine sulfoximine during sustained attention: Possible compensatory mechanisms?

Authors: *A. D. CASCONE[1], S. ZHANG[1], W. XIE[1], E. KHARITONOVA[1], M. LEONG[1], L. A. NEWMAN[2];
[2]Dept. of Psychological Sci., [1]Vassar Col., Poughkeepsie, NY

Abstract: Healthy astrocytes are known to recycle glutamate and GABA, the major excitatory and inhibitory neurotransmitters, from synapses. Glutamine synthetase (GS) in astrocytes is responsible for the synthesis of glutamine from glutamate and the transportation of glutamine into neuron, where it is resynthesized into glutamate or GABA. The present study examined the importance of glutamate recycling mediated by GS in a sustained attention task (SAT). The task evaluated sustained attention performance in Long-Evans rats utilizing light signals of varying length (500ms, 100ms, 25ms), non-signal trials, and a variable intertrial interval. After the subjects reached criterion performance, they underwent bilateral prelimbic cortex cannulae implantation which accommodates microinjections of methionine sulfoximine (MSO). Since MSO is an irreversible inhibitor of GS and the enzyme must be replaced after inhibition, we collected data for a week after injection to assess the recovery from MSO. We also waited for recovery of criterion performance before giving a new injection. After recovering from surgery and reaching criterion performance, each subject received 0.5 µL of 10mM, 2mM, or 0.4mM of MSO or the vehicle, 0.9% saline, in a counterbalanced order 15 minutes prior to the SAT. We hypothesized that there would be a dose-dependent effect of MSO on SAT performance, with the greatest performance decline occurring after 10mM injection, a milder decline after 2mM injection, and a slight improvement after 0.4mM injection. Repeated-measures ANOVAs did not reveal any significant dose-dependent effects of MSO on SAT performance. However, analysis of the first exposure to MSO suggested a significant effect of initial MSO exposure on SAT performance. For the 500ms signal trials, subjects who have previously been exposed to MSO performed significantly better on SAT than those who are naïve to MSO, regardless of injection dosage. This finding suggests compensatory mechanism(s) in response to MSO administration. Future studies will examine the initial effect of MSO exposure on SAT performance and compensatory mechanisms, such as overproduction of GS after inhibition with MSO.

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Poster

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Program #/Poster #: 418.10/Y41

Topic: H.01. Animal Cognition and Behavior

Support: Ro1AG050518

Title: Effects of medial prefrontal cortical administration of the orexin-1 receptor antagonist, SB-334867, on attentional performance in rats

Authors: *J. A. BURK[1], R. PATEL[1], E. MANESS[1], S. BLUMENTHAL[1], J. R. FADEL[2]; [1]William and Mary, Williamsburg, VA; [2]Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Orexin neurons project from the lateral hypothalamus to numerous brain regions where orexin receptors (Ox1R and Ox2R) reside. The medial prefrontal cortex (mPFC), specifically, is important for executive attention, allowing for the processing of different sources of information. Since the mPFC is dense with both orexin-1 and orexin-2 receptors (Ox1R, Ox2R), orexin may play a key regulatory role in mPFC-mediated attention. Male Fischer Brown Norway F1 (FBNF1) hybrid rats were used in this experiment. Each rat received 6 infusions total—two mock saline infusions and four drug doses (vehicle, 0.25ug/uL, 0.5ug/uL, 1ug/uL). To avoid confounding effects, the order of drug dose administration was random and each infusion was at least 48-hrs apart. After infusing the drug into the left or right mPFC, the rats were tested on a modified attention task, where a distracter (flashing houselight) was presented. We observed trends for improvement in post-distracter performance, when the lowest dose (0.25ug/uL) was administered to the right mPFC. Also, accuracy on non-signal trials following the distracter task improved when the lowest dose (0.25ug/uL) was administered to either hemisphere of the mPFC. These results indicate that orexin-1 receptors in the medial prefrontal cortex contribute importantly to attentional performance.

Disclosures: **J.A. Burk:** None. **R. Patel:** None. **E. Maness:** None. **S. Blumenthal:** None. **J.R. Fadel:** None.

Poster

418. Attention and Neuromodulation

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Program #/Poster #: 418.11/Y42

Topic: H.01. Animal Cognition and Behavior

Support: Cole Fund

Title: Dissociable attentional effects of dopaminergic and cholinergic lesions to the anterior cingulate cortex

Authors: *M. K. CLEMENT, C. S. PIMENTEL, J. A. SWAINE, A. J. PIMENTEL, D. HUTCHINS, J. A. MCGAUGHY;
Psychology, Univ. of New Hampshire, Durham, NH

Abstract: Prior work from our lab has shown that excitotoxic lesions to the anterior cingulate cortex (ACC) impairs the ability of rats to filter certain types of distracting stimuli (Newman and McGaughy 2011). Specifically, rats with lesions of the ACC cannot filter distractors that have been made salient through pairing with reinforcement. In contrast, these same subjects can filter distracting stimuli that have not been predictive of reward. The present study investigates the effects of neuromodulator specific lesions of the same region to determine how specific neuromodulators contribute to the attentional function of ACC. Cholinergic or dopaminergic deafferentation of the ACC was achieved using either 192 IgG saporin (n=10) or dopamine transporter saporin (n=10). Lesions were restricted to the rostral portion of the area and did not spread to nearby prefrontal sub-regions e.g prelimbic cortex. After lesioning, subjects were tested in an attentional set-shifting task (Birrell and Brown 2000). While both cholinergic and dopaminergic lesions increased distractibility, these deficits were not as severe as those produced after excitotoxic lesions (n= 8). In contrast to excitotoxic lesions, both cholinergic and dopaminergic lesions also impeded formation of an attentional set. Because dopaminergic lesions produced impairments in many stages of the tasks, we hypothesized that these subjects had a more general impairment in stimulus processing. In order to address these broader processing impairments, we analyzed the data to determine whether lesioned rats showed more sensitivity to novel stimuli, or made more perseverative errors. The implications of these data for understanding the unique contributions of acetylcholine and dopamine to attentional processing in the ACC will be discussed.

Disclosures: M.K. Clement: None. **J.A. McGaughy:** A. Employment/Salary (full or part-time):; University of New Hampshire, Full-time. **C.S. Pimentel:** None. **A.J. Pimentel:** None. **D. Hutchins:** None. **J.A. Swaine:** None.

Poster

418. Attention and Neuromodulation

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Topic: H.01. Animal Cognition and Behavior

Support: Cole Fund at the University of New Hampshire

Title: The effects of lesions to anterior cingulate cortex or mid-cingulate cortex on distractibility in rats

Authors: M. K. CLEMENT, C. S. PIMENTEL, ***J. A. MCGAUGHY**;
Univ. of New Hampshire, Durham, NH

Abstract: Dysfunction in the anterior cingulate cortex (ACC) has been linked to cognitive impairments in many neuropsychiatric disorders including but not limited to depression, schizophrenia and addiction. Studies in humans, non-human primates and rats have shown this region is critical to many aspects of attention including ignoring salient distractors and conflict monitoring. Unfortunately, many brain imaging studies define the ACC broadly confounding the function of ACC and the more posterior portion of the gyrus, the mid-cingulate cortex (MCC). This failure to maintain consistent anatomical boundaries has impeded progress in understanding the function of both regions. Recent advances in the cross-species definitions of the both the ACC and MCC by Vogt and Paxinos (2014) provides an important foundation to begin to explore the function of these regions using rodents. The present study used Long-Evans rats to compare the function of the ACC (n =8) and MCC (n= 6) in an attentional set-shifting task (Birrell and Brown 2000). Lesions to the ACC, but not the MCC, increased distractibility to salient distractors previously predictive of reinforcement. The dissociable effects of MCC and ACC lesions will be discussed in the context of connectivity differences between the two regions. Moreover the implications for translational neuroscience of functional distinctions along the rostro-caudal gradient of the cingulate cortex in rats will be discussed.

Disclosures: M.K. Clement: A. Employment/Salary (full or part-time):; University of New Hampshire Durham, NH. **C.S. Pimentel:** None. **J.A. McGaughy:** A. Employment/Salary (full or part-time):; University of New Hampshire Durham, NH.

Poster

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Support: NIH Grant P50NS091856

Title: Silencing of striatal cholinergic interneurons disrupts attentional-motor interactions

Authors: *C. AVILA[1], A. J. KUCINSKI[2], M. SARTER[1];
[2]Psychology, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Falls in patients with Parkinson's disease (PD) are associated with loss of basal forebrain (BF) cholinergic neurons and thought to reflect impairments in the attentional supervision of slow and low-vigor movement, the latter resulting from striatal dopamine (DA) loss. We previously established a rat model of PD falls which reproduce the combined cholinergic-dopaminergic losses seen in patients (dual, DL rats), and which exhibit a high propensity for falls during the traversal of dynamic surfaces (Michigan Complex Motor Control Task; MCMCT). Moreover, similar to PD fallers, these rats were impaired in executing cue-triggered turns, but not cue-triggered stops, while walking on a treadmill (Cue Triggered Turning Task; CTTT). Collectively, these results have suggested that BF cholinergic loss deprives the striatum of information about extero- and interoceptive movement cues, including stepping errors. Loss of such information interacts with impaired striatal movement sequencing and selection to produce falls and impaired cued turning behavior. In the striatum, cholinergic interneurons (ChIs) are positioned to integrate cortical and thalamic afferent information about movement cues with the dopaminergic mediation of action selection and sequencing. We therefore predicted that silencing these interneurons, specifically in the dorsomedial, striatum, is sufficient to reproduce high fall rates and cued turning impairments seen in DL rats. Cre-recombinase-dependent AAV expressing the inhibitory M4-type DREADD receptor, or an m-Cherry expressing control construct, was bilaterally infused into the striatum. Following surgeries, rats first underwent MCMCT testing, consisting of a total of 48 traversals of the rotating rods. In rats expressing the inhibitory DREADD, CNO (5.0 mg/kg) nearly completely reproduced the fall rates seen previously in DL rats. Rats then underwent 14 days (20 trials per day) of testing on the CTTT. CNO reduced the rate of cue-evoked turns but had no effects on cue-evoked stops, again mirroring effects found in DL rats. Additional tests confirmed that administration of CNO neither disrupted the animals' ability to turn *per se* nor induced a preferred turning direction. These results support the hypothesis that striatal ChIs mediate the integration of external and proprioceptive cues into complex movement sequences such as those required to traverse complex surfaces or turning. Furthermore, restoring ChI function in PD

fallers may be a useful therapeutic target to reduce falls and complex movement deficits in PD patients.

Disclosures: C. Avila: None. **M. Sarter:** None. **A.J. Kucinski:** None.

Poster

418. Attention and Neuromodulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 418.14/Z1

Topic: H.01. Animal Cognition and Behavior

Title: Reduced choline clearance *in vivo* in mice expressing a choline transporter subcapacity variant associated with low attentional control in humans

Authors: *E. DONOVAN[1], C. AVILA[1], V. V. PARIKH[3], A. ANTCLIFF[2], R. D. BLAKELY[4], M. SARTER[5];
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Abstract: Cholinergic signaling in cortex mediates the detection of cues in attention-demanding contexts. Spontaneous, genetically-imposed or degeneration-induced loss of cholinergic function in humans and rodents reliably results in detection deficits and associated impairments in attentional control, including distractor vulnerability. The high-affinity choline transporter (CHT) is an essential step regulating the capacity of cholinergic terminals to synthesize and release acetylcholine. We previously demonstrated that humans who express a subcapacity CHT variant (rs1013940 of SLC5A7; I89V) exhibit increased distractor vulnerability and attenuated right frontal activation during performance. When expressed in COS-7cells, this variant reduced choline clearance by about 50% (Okuda et al., 2002). Mice encoding an Ile89Val substitution were generated using CRISPR/Cas9 methods and continued to be backcrossed onto C57BL/6 female. Because synaptosomal choline uptake assays unexpectedly failed to reveal the impact of this variant, experiments were designed to generate presumably more sensitive, high-temporal resolution measures of choline clearance *in vivo*. Choline oxidase was immobilized on the surface of Platinum electrodes fabricated onto ceramic backbones (Quanteon), inserted into the cortex of anesthetized mice, and choline currents were measured amperometrically (+0.7 V vs. an Ag/AgCl reference). To determine the capacity for choline clearance, pressure ejections of 5.0 mM choline, via glass micropipettes with tips positioned between choline-sensitive and control electrodes. We previously demonstrated that the time required for the choline current to decline between 40-80% of peak amplitude ($T_{40\text{-}80}$) is significantly lengthened in the presence of hemicholinium-3 (HC-3) and thus indicative of CHT capacity (Parikh et al. 2006). Consistent with previous data, $T_{40\text{-}80}$ in wild type mice was 1.3 s. In contrast, in heterozygous I89V mice,

such clearance required 2-fold more time (2.2 s). Additionally, T_{40-80} choline clearance was robustly slower in homozygous I89V mice compared to wild-types (6.2 s). Ongoing experiments determine choline clearance in the presence of HC-3, the capacity for ACh release evoked by trains of depolarizing stimuli, and the subcellular distribution of CHTs. Moreover, the attentional capacities of I89V mice will be determined. This evidence forms the basis for I89V mice as a model to determine the neurobiological mechanisms via which such CHT variants produce phenotypes in healthy humans, and increased risk for traumatic brain injury and a range of psychiatric and neurodegenerative disorders.

Disclosures: E. Donovan: None. **C. Avila:** None. **V.V. Parikh:** None. **A. Antcliff:** None. **R.D. Blakely:** None. **M. Sarter:** None.

Poster

418. Attention and Neuromodulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 418.15/Z2

Topic: H.01. Animal Cognition and Behavior

Support: Israel Scientific Foundation

Title: Behavioral and neural evidence for inhibition of return in barn owls (tyto alba)

Authors: *T. LEV-ARI[1], Y. ZAHAR[2], A. AGARWAL[2], Y. GUTFREUND[3];
[1]Neurosci., Technion - Israel Inst. of Technol., Haifa, Israel; [2]Neurosci., Technion, Haifa, Israel; [3]Neurosci., The Technion, Haifa, Israel

Abstract: Inhibition of return (IOR) is the reduction of detection speed and/or detection accuracy of a target that is in a previously attended location. This phenomenon, discovered and studied thoroughly in humans, is believed to reflect a brain mechanism to control the allocation of spatial attention in a manner that enhances efficient search. Findings that IOR is robust, apparent at a very early age and seems to be dependent on midbrain activity suggest that IOR is a universal attentional mechanism in vertebrates. However, studies in non-mammalians species are lacking. To comparatively explore this hypothesis we tested for IOR in barn owls using the classical Posner cueing paradigm. Two barn owls were trained to initiate a trial by fixating on a fixation point at the center of a computer screen and then turn their gaze to the location of a target when it appears. In all experimental trials a short, non-informative cue appeared before the target, with a cue to target onset asynchrony (CTOA) varying from 66 to 633ms. The cue was either at a location predicting the target (valid) or in a location not-predicting the target (in-valid). Using infrared reflectors tracking system (OptiTrack) we measured the owl's gaze position on screen throughout the trial. The barn owls response times (RT) to the valid targets compared to the in-valid targets shifted from facilitation (lower RTs) to inhibition (higher RTs)

when increasing the CTOA. In one owl the shift to inhibition occurred at a CTOA of 433ms and in the second at a CTOA of 633ms. To facilitate comparison with humans we measured in 15 human subjects the RTs when performing the same task. Results were comparable between humans and owls. In addition, we recorded multiunit responses in the optic tectum (OT) of head-fixed owls, passively viewing a similar cueing paradigm as in the behavioral experiments. At short CTOAs (<250ms) neural responses to the target in the receptive field (RF) were usually enhanced if the cue appeared earlier in the RF (valid) and suppressed if the cue appeared outside the RF (in-valid). This was reversed at longer CTOAs where neural responses were suppressed in the valid conditions and enhanced in the in-valid conditions. Thus, our results support the notion that IOR is a basic mechanism in the evolution of vertebrate behavior and suggest that the effect appears as a result of interaction between lateral and forward inhibition in the tectal circuitry.

Disclosures: T. Lev-Ari: None. **Y. Zahar:** None. **A. Agarwal:** None. **Y. Gutfreund:** None.

Poster

418. Attention and Neuromodulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 418.16/Z3

Topic: H.01. Animal Cognition and Behavior

Title: Comparative animal consciousness is multimodal and nonbinary

Authors: *L. N. IRWIN;
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Abstract: Speculations about the capacity for consciousness in different animals is usually expressed as a binary possibility – either it exists or it doesn't. And among those animals in which it is presumed to exist, consideration is seldom given to varying degrees or alternative modes of consciousness. I agree with the likelihood that consciousness has deep evolutionary origins, but assume with most behavioral scientists and philosophers that different species experience it in radically different ways. As an exploratory attempt to construct a taxonomy of modes of consciousness, I propose that it can be studied through proxy behaviors that fall into four categories: Attentive (A), Intentional (I), Interactional (X), and Reflexive (R). Type A behavior is composed of sedentary awareness of surroundings and alertness to exteroceptive input. Type I behavior consists of goal-directed action. Type X behavior requires interaction with other conspecific or allospecific individuals. Type R behavior is activity in which the animal attends to or acts upon its own body, generally with a greater focus on interoceptive input. To test the feasibility of this taxonomy, a variety of vertebrates and invertebrate were observed in 10-20 minute segments in a non-intrusive manner under natural or semi-natural (e.g. zoo and aquarium) conditions. The average percent of total observational time each animal devoted to

each of the four proxy behaviors was tabulated (summing to more than 100% since modes frequently overlapped). In some species, Type A behavior predominated, such as the sea urchin (100% A, 0% I, X, or R) and komodo dragon (100% A, 30% I, 0% X, 5% R). In others, behavior I was most frequent, as displayed by both humans (83% I, 12% A, 60% X, 5% R) and the seahorse (100% I, 25% A, 19% X, 25% R). A tendency toward substantial interactive behavior, Type X, was seen in humans (83% I, 12% A, 60% X, 5% R) and octopi (60% X, 70% A, 40% I, 0% R). While reflexive behaviors, Type R, were displayed by nearly all observed animals, it was never the highest frequency activity. To the extent that these proxy behaviors reflect different modes of consciousness, these results illustrate that they are differentially invoked, without strict phylogenetic correlation, according to the specific lifestyle and ecological niche requirements of each species. A taxonomy such as this, if expanded to include measures of behavioral variety and complexity, and brain metrics associated with cognitive capacity such as brain size and number of neurons, could advance the study of comparative animal consciousness to a more nuanced, detailed, and objective level.

Disclosures: L.N. Irwin: None.

Poster

418. Attention and Neuromodulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 418.17/Z4

Topic: H.01. Animal Cognition and Behavior

Support: NIMH NRSA F32MH118794
NIH R00DA037279
MQ Fellows Award
NARSAD Young Investigator Grant

Title: D1 and D2 medium spiny neurons in the nucleus accumbens bi-directionally modulate attentional control behavior of mice

Authors: *M. T. PISANSKY, D. W. LEIPOLD, P. E. ROTHWELL;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Attention is a critical cognitive capacity for organizing and directing motivated behavior. Deficits in attentional control epitomize several neuropsychiatric disorders, including schizophrenia and attention-deficit/hyperactivity disorder (ADHD). Prior research (e.g., Pezze et al., Neuropsychopharmacology, 2007) suggest dopamine signaling in the nucleus accumbens (NAc) modulates attentional performance. Here, we examined the primary cell types of the NAc - D1- or D2-receptor-expressing medium spiny neurons (MSNs) - in attentional control using male and female D1-Cre or A2a-Cre transgenic mice, respectively. Mice were trained on the

five-choice serial reaction time (5-CSRT) task, in which an operant response is withheld until it can be directed to one of five locations indicated by a brief visual cue. We first expressed the Gi-coupled hM4D DREADD in D1- or D2-MSNs, and found that chemogenetic inhibition of D1-MSN activity reduced attention, whereas chemogenetic inhibition of D2-MSN activity enhanced attention. To monitor the activity of these two cell types in real time during 5-CSRT behavioral performance, we are currently using fiber photometry to image spectrally distinct red and green calcium indicators expressed in D1- and D2-MSNs. Preliminary results suggest D1-MSN activity increases on correct trials, whereas D2-MSN activity does not change. These findings highlight a role of NAc microcircuitry in attention and identify cell-type specific targets for treating related impairments in neuropsychiatric disorders.

Disclosures: M.T. Pisansky: None. **P.E. Rothwell:** None. **D.W. Leipold:** None.

Poster

418. Attention and Neuromodulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 418.18/Z5

Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust 093104

Title: Effects of attention on Granger causal interactions between cortical layers and cortical areas V1 and V4

Authors: *D. FERRO[1,2], J. VAN KEMPEN[3], M. BOYD[3], S. PANZERI[1], A. THIELE[3];
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Abstract: Visual attention improves sensory processing as well as perceptual readout and behavior. How attention shapes interactions between cortical layers within and between sensory areas is poorly understood [1-3].
To investigate this we trained macaque monkeys on a covert feature based spatial attention task, and recorded simultaneously from laminar electrodes in areas V1 and V4 (16 contacts, 150 μm inter-contact spacing). Electrodes were inserted normal to the cortical surface. Receptive Fields (RFs) between V1 and V4 were overlapping. Channel alignment relative to layer 4 was based on current source density and latency analysis.
For all analyses the LFP was locally re-referenced (bipolar LFP) offline. Spectral power in different frequency bands showed relatively small differences along cortical depths (V1 and V4). For V1 LFPs, attention to the RFs resulted in a shift of the low-gamma (~30-50Hz) spectral power peak towards higher frequencies (2-4Hz shift). For V4 LFPs attention to the RFs caused a

decrease in power for frequencies <20Hz and a broad band increase for frequencies >20Hz. Attention affected spectral coherence within V1 and within V4 layers similarly to the spectral power modulation reported above. Spectral coherence across V1 and V4 pairs was increased by attention in beta band (~15-30Hz) and the low-gamma range (30-50Hz).
Attention affected Granger causal interactions (GCI) in a layer and frequency dependent manner in complex ways. These often failed to follow predictions made by feed-forward and feedback models [1,4]. Within V1, attention increased feed-forward efficacy across different frequency bands (2-50 Hz). Within V4, attention mostly increased GCIs in the low and high gamma frequency in a 'downwards' direction within the column, i.e. from supragranular to granular and to infragranular layers. Increases were also evident in an upward direction from granular to supragranular layers. The dominant changes in V1-V4 CGIs were an increase in the gamma frequency range from V1 granular and infragranular layers to V4 supragranular and granular layers, as well as an increase from V4 supragranular layers to all V1 layers.
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[3] A. S. Nandy, J. J. Nassi, and J. H. Reynolds, Neuron 93, 235 (2017).
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Disclosures: **D. Ferro:** A. Employment/Salary (full or part-time):; Center for Mind/Brain Sciences (CIMeC), University of Trento, Rovereto, Italy. **J. Van Kempen:** None. **M. Boyd:** None. **S. Panzeri:** None. **A. Thiele:** None.

Poster

418. Attention and Neuromodulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 418.19/Z6

Topic: H.01. Animal Cognition and Behavior

Support: Research Division of the Universidad Iberoamericana A. C.

Title: The effect that selective and nonselective cannabinoid receptor agonists and antagonists have on the attentional system

Authors: *M. CHAVEZ HERNANDEZ[1], M. H. BUENROSTRO-JAUREGUI[1], M. MENDEZ-DIAZ[4], H. SANCHEZ-CASTILLO[5], C. A. HERNANDEZ-GUERRERO[2], E. R. BOJORGES-VALDEZ[3], L. F. S. HERNANDEZ-GONZALEZ[1], I. LOPEZ-CORTINA[1], O. R. GALICIA-CASTILLO[1];
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[4]Univ. Nacional Autonoma De Mexico, Mexico DF, Mexico; [5]Univ. Nacional Autonoma De Mexico. Fac Psicologia, Mexico City, Mexico

Abstract: The attentional system (AttS) is important for the adequate survival and behavioral performance of species. Because of legislation changes, it has become a research interest to have a deeper understanding of the effects of cannabinoids (CBs) can have on the SNC. These substances bind to CB1 and CB2 receptors (Rs) of the endocannabinoid system. Studies have reported that the use of CBs can have a negative effect in the AttS. There is little known about the effect that agonism/antagonism selective to CB2 receptor has in the SNC and on the AttS. The aim of this exploratory study is to observe the effect CBs can have on a temporal bisection attentional task (TBAT) using selective agonists (Anandamide -AEA- and HU308) and antagonists (SR141716A and SR144528), as well as the effect of non-selective agonism (2AG) and antagonism (Co-adm) of CB1/CB2 cannabinoid Rs. Male adult wistar rats were trained in a TBAT using Skinner operant conditioning chambers. The TBAT has 2 phases: Training phase (TrP) and Testing phase (TsP). TrP: trials initiated presenting a tone of either 2s (short -S-) or 8s (long -L-), each of which associated to a specific lever. Correct responses resulted in reinforcer delivery. TsP: sessions included the previous two S and L sounds, and 5 probe duration values (2.52, 3.17, 4.00, 5.04, and 6.35s). TsP registers how many of the 5 probe durations the rat discriminates as a L/S stimulus. After 15 base-line TsP sessions, rats were injected i.p. with previously reported effective doses of either selective or non-selective agonist or antagonist. Rats were evaluated in TsP under influence of these substances for 3 consecutive sessions. Results include: performance in the TBAT (pTBAT), omission errors (OE), bisection point (BP) and Weber's Fraction (WF); they show that, when comparing to control group: experimental groups show tendency to reduce pTBAT, except antagonism selective to CB2, which shows a tendency to improve pTBAT and reduce OE; both AEA and non-selective antagonism significantly increase the number of OE. Both the BP and WF of experimental groups do not significantly differ when compared to the control group. These findings show that CBs have a behavioral effect in the AttS; of particular interest is the effect that agonism and antagonism selective to CB2 has on the AttS.

Disclosures: M. Chavez Hernandez: None. **M.H. Buenrostro-Jauregui:** None. **M. Mendez-Diaz:** None. **H. Sanchez-Castillo:** None. **C.A. Hernandez-Guerrero:** None. **L.F.S. Hernandez-Gonzalez:** None. **I. Lopez-Cortina:** None. **O.R. Galicia-Castillo:** None. **E.R. Bojorges-Valdez:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.01/Z7

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant SC3GM111188

Title: Characterization of the nitric-oxide signaling cascades underlying short-term and long-term cellular correlates of feeding suppression induced by sensitization training in *aplysia*

Authors: *R. MOZZACHIODI, R. CHATTERJI, S. KHOURY, M. WAINWRIGHT;
Texas A&M Univ. Corpus Christi, Corpus Chrsiti, TX

Abstract: In the mollusk *Aplysia*, exposure to aversive stimuli not only causes a learned enhancement of defensive responses, known as sensitization, but also triggers a concurrent suppression of feeding. Similar to sensitization, feeding suppression can be expressed in the short-term (15 min) or in the long-term (24 h, Acheampong et al. 2012) timeframe, depending on the amount of training. Recent work in our lab revealed that nitric oxide (NO) is required for both short-term and long-term feeding suppression as well as for the underlying decreased excitability of feeding decision-making neuron B51, which is induced by *in vitro* sensitization training (Farruggella et al. 2019).

In this study, we began to characterize the downstream targets of NO modulation of B51 excitability triggered by *in vitro* sensitization training. Because a NO-cGMP pathway is functionally active in B51 and can downregulate its excitability (Goldner et al. 2018), we focused on the contributions of the soluble guanylyl cyclase (sGC) and protein kinase G (PKG) to B51 decreased excitability induced by *in vitro* training. Patterns of electrical shocks delivered simultaneously to pedal nerves P8 and P9 (10-s, 1 Hz, train of 10 500-ms, 60-Hz, 60-V impulses) were used as *in vitro* sensitizing stimuli. Because sGC and PKG activity may selectively contribute to distinct forms of plasticity, we employed single-trial (ST) and four-trial (FT) *in vitro* training protocols, which induce short-term and long-term B51 decreased excitability, respectively (Weisz et al. 2017). B51 excitability was measured prior to and 15 min after ST *in vitro* training, and prior to and 24 h after FT *in vitro* training. The following inhibitors were applied in the recording chamber prior to *in vitro* training: ODQ to block sGC (final concentration: 25 μM in 0.1% DMSO) and KT5823 to block PKG (final concentration: 2 μM in 0.1% DMSO).

ODQ and KT5823 both prevented the long-term B51 decreased excitability. This result indicates that sGC and PKG are necessary for the induction of long-term B51 decreased excitability, thus implicating the recruitment of a NO-cGMP-PKG pathway by FT *in vitro* training. Conversely, ODQ and KT5823 partially blocked the short-term B51 decreased excitability, indicating that sGC and PKG contribute only marginally to short-term plasticity in B51. This finding suggests that NO-mediated mechanisms independent from cGMP are recruited by ST *in vitro* training. Overall, these results indicate that although NO is necessary for training-induced plasticity in B51, distinct downstream cascades are activated in short-term and long-term decreased excitability.

Disclosures: R. Mozzachiodi: None. **R. Chatterji:** None. **S. Khoury:** None. **M. Wainwright:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.02/Z8

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant SC3GM111188
Texas Comprehensive Research Fund

Title: Characterization of the cellular mechanisms underlying memory impairment caused by food deprivation in an invertebrate model

Authors: *X. DENG, R. MOZZACHIODI;
Life Sci., Texas A&M University-Corpus Christi, Corpus Christi, TX

Abstract: Malnutrition can cause severe consequences to brain functions include memory deficits. Using the marine mollusk *Aplysia* as animal model, the mechanisms of an aversive form of learning known as sensitization have been extensively investigated (Kandel 2001). In normally-fed *Aplysia* (i.e., animals fed every two days, 2DFD), strong electrical shocks to the body wall, which mimic the attack of a predator, increase defensive responses, including the tail-induced siphon withdrawal reflex (TSWR). Such stimuli also reduce a non-defensive response, feeding (Acheampong et al. 2012). Sensitization and feeding suppression are concurrently expressed in *Aplysia*. The co-expression of sensitization and feeding suppression is disrupted by fourteen days of food deprivation (14DFD; Mac Leod et al. 2018). Under 14DFD, *Aplysia* completely lack sensitization (Mac Leod et al. 2018). In 14DFD animals, feeding suppression is maintained, but the amount of suppression is attenuated compared to 2DFD animals (Mac Leod et al. 2018). This project aimed to investigate the cellular mechanisms underlying the memory deficits caused by 14DFD. An *in vitro* analog was used to study sensitization and feeding suppression at the cellular level (Weisz et al. 2017). Sensitization training can be mimicked *in vitro* by electrical stimulation of afferent nerves from the body wall (Weisz et al. 2017). Correlates of sensitization and feeding suppression include increased excitability of the tail sensory neurons (TSNs) in the TSWR neural circuit, and decreased excitability of the decision-maker neuron B51 in the feeding neural circuit, respectively (Weisz et al., 2017). TSN and B51 excitability was tested before and 15 min after *in vitro* sensitization training. Four groups of preparations were used: 2DFD with or without training, 14DFD with or without training. Under 14DFD, no difference was observed in the excitability of either TSNs or B51 between the trained and untrained groups. These data suggest that in the starved *Aplysia*, prevented sensitization at the behavioral level may result, at least in part, from the lack of increased TSN excitability at the cellular level. The discrepancy between the absence of decreased excitability in B51 at the cellular level and the presence of an albeit attenuated feeding suppression *in vivo* suggests that

the feeding suppression under 14DFD may be contributed by additional neurons. These results offer insights about the cellular mechanisms of memory deficits caused by food deprivation. Data obtained from *Aplysia* can be used as foundation to elucidate the mechanisms of malnutrition-induced memory deficits and ultimately be used in higher organisms.

Disclosures: X. Deng: None. **R. Mozzachiodi:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.03/Z9

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 NS019895

Title: Complex interactions of PKA and MAPK pathways contribute to 5-HT-induced dynamics of RSK activation in *Aplysia* sensory neurons

Authors: *Y. ZHANG, R.-Y. LIU, L. J. CLEARY, J. H. BYRNE;
McGovern Med. Sch. of UTHSC at Houston, Houston, TX

Abstract: The protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) pathways play critical roles in mediating serotonin (5-HT)-induced long-term synaptic facilitation (LTF) of the *Aplysia* sensorimotor synapse. To better understand the regulation of kinases by 5-HT, we examined the dynamics of three key elements of MAP kinase cascades: extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK) and 90 ribosomal S6 kinase (RSK) after 5-HT treatment, and investigated the interactions among these kinases. Biphasic regulation of p38 MAPK by a single pulse of 5 min 5-HT was reported in our previous study (Zhang et al. 2017). Here, we used immunofluorescence to examine the phosphorylation (i.e., activation) of RSK after one pulse of 5-HT applied to isolated sensory neurons in culture. We found that 5-HT also induced a complex pattern of changes in RSK activity. RSK phosphorylation increased immediately after treatment, returned to basal level at 15 min, followed by a delayed increase at about 45 min, and then a return to basal level at 60 min. RSK activation was blocked by U0126, an inhibitor of MEK, the kinase upstream of ERK, suggesting that *Aplysia* RSK is downstream of the Ras/MEK/ERK pathway (Liu et al. 2018). However, ERK activity did not increase until about 45 min after one pulse of 5-HT. This difference between ERK and RSK dynamics suggests that 5-HT might induce multiple pathways to activate RSK in *Aplysia*. A recent study indicates that a PKA-dependent feedback loop in *Aplysia* sensory neurons activates ERK (Jin et al. 2018). To investigate the role of the PKA pathway in activation of ERK/RSK after one pulse of 5 min 5-HT, we applied the PKA inhibitor KT5720 30 min before and during 5-HT treatment. KT5720 blocked the activation of ERK 45 min after

application of 5-HT. Moreover, it also blocked the activation of RSK immediately after 5-HT stimulation. At that time point, ERK activity still remained near the basal level, suggesting that the PKA pathway contributes to the immediate activation of RSK as well as to the delayed activation of ERK. We also found that BI-D1870, a specific inhibitor of RSK, blocked the activation of p38 MAPK 45 min after application of 5 min 5-HT, suggesting that the delayed activation of that kinase reported in Zhang et al. (2017) may be induced by the ERK/RSK pathway. These data begin to delineate the complex interactions between second messenger pathways after 5-HT treatment. Further study is needed to investigate how they contribute to the efficacy of training protocols to form LTF.

Disclosures: Y. Zhang: None. **R. Liu:** None. **L.J. Cleary:** None. **J.H. Byrne:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.04/Z10

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grants R01NS019895

Title: Ribosomal S6 kinase is essential for long-term enhancement of excitability of sensory neurons of *Aplysia*

Authors: *R.-Y. LIU, Y. ZHANG, P. D. SMOLEN, L. CLEARY, J. H. BYRNE;
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Abstract: Analyses of long-term synaptic facilitation (LTF) of the monosynaptic connections between sensory neurons (SNs) and motor neurons (MNs) of *Aplysia* and long-term enhancement of neural excitability (LTEE) of SNs have provided key insights into cellular mechanisms of memory. Ribosomal S6 kinase (RSKs) are a family of serine-threonine kinases that are activated by mitogen-activated protein kinase (MAPK) and consequently activate cAMP response element-binding protein (CREB). Our recent studies in *Aplysia* (Liu et al., 2018) reveal roles for the MAPK isoform ERK, and RSK, in the activation of CREB1 and the induction of LTF. In addition, inhibition of RSK expression or its activation significantly reduced LTF, and these impairments were rescued by the Enhanced protocol, a computationally designed protocol with stimuli irregularly spaced in time. This protocol was previously shown to augment normal LTF and long-term memory (LTM) (Zhang et al. 2012). These results illustrate a previously underappreciated role for RSK as a CREB1 kinase and thus as a factor in long-term synaptic plasticity in *Aplysia*. Another cellular correlate of LTM is modulation of the intrinsic excitability of presynaptic neurons. The role of RSK in this form of plasticity has not yet been examined. Therefore, we applied an inhibitor of RSK, BI-D1870 (BID), expecting it to inhibit LTEE. LTEE

was induced by a Standard protocol of regularly spaced 5-HT applications (S). Excitability was examined in four groups: 1) Vehicle (Veh), 2) S alone, 3) BID alone, and 4) BID + S. One-way ANOVA followed by post-hoc comparisons revealed that 5-HT treatment produced a significant increase in the number of stimulus-evoked action potentials at 24 h post-treatment, as compared to Veh, or BID alone. LTEE induced by BID+S group was significantly less than that induced by S group, suggesting that inhibition of RSK impaired LTEE. Next, we examined whether the Enhanced protocol could rescue the LTEE impairment. Four groups were used: 1) S, 2) BID + S, 3) E (Enhanced protocol), and 4) BID + E. One-way ANOVA revealed a significant interaction between groups at 24 h post-treatment. Post-hoc comparisons revealed that LTEE induced by E was significantly greater than that induced by S. LTEE in the BID + E group was significantly greater than in the BID + S group and significantly greater than in the S group. These results indicate that the Enhanced protocol rescued the BID-induced impairment in LTEE. In summary, our results suggest that *Aplysia* RSK is required for both LTEE and LTF. The computationally designed Enhanced protocol not only improves normal LTF and LTEE, but also rescues deficits in LTF and LTEE induced by impaired RSK signaling.

Disclosures: R. Liu: None. **Y. Zhang:** None. **P.D. Smolen:** None. **L. Cleary:** None. **J.H. Byrne:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.05/Z11

Topic: H.01. Animal Cognition and Behavior

Support: NIH 1R15MH107892-01

Title: Transcriptional changes fade prior to long-term memory in sensitization of the *Aplysia* siphon-withdrawal response

Authors: T. ROSILES, M. NGUYEN, I. E. CALIN-JAGEMAN, *R. CALIN-JAGEMAN; Neurosci., Dominican Univ., River Forest, IL

Abstract: Forming a long-term memory requires changes in neuronal transcription. What happens, though, as the memory is forgotten? And how does the transcriptional state relate to the maintenance and recall of the long-term memory? To answer these questions we have been systematically tracing the time-course of transcriptional changes evoked by long-term sensitization in the marine mollusk *Aplysia californica*. Our approach captures transcriptional changes in neurons of known behavioral relevance using a within-subjects design, delineating patterns of transcriptional change that are comprehensive and reproducible. We have previously reported that within 24 hours of long-term sensitization training there is a widespread

transcriptional response involving robust changes in over 5% of tested transcripts (about 1,200 of ~22k; Conte, 2017). Within 1 week, however, memory strength fades and nearly all transcriptional changes relapse to baseline (Perez, 2018). Here we report microarray analysis (N = 16) of transcriptional changes 5 days post-learning, a time-point when memory strength has weakened but is still robust. Remarkably, we find that at this intermediate behavioral stage nearly all transcriptional changes have already fully decayed. Thus, most transcriptional changes seem to decay more rapidly than memory expression. This suggests either a) that there is considerable time-lag between transcriptional and behavioral changes (perhaps due to the persistence of translated proteins) or b) that the transcriptional changes evoked after learning are not required for all phases of memory maintenance.

Disclosures: **T. Rosiles:** None. **M. Nguyen:** None. **I.E. Calin-Jageman:** None. **R. Calin-Jageman:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

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Program #/Poster #: 419.06/Z12

Topic: H.01. Animal Cognition and Behavior

Support: HHMI Grant
A*STAR scholarship

Title: A trial-by-trial associative-learning paradigm is modulated by biogenic amines and can result in trace-conditioning in C. elegans

Authors: E. LEE[1], H. R. HORVITZ[2];
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Abstract: Associative learning allows animals to adapt to multiple environmental stimuli that occur proximally in space and time. How molecular and cellular interactions control the formation, maintenance and degradation of learned memories in precise spatiotemporal terms under various contexts remains under active investigation. The nematode *C. elegans* can be trained to associate multiple cues and exhibit learned locomotor responses. Comprehensive genetic and cellular manipulation tools and a deep understanding of its neural circuits allow a single-cell level resolution of analysis to be applied to *C. elegans* learning and memory. Short wavelength light is an aversive stimulus that triggers an escape response by *C. elegans* and also causes worms to stop feeding. We have developed a novel trial-by-trial conditioning paradigm for *C. elegans* that utilizes the pairing of a noxious light stimulus and a neutral odor stimulus. After training, worms not only learned to reverse to the once-neutral smell but also learned to stop feeding - a new learned response that has not been previously described. As

neuromodulators are crucial factors in learning and memory processes, we conducted a screen of neuromodulator mutants. Mutants defective in dopamine and octopamine exhibited defects in learning efficiencies. Strikingly, mutants defective in serotonin learned faster and more consistently than wild-type worms. An animal's physiological state could also influence its learning capabilities. For example, worms that were food-deprived for varying amounts of time had a U-shaped curve of learning efficacy. Additionally, serotonin-defective worms that were food-deprived had an altered learning efficacy curve compared to wild-type worms. Finally, temporal order and contiguity of stimuli presentation is a key feature of associative learning. Worms were even able to learn associations in a trace-conditioning procedure in which the light and odor stimuli presentation was separated in time. By studying how temporal variables in learning and physiological states of an animal are modulated and interact with each other at a single-cell level resolution of analysis, we hope to gain a detailed understanding of the molecular, cellular and circuit mechanisms that underlie learning behavior.

Disclosures: E. Lee: None. **H.R. Horvitz:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

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Topic: H.01. Animal Cognition and Behavior

Support: BBSRC Grant BB/P00766X/1

Title: Learning-induced switch in a sensory response that anticipates changes in behavioural outcomes

Authors: *P. R. BENJAMIN, Z. PIRGER, Z. LÁSZLÓ, G. KEMENES, I. KEMENES; Neurosci., Univ. Sussex, Brighton, United Kingdom

Abstract: The decision to respond to a sensory stimulus depends on variables such as prior experience. For instance, aversive learning may lead to a change in the anticipated outcomes that reverse the behavioural response to a stimulus. However, the neuronal mechanisms of how this type of learning can alter the decision to perform a certain behaviour is much less understood. We use the model system of *Lymnaea stagnalis* to address the question of how anticipated aversive outcomes can alter the behavioral response to a previously effective feeding stimulus. We found that applying an aversive conditioning paradigm reverses the decision so that the same stimulus inhibits feeding, rather than activating it. By pairing a food stimulus, the CS (conditioned stimulus), with touch, an aversive US (unconditioned stimulus), the feeding stimulatory response to sucrose was reversed to an inhibition of feeding. Key to the understanding of the neural mechanism underlying the switch in the behavioural response is the

PlB (pleural buccal) extrinsic interneuron of the feeding network whose modulatory effects on the feeding circuit inhibit feeding. PlB is excited by touch to reverse the effects of sucrose on the feeding response. Aversive associative learning induces a persistent change in the electrical properties of PlB that is both sufficient and necessary for the switch in the behavioural output.

Disclosures: P.R. Benjamin: None. **Z. Pirger:** None. **Z. László:** None. **G. Kemenes:** None. **I. Kemenes:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.08/Z14

Topic: H.01. Animal Cognition and Behavior

Support: DFG grant

Title: CRISPRCas9 based genome editing of the FOXP locus in *Drosophila melanogaster*

Authors: *O. PALAZZO[1], B. BREMBS[2], A. EHWEINER[1];
[1]Inst. für Zoologie - Neurogenetik, [2]Univ. Regensburg, Regensburg, Germany

Abstract: The human FOXP2 (Forkhead Box P2) gene has been identified as a key component for the development of language. Such vocal learning is a form of motor learning that proceeds slowly from babbling in toddlers (or subsong in songbirds) towards speech and language in adults (or crystallized song in birds). This particular motor learning process can be conceptualized as operant self-learning, in which the organism learns the correct actions only by evaluating the outcomes of its previous behavior, in the absence of other sensory cues beyond the feedback. In the fruit fly *Drosophila*, the *dFoxP* orthologue has been shown to also be involved in operant self-learning of yaw torque (attempted rotations around the vertical body axis) in tethered flies, an experiment conceptually analogous to vocal learning. The expression, function and mechanisms of action of *dFoxP* remain to be elucidated. In this work, we generated three transgenic lines using the CRISPR/Cas9 technique: a conditional knockout, an isoform-b-specific GAL4 knock-in and a LexA knock-in that targets all three known isoforms. The GAL4 knock-in confirmed previous results of FoxP expression in neurons, but not in Glia. Moreover, strong expression was found in the protocerebral bridge, saddle, vest and superior medial protocerebrum. Interestingly, the mushroom bodies were not labeled by this technique. Antibody stainings and the LexA knock-in revealed a much broader FoxP expression with only ~40% of FoxP neurons expressing isoform b. The conditional knock-down lines yielded weaker behavioral phenotypes in a locomotor experiment (Buridan's paradigm) when the gene was knocked-out in the adult stages compared to knock-out throughout development, confirming the

developmental role of the transcription factor. The three lines were also used in different knock-out and silencing strategies and tested for operant self-learning at the torque meter.

Disclosures: O. Palazzo: None. **B. Brembs:** None. **A. Ehweiner:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

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Program #/Poster #: 419.09/Z15

Topic: H.01. Animal Cognition and Behavior

Support: University of Padova DOR Funds to AM and MAZ

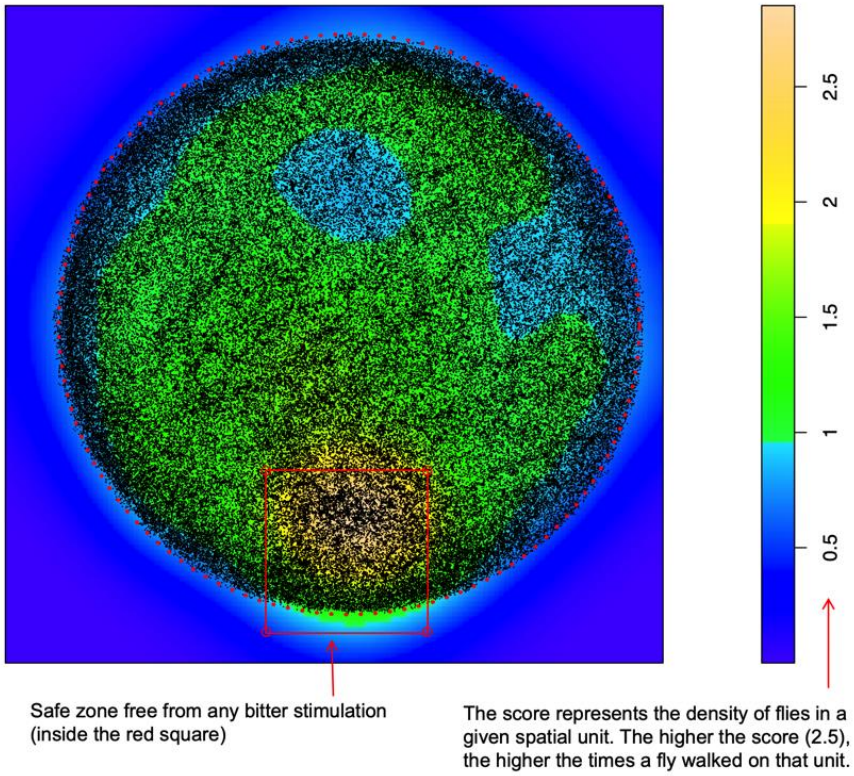
Title: Bitter taste drives pain relief place learning in *drosophila melanogaster*

Authors: N. MEDA[1], G. FRIGHETTO[2], *A. MEGIGHIAN[1], M. A. ZORDAN[3];
[1]Dep of Biomed. Sci. and Padova Neurosci. Ctr., [2]Dep of Gen. Psychology, [3]Dep of Biol. and Padova Neurosci. Ctr., Univ. of Padova, Padova, Italy

Abstract: Place learning is an adaptive behavior which animals use to locate salient stimuli in space. If such stimuli are life-threatening or detrimental, pain-relief is a type of learning used to assess whether a certain action can weaken or abolish noxious stimuli. In analogy to Morris' water-maze and Ofstad's heat-maze, we show how optogenetically-induced bitter-sensing neurons activation urges *Drosophila melanogaster* to find a safe zone free of any bitter taste stimulation.
Adult fruit fly males (8-10 days) expressing, via Gal4/UAS system blue-light sensitive ChannelRhodopsin2 (ChR2) in bitter-sensing neurons were tested in a plexiglass arena encircled by led panels displaying Buridan's paradigm visual stimuli. Eight pc controlled blue-emitting leds, were used to activate ChR2 according to fly position in the arena. An infrared camera online tracked fly movements at 11 fps.
The first experimental step consisted in a training session of 16, 3', trials. During odd trials (trial 1,3,…,15), the fly could find relief from the bitter taste close only one of the two identical Buridan's stripes; during even trials the safe zone was moved to the opposed stripe.
A probe session, without any safe zones, followed immediately the training session.
Single-blind bayesian analysis evidenced that flies perceived bitter taste as a punishing stimulus, and were able to search for a safe zone alternatively between two identical visual markers to relieve the unpleasant stimulation. As our work suggests, optogenetics can be used to assess the behavioural response of an experimental model to a precise and specific sensitive stimulation.

Spatial distribution of bitter–sensing flies during training

Figure 1. Spatial distribution of bitter-sensing flies during odd number trials



Disclosures: **N. Meda:** None. **G. Frighetto:** None. **A. Megighian:** None. **M.A. Zordan:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.10/Z16

Topic: H.01. Animal Cognition and Behavior

Title: The appetitive long term memory reacts differently to extinction wihtin a narrow time window

Authors: *L. L. WANG[1], Q. YANG[1], B. LU[2], Q. LI[1];
[1]Tsinghua Univ., Beijing City, China; [2]Inst. of Botany, the Chinese Acad. of Sci., Beijing City, China

Abstract: Extinction is widely used as the basis of exposure therapy to treat some psychiatric disorders while the risk of relapse of the enduring traumatic memories after extinction perplexes scientists a lot. Extinction given during the relatively labile period of memory seems attenuate it for a longer time but the exact mechanisms still need to be elucidated. To enhance the extinction effect for the translational purpose, study the molecular and cellular mechanisms of how and how can not the recovery happens is of great importance. Drosophila has been proven to be a powerful genetic model to study neural mechanisms of learning and memory. Here we find different periods of drosophila LTM show different reactions to extinction; extinction given at the late stage of LTM can spontaneously recover with the time decay while the same extinction paradigm given at the early stage of LTM shows no spontaneous recovery. Thus we provide a simpler and operable model to study the spontaneous recovery of extinction. The different reactions of different periods of LTM to extinction indicate a narrow dynamic window of the consolidated *Drosophila* LTM. Further studies about the mechanisms of how extinction recovers and why the early stage LTM shows no recovery may help us find better ways to enhance the extinction effect and make good contributions to the treatment of maladaptive behaviors.

Disclosures: **L.L. Wang:** None. **Q. Yang:** None. **B. Lu:** None. **Q. Li:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

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Topic: H.01. Animal Cognition and Behavior

Support: NIDCD 5R01DC013071-06
DART NeuroScience LLC
NSF DBI-0735191
NSF DBI-1265383
NSF DBI-1743442
NSF ACI-1548562

Title: Single-cell RNA-seq identifies differential expression in response to memory formation in *Drosophila*

Authors: *M.-F. M. SHIH[1], T. HIGE[3], G. C. TURNER[4], J. T. DUBNAU[2];
[1]Anesthesiol., [2]Anesthesiology, Neurobio. and Behavior, Stony Brook Sch. of Med., Stony Brook, NY; [3]Biology/Cell Biol. and Physiol., Univ. of North Carolina, Chapel Hill, NC; [4]Janelia Res. Campus, Ashburn, VA

Abstract: Abstract
Drosophila melanogaster is a favorable animal model for neuroscience studies because of (1) relatively simpler circuits orchestrating complex behaviors and (2) powerful genetic tools to characterize and manipulate gene expression and neural activity at high spatiotemporal resolution. A wealth of functional information about circuits-to-behavior has paved the way for single-cell RNA sequencing (scRNA-seq) technologies to identify differential expression within functionally characterized neuronal cell types, in response to different internal states and behavioral manipulations. Here, we established a fly scRNA-seq pipeline by adapting the design of whole-cell in vivo patch-clamping to physically remove single, identified neuronal cell somata, followed by CEL-Seq library preparation. Using this pipeline, we have acquired high-quality transcriptome profiles of 31 dorsal medial paired (DPM) neurons from female adult flies subjected to different olfactory memory training protocols. With this method, we achieve a median detected gene count of 8205 per DPM neuron. The scRNA-seq signal for DPM marker genes, including the reporter *GFP* and the neuronal marker gene *elav*, is strong, while the glial marker gene *repo* is barely detectable. Expression analysis at baseline identified multiple DPM expressed transcripts, and analysis of the effects of behavioral training identified dozens of up- or down-regulated genes. We have made use of the arsenal of genetic tools in *Drosophila*, to perform subsequent manipulations of gene function with this cell type. This provides the means to functionally validate our findings from scRNA-seq. Our results establish proof of concept for a scRNA-seq pipeline to profile gene expression changes after behavioral training in each of the cell types that participate in memory formation and storage in this olfactory task.

Disclosures: **M.M. Shih:** None. **T. Hige:** None. **G.C. Turner:** None. **J.T. Dubnau:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

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Program #/Poster #: 419.12/Z18

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 5R35NS097224

Title: Aversive training induces both pre and postsynaptic suppression in *Drosophila*

Authors: *X. ZHANG, N. NOYES, R. L. DAVIS;
Neurosci., Scripps Res., Jupiter, FL

Abstract: The α'β' subtype of *Drosophila* mushroom body neurons (MBn) is required for memory acquisition and early memory retrieval after aversive olfactory conditioning. However, prior *in vivo* functional imaging studies have failed to detect an early forming memory trace in these neurons as reflected by an enhanced G-CaMP signal in response to presentation of the learned odor. Moreover, the status of any potential early cellular memory trace in the mushroom body output neurons (MBOn) downstream of the α'β' MBn remains unknown. Here, we show that aversive olfactory conditioning suppresses the calcium responses to the learned odor in both α'3 and α'2 axon segments of α'β' MBn and in the dendrites of α'3 MBOn immediately after conditioning. Notably, the cellular memory traces in both α'3 MBn and α'3 MBOn are short-lived and persist for less than 30 min. The suppressed response in α'3 MBn is accompanied by a reduction of acetylcholine (ACh) release, suggesting that memory trace in postsynaptic α'3 MBOn may simply reflect the suppression in presynaptic α'3 MBn. Furthermore, we show that the α'3 MBn memory trace does not occur from the inhibition of GABAergic neurons via $GABA_A$ receptor activation. Because activation of α'3 MBOn drives approach behavior, our results demonstrate that aversive conditioning promotes avoidance behavior through suppression of the α'3 MB-MBOn circuit.

Disclosures: X. Zhang: None. **N. Noyes:** None. **R.L. Davis:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.13/Z19

Topic: H.01. Animal Cognition and Behavior

Title: The clock neurons acutely regulate memory retrieval in *Drosophila*

Authors: *Y. ZHOU, Y. ZHANG, Y. ZHONG;
Tsinghua Univ., Beijing City, China

Abstract: Time and memory are inextricably linked. Although circadian rhythms have been widely reported to regulate learning and memory, it remains wide open as to how individual or subgroups of clock neurons are acutely involved in encoding or regulating temporal nature of memory. Here, we take advantage of the well-defined circadian system of *Drosophila Melanogaster*, to dissect such mechanism in neural circuit levels. We found that acute activation of clock neurons exerted no effects on memory formation and maintenance, but attenuated retrieval of long-term memory while had no impact on every aspects of short-term memory tested. Genetic dissection allowed us to attribute such acute effects to a subgroup of clock

neurons. We are continuing to make efforts in elucidating the circuits that mediate this effect in an attempt to gaining insights into its mechanisms and functions. Thus, this work identifies a novel function for clock neurons in acute regulation of retrieval of long-term memory.

Disclosures: Y. Zhou: None. **Y. zhang:** None. **Y. zhong:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

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Program #/Poster #: 419.14/Z20

Topic: F.01. Neuroethology

Title: Screening for neuronal substrate of the inhibitory interaction between two genetically different learning systems in *Drosophila*

Authors: *A. E. ERIKSSON, B. BREMBS;
Inst. für Zoologie - Neurogenetik, Univ. Regensburg, Regensburg, Germany

Abstract: Learning is an essential component for an animal's survival as it enables for better future decisions: such as finding more nutritious food or avoiding harmful stimuli. While the molecular mechanisms underlying learning and memory have been studied extensively, only little is known about the neuronal architecture and the mechanisms for how the different learning systems interact in composite learning tasks. It has been proposed that at least two types of learning are mediated by distinct molecular mechanisms in humans, mice, songbirds, snails and in fruit flies, where one is involved in the effects of the animal's own behavior on the world (self-learning) and a separate one for the relationship of events in the world (world-learning). A fly primarily uses a classical learning system as it explores its environment and learns about the stimuli associated with it. For this initial phase, the world-learning system inhibits self-learning ensuring a flexible memory, independent of the behavioral context within which it was acquired. This inhibition is mediated by the mushroom bodies (MB) in fruit flies: blocking the synaptic output of the mushroom bodies impairs their ability to inhibit self-learning, leading to premature habit formation. For the purpose of this study, we used a two-phase experimental design. The first phase consists of a composite learning task containing both self- and world-learning components, followed by a test phase that isolates the operant component. The MB is composed of 21 different types of output neurons (MBONs) which receive the neuronal activity from the MB for further processing. To map the MBON pathway through which the Mbs inhibit self-learning, we performed a comprehensive screen blocking each of the 21 different types of MBONs and testing them for a loss of the ability to inhibit self-learning. The results from this screen allow us to propose downstream MBON targets which must be associated with the neurons mediating operant self-learning.

Disclosures: **A.E. Eriksson:** None. **B. Brembs:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

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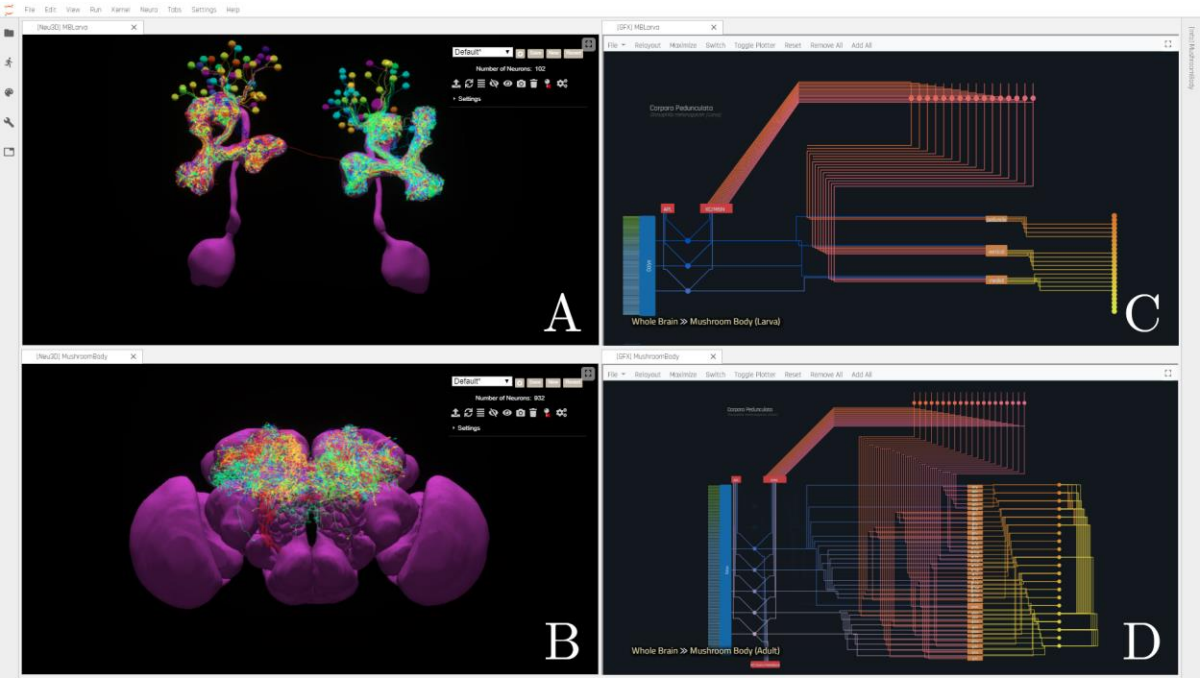
ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: I.06. Computation/ Modeling/ and Simulation

Support: NSF Grant 1544383
AFOSR Grant FA9550-16-1-0410

Title: FlyBrainLab: An interactive computing platform for the fruit fly brain

Authors: ***M. K. TURKCAN**, T. LIU, C.-H. YEH, Y. ZHOU, A. A. LAZAR;
Dept. of Electrical Engin., Columbia Univ., New York, NY

Abstract: To address the challenge of building a functional map of the fruit fly brain we devised an open-source interactive computing platform called (https://github.com/FlyBrainLab) FlyBrainLab (FBL). FBL is a first-of-its-kind platform built for studying the function of executable circuits constructed from fly brain data available worldwide; its tools and libraries will benefit neurobiologists, computational neuroscientists and computer scientists in their quest of understanding the function of the brain of the fly and other model organisms. FBL integrates the key innovations of the Fruit Fly Brain Observatory ecosystem [1]. Unlike conventional simulation platforms focusing on model execution, FBL champions a synergistic integration of neurobiology and circuit model data into a common NeuroArch graph database. NeuroArch currently features cell-type, connectome, synaptome, neurogenetics, and neurophysiology data from the FlyCircuit, Janelia FlyEM and spike recordings including our own [2]. For brain circuit execution FBL also provides access to the Neurokernel engine operating on GPUs. A high level view of these components is provided by NeuroMynerva, a modern integrated development environment that is built upon and substantially extends JupyterLab. Together, these 3 open-source components enable easy management, rapid loading, effective visualization, and intuitive exploration of neural data (Fig. 1A,B), exploration of circuit diagrams (Fig. 1C,D), code libraries for execution, analysis and biological validation. Furthermore, FBL significantly accelerates the development cycle of models of Drosophila brain circuits. Finally, FBL is scalable to the flood of fly brain data anticipated in the near future. We applied FBL for an extensive comparative analysis of the mushroom body in the adult and larva and an exhaustive comparison of recently published models of the central complex.
[1] Ukani et al., bioRxiv doi: 10.1101/580290, 2019
[2] Kim et al., eLife doi: 10.7554/eLife.06651, 2015



Disclosures: **M.K. Turkcan:** None. **T. Liu:** None. **C. Yeh:** None. **Y. Zhou:** None. **A.A. Lazar:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant 1R15AG045820-01A1
DoD Grant ONR#000141010198

Title: Aversive conditioning in the tardigrade, Dactylobiotus dispar

Authors: *T. C. DUMAS[1], S. Y. ZHOU[2], J. P. DEFRANCO[1], N. T. BLAHA[1], A. M. KABBANI[1];
[1]George Mason Univ., Fairfax, VA; [2]Univ. of Virginia, Charlottesville, VA

Abstract: Tardigrades, often colloquially referred to as “water bears”, are microscopic octopedal organisms found in aquatic and terrestrial habitats all over the world. They are well known for their uncommon ability to thrive in extreme conditions as they can enter into a cryptobiotic state known as a “tun”. In the tun state, protective sugars accumulate, glycerol is produced, intrinsically disordered proteins are expressed, and metabolism is effectively terminated. Given

this unique ability, tardigrades present an opportunity to study metabolic constraints on memory consolidation and maintenance. To investigate this further, it was necessary first to determine if tardigrades could be classically conditioned. Four to nine week old tardigrades of the species *Dactylobiotus dispar* were used for all experiments. The sex of the animals was unknown. Tardigrades were trained and tested on a microscope slide with two pieces of wire laid with ends 5 mm apart. A droplet of water was used to connect the two wires and to contain a single tardigrade. An electrical stimulator was connected to the wires to complete the circuit. Naive tardigrades will curl their bodies inward in response to a sufficient electrical shock but will not do so in response to a blue light stimulus. Tardigrades were trained by pairing an electrical shock with blue light. A time interval of either one or four hours passed and the animals were exposed to the conditioned stimulus alone and behavior was recorded. Control conditions during training included shock alone, blue light alone, backward pairing, random pairing, and forward pairing with green or red light. It was found that when forward pairing of shock with blue light was applied to naive tardigrades, an associative memory was retained for at least one hour. When tardigrades were tested four hours after training there was no significant response to the blue light, indicating a time dependent decline in memory performance.This study was able to show that tardigrades are capable of learning and memory and that they can retain an associative memory for one hour after a single pairing of a US and CS. Current experiments involve multiple pairings of the US and CS to induce longer lasting memories. In future experiments, half of the tardigrades will undergo tunning and revival prior to memory testing to investigate the metabolic requirements for memory storage and the possibility of attenuated memory decay in the tun state.

Disclosures: **T.C. Dumas:** None. **S.Y. Zhou:** None. **J.P. DeFranco:** None. **N.T. Blaha:** None. **A.M. Kabbani:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.17/Z23

Topic: H.01. Animal Cognition and Behavior

Support: NSF

Title: Quest for neuronal cell analogs in nerveless animals: Ultrastructural atlas of cell types and behaviors in Placozoa

Authors: D. Y. ROMANOVA[1], F. VAROQUEAUX[2], M. EITEL[3], *L. L. MOROZ[4];
[1]Russian Acad. of Sci., Moscow, Russian Federation; [2]Univ. of Lausanne, Lausanne, Switzerland; [3]Univ. of Munich, Munich, Germany; [4]Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Placozoans are the simplest free-living animals without neurons and muscles, but with complex behaviors. They are descendants of earlier branching basal metazoan lineages and, therefore, critical to reconstructing the genealogy of cell types in Metazoa. For more than 100 years, only one species of Placozoa was known - *Trichoplax adhaerens*. However, Eitel *et al*. (2018) and Osigus *et al*. (2019) described two more placozoan species *Hoilungia hongkongensis and Polyplacotoma mediterranea*. Here, we characterized the ultrastructural organization of *H. hongkongensis* and compared it to known haplotypes of *Trichoplax*. Our data confirm the organizations of ventral and dorsal epithelia and the existence of six major morphological cell classes across Placozoa including crystal and fiber cells, as well as a remarkable diversity of cellular subtypes suggesting both unique lineage-specific adaptations and ecological/functional diversification across Placozoa. Next, we established a culture for *H. hongkongensis* and other placozoans as well as developed standardized assays to reveal both innate and pharmacologically induced behavioral patterns among various haplotypes. Our analyses, combined with molecular profiling of specific cell types and signal molecule candidates, provide novel insights in cellular bases of behaviors in organisms without neurons and muscles.
References:
(1) Eitel M. *et al*. (2018). Comparative genomics and the nature of placozoan species. *PLoS Biol.* 2018; 16: e2005359;
(2) Osigus *et al*. (2019). *Polyplacotoma mediterranea* is a new ramified placozoan species. *Current Biology*, 29 (5), R148-R149.

Disclosures: D.Y. Romanova: None. **F. Varoqueaux:** None. **M. Eitel:** None. **L.L. Moroz:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.18/Z24

Topic: H.01. Animal Cognition and Behavior

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Title: *De novo* transcriptome assembly and differential expression analysis in the ventral nerve cord of Manduca sexta after nociceptive sensitization

Authors: *R. L. MELTON, D. R. TABUENA, M. FUSE;
Biol., San Francisco State Univ., San Francisco, CA

Abstract: Chronic pain is a prevailing health problem ravaging the United States. Research in this field is primarily limited to vertebrate models. With the extensive ethical and financial limitations of vertebrate research, the development of an invertebrate model for preliminary therapeutic research would greatly broaden our understanding and treatment of chronic pain. The tobacco hornworm, *Manduca sexta*, has a well-defined behavioral defensive strike to noxious or harmful stimuli that provides a gauge of a pain-like state. This state becomes hypersensitized after presentation of a harmful stimulus – assessed by a reduction in the force required to elicit a strike – and this can last up to 19 hours. We use this as a model for injury-induced sensitization, and this has been shown to arise centrally. We have also previously shown that protein synthesis is required within 3 hours of injury in order to maintain sensitization. The goal of this study was to assess transcriptional changes in the CNS associated with long-term sensitization. We first determined whether the brain was required for sensitization, using our previously established *in vitro* electrophysiological preparation. We removed the brains of 5^{th} stage larvae 24 h prior to injury by a pinch. Pinched animals with or without brains showed an increased firing frequency compared to non-sensitized controls, indicating that sensitization occurred without the brain. We therefore used abdominal ganglia to produce *de novo* transcriptomes for control and sensitized states. This transcriptome was developed through Trinity, resulting in 211,719 predicted 'trinity genes' and 2,768,665 transcripts, with a Bowtie2 alignment of 94.56%. Using Trinotate, we annotated 87.49% and 88.45% of our transcriptome with UniProt and TrEMBL, respectively. Differential expression was noted in the transcriptome after injury, including 110 up-regulated transcripts and 38 down-regulated transcripts. To begin characterizing proteins involved in injury-induced sensitization, we mined *in silico* for classical learning and memory/sensitization-induced genes. This resulted in the presence of 2,364 of the 7,078 target genes derived from nervous system gene ontology categories within our transcriptome. Additionally, 68 differentially expressed transcripts were found to be within these gene ontology categories, including Ras GTPase, CASK, and integrin alpha-8. The presence and expression of these classical neuroplasticity genes are a promising start to examining the underlying conservation of long-term injury-induced sensitization in *M. sexta.*

Disclosures: R.L. Melton: None. **D.R. Tabuena:** None. **M. Fuse:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.19/Z25

Topic: H.01. Animal Cognition and Behavior

Support: Programa UNAM-DGAPA-PAPIIT IN224417

Title: Learning and memory in crayfish

Authors: A. JUÁREZ-JARAMILLO[1], A. DE LA O-MARTÍNEZ[1], M. OSORIO-PALACIOS[1], I. OLIVER-DOMÍNGUEZ[1], K. MENDOZA-ANGELES[2], *J. HERNANDEZ-FALCON[1];
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Abstract: Crayfish has been used as a biological model in neurosciences for many years. A relatively “simple” brain and fine adaptation to laboratory conditions make this animal a suitable biological model for studying sensory systems and memory. Recently we demonstrated that this crustacean uses individual recognition memory during the establishment of hierarchical relationships and we are interested in the mnemic capabilities of this crustacean. We used two protocols to investigate if this animal is able to learn through classical conditioning and/or operant conditioning. In the first one we recorded the heartbeat (through an ECG) while applying a light pulse each 40 s. After 6 repetitions of the light pulse we applied a mechanical stimulus (droping of a heavy object in the aquarium). We found that the heart rate diminishes with the mechanical stimulus and that after 14 repetitions the light pulse is enough to induce bradycardia. This association disappeared after 3 days and was sensitive to cold anesthesia. Operant conditioning consisted in the application of an electric shock in the walking legs of a crayfish placed in a conditioning cage. When the crayfish learned to escape the electrical stimulus, we associated an intense light 5 s before the electrical shock. The crayfish learned to avoid the electric shock by escaping to its aquarium when the light was turned on. This response disappeared after 3 days without reinforcement. These experiments show that the crayfish is capable to associate stimuli under classic and operant conditioning.

Disclosures: A. Juárez-Jaramillo: None. **A. de la O-Martínez:** None. **M. Osorio-Palacios:** None. **I. Oliver-Domínguez:** None. **K. Mendoza-Angeles:** None. **J. Hernandez-Falcon:** None.

Poster

419. Mechanisms Underlying Learning and Memory in Invertebrates

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 419.20/Z26

Topic: F.01. Neuroethology

Title: Laboratory studies of spatial learning in Fiddler crabs (Uca pugilator)

Authors: *F. W. GRASSO[1], E. ISAACS[2], R. TROISE[2];
[2]Psychology, [1]Brooklyn College, CUNY, Brooklyn, NY

Abstract: The use of path integration mechanisms in arthropods, particularly eusocial insects, for navigation appears to be ubiquitous and well-studied. The propensity of fiddler crabs to return their home burrows after foraging trips makes them amenable subjects for studies of spatial navigation in non-eusocial species. Several clever studies of spatial memory in Fiddler crabs in the field suggest that fiddler crabs may navigate using mechanisms in addition to path integration. In a series of behavioral experiments conducted under controlled laboratory conditions, we examined their ability to use allocentric cues to learn spatial locations in spatial tasks that do not involve a home burrow. A study conditioned place preference learning using visual cues showed no evidence for place preference learning but a strong preference for specific types of visual stimuli. A conditioned place preference study that controlled for visual cues found an interaction effect confirming field studies that indicated The results do not provide unequivocal evidence for these abilities but do indicate learning of space in these artificial task in more complex processes, and indicate interference in learning processes by predispositions.

Disclosures: F.W. Grasso: None. **E. Isaacs:** None. **R. Troise:** None.

Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 420.01/Z27

Topic: H.01. Animal Cognition and Behavior

Support: Medical Research Council, UK (MC_UU_12024/1)
Wellcome Grant, 109030/Z/15/Z

Title: Motor thalamic innervation of NDNF-expressing interneurons in layer 1 motor cortex

Authors: *N. S. M. BERRY[1], R. SHAH[1], L. WAITE[1], K. MELETIS[2], A. SHAROTT[1];
[1]MRC Brain Network Dynamics Unit, Dept. of Pharmacol., Univ. of Oxford, Oxford, United Kingdom; [2]Karolinska Inst., Stockholm, Sweden

Abstract: Basal ganglia (BG) and cerebellar circuits (CB) exert control over ongoing behaviour through their connections with brainstem motor centres and distinct populations of neurons comprising adjacent nuclei in the motor thalamus (BG.MThal and CB.MThal). CB.MThal (the ventral lateral nucleus in rodents) neurons resemble “first order” thalamic neurons, which faithfully ‘relay’ information to the deep layers of sensorimotor cortex. By contrast, BG.MThal (the ventral anterior and ventral medial nuclei in rodents) neurons have the characteristics of “higher order” thalamic neurons, which target the superficial layers of widespread functionally-related cortical areas, particularly Layer 1 (L1). As the apical dendrites of both deep and superficial pyramidal neurons reside in L1, this laminar specificity does not necessarily define the neuron types targeted by thalamocortical synapses. For both parts of MThal, there has been

an assumption that the main postsynaptic targets of thalamocortical neurons in motor cortex are Layer 5 (L5) pyramidal neurons. However, recent evidence has suggested that in L1 of sensory areas, extended neurogliaform (eNGF) interneurons, which are defined by the expression of neuron-derived neurotrophic factor (NDNF), are also an important thalamic target for higher order nuclei. We tested the hypothesis that motor cortical L1 eNGF interneurons receive direct innervation from neurons in motor thalamus. Using an NDNF-Cre mouse, with stereology we demonstrate that the majority of motor cortical L1 NDNF-expressing interneurons coexpressed GAD67 and reelin, consistent with being eNGF interneurons. In addition, we found that many of these interneurons express chicken ovalbumin upstream promotor transcription factor II (COUP-TFII). To further explore the thalamic connectivity and function of these interneurons, we developed an injection technique to restrict viral labelling of neurons mostly to L1. Monosynaptic retrograde rabies viral tracing demonstrated that motor cortical L1 NDNF+ neurons receive synaptic input from several thalamic nuclei. Our preliminary data suggests that BG.MThal neurons provide the largest innervation, consistent with their dense projections to this cortical layer. In contrast, the same experiments using retinol-binding protein 4 (RBP4)-Cre mice demonstrated motor cortical L5 pyramidal neurons receive more inputs from CB.MThal than BZ.MThal neurons. These results suggest that not only do motor thalamic nuclei differ in their preferential laminar targeting of motor cortex, but also in their relative innervation of specific populations of pyramidal neurons and interneurons.

Disclosures: **N.S.M. Berry:** None. **R. Shah:** None. **L. Waite:** None. **K. Meletis:** None. **A. Sharott:** None.

Poster

420. Thalamic and Brainstem Circuits

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Commissariat à l'Energie Atomique
Collège de France
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Title: Thalamic stimulation modulates consciousness in anesthetized macaques by restoring spontaneous and evoked fMRI activity in a cortical global neuronal workspace

Authors: *J. TASSERIE[1], L. UHRIG[1], J. D. SITT[2], M. DUPONT[1], S. DEHAENE[1,3], B. JARRAYA[1,4,5];
[1]INSERM, CEA - Neurospin Ctr., Paris Metropolitan Area, France; [2]ICM - Hôpital Pitié Salpêtrière, Paris, France; [3]Collège de France, Paris, France; [4]Foch Hosp., Paris, France; [5]Univ. de Versailles Paris-Saclay, Versailles, France

Abstract: Consciousness can be studied at two different levels: arousal, modulated in part by the central thalamus, and awareness which is characterized by the conscious access to a specific piece of information, thought to be related to a long-range cortical global neuronal workspace. It has been reported that electrical Deep Brain Stimulation (DBS) of central thalamic nuclei could restore arousal in rodents (Shirvalkar et al., 2006) and ameliorate behavior in minimally conscious state patients (Schiff et al., 2007). Here, we use functional MRI and thalamic DBS in macaque monkeys to evaluate the hypothesis that DBS can causally modulate both arousal and awareness through the reorganization of prefronto-parietal networks.
Two Rhesus macaque monkeys were chronically implanted with DBS leads targeting central thalamus. To manipulate the state of consciousness, we applied finely tuned, EEG-monitored propofol anesthesia as previously developed in macaques (Uhrig et al., 2016). We first tested the behavioral effects of thalamic DBS during anesthesia. Then, we performed a series of fMRI experiments at 3T. We studied the effects of thalamic DBS on resting-state brain activity, particularly focusing on a previously described signature of consciousness in the dynamics of resting-state fMRI (Barttfeldt et al., 2015). We also examined task-related fMRI in the ON-DBS and OFF-DBS conditions using the 'Local-Global' auditory paradigm, in which a signature of the conscious processing of auditory regularities was previously described in humans (Bekinschtein et al, 2009) and macaques (Uhrig et al, 2014).
Thalamic DBS robustly induced arousal in anesthetized macaques, with spontaneous motor behavior and eye blinking. When thalamic DBS was switched on, fMRI activity was seen in several cortical areas including in the prefrontal, parietal and cingular cortices. This DBS-induced fMRI activity gradually returned to baseline seconds after the stimulator was turned off. Moreover, thalamic DBS led to a vast reconfiguration of resting-state cortical dynamics by decreasing the function-structure similarity, as previously described for the transition from anesthesia to awake state (Barttfeld et al., 2015). Finally, thalamic DBS restored a broad hierarchical cortical response to global auditory regularities, as previously observed in the conscious state (Uhrig et al., 2014).
Our data demonstrate that thalamic DBS can restore both arousal and awareness in a non-human primate model of loss of consciousness, paving the way to its therapeutical translation in patients with disorders of consciousness.
JT and LU equally contributed to the study.

Disclosures: J. Tasserie: None. **L. Uhrig:** None. **J.D. Sitt:** None. **M. Dupont:** None. **S. Dehaene:** None. **B. Jarraya:** None.

Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 420.03/Z29

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH112746
Swartz Foundation

Title: A computational model of thalamic reticular circuits: Gating of signal processing by top-down control and effects of inhibitory dysfunction

Authors: Q. GU[1], N. H. LAM[2], *J. D. MURRAY[1];
[1]Psychiatry, [2]Physics, Yale Univ., New Haven, CT

Abstract: The thalamus plays a key role in long-range communication in the mammalian brain. In addition to its role as a relay of peripheral sensory signals to cortex, a growing literature has characterized its flexible engagement in higher-order cognitive functions according to task demands. For instance, attentional goals can exert top-down control of thalamic gain to amplify task-relevant signals and filter task-irrelevant signals depending on cognitive needs. However, the thalamic circuit mechanisms supporting this cognitive flexibility are poorly understood. Furthermore, thalamus is thought to be a key site of alteration in multiple neuropsychiatric disorders, in particular through disruption of inhibitory regulation by the thalamic reticular nucleus (TRN). Yet it is mechanistically unclear how synaptic or cellular-level dysfunction can induce behavioral deficits in cognitive tasks.

To study these questions, we developed a biophysically detailed computational model of a thalamic reticular microcircuit, composed of thalamocortical and TRN cells. Each cell type is modeled through Hodgkin-Huxley equations capturing key voltage-dependent active conductances. Circuit dynamics arise through an interplay between the active conductances and regulation by inhibitory synaptic feedback. The *in vivo*-like regime can be constrained through comparison by range of experimental results. Our model can capture *in vivo* circuit dynamics consistent with experiments, including burst and spindle statistics under spontaneous and evoked conditions. Perturbation analysis indicates TRN is crucial in modulating the dynamical regime of the thalamus. In addition, we examined the input-output characteristics of the circuit model, demonstrating how key parameters control the tonic and burst modes of the network, and ultimately the information transmission process. Finally, we altered the circuit models based on hypothesis microcircuit disruptions and functional alterations associated with psychiatric disorders including schizophrenia. We identified specific alterations to coupling strengths and background thalamic inputs as potential candidates to drive the circuit impairments.

In summary, we presented a thalamic circuit model which reproduced experimental observations,

and allowed a circuit-level understanding of the thalamus. The model makes dissociable predictions for optogenetic and pharmacological disruptions, potentially informing future experiments and providing insights to brain disorders and potential therapeutic interventions.

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Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 420.04/Z30

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01MH110311
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Title: Mediodorsal and ventroanterior thalamic neurons are selective for rules and goals

Authors: *J. M. PHILLIPS[1], N. A. KAMBI[1], M. J. REDINBAUGH[1], E. R. JOHNSON[2], S. MOHANTA[1], Y. B. SAALMANN[1];
[1]Psychology, [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract: The lateral prefrontal cortex (PFC) enables cognitive control in primates, but its extensive connectivity with subcortical structures emphasizes that the PFC does not act in isolation. The lateral PFC is reciprocally connected with at least two thalamic nuclei, forming both closed and open loop circuits in a hierarchically organized cortico-thalamo-cortical network. The projections from distinct PFC regions overlap in both the mediodorsal thalamic nucleus (MD) and ventroanterior thalamic nucleus (VA), especially its medial magnocellular compartment (VAmc). MD and VA are thus well placed to influence information transmission across PFC according to cognitive control demands. In mice, MD synchronizes frontal cortical neurons, increasing their functional connectivity, and supporting their rule maintenance, without expressing rule selectivity itself. In monkeys, lateral PFC and MD show similar responses during spatial working memory tasks, but the contribution of MD to more complex rule-guided behavior, and its interaction with lateral PFC neurons in this context, have not been explored. There is even less information available regarding VA function. How do VA and MD uniquely contribute to PFC neural activity and cognitive control?
To address these questions, we used linear multielectrode arrays to simultaneously record from central MD, medial VA, anterior and posterior area 46 of two monkeys performing a hierarchical

rule-based task. We used diffusion MRI and probabilistic tractography to target electrode arrays to interconnected cortical and thalamic sites. Tractography between the substantia nigra and VA aided in delineating and targeting VAmc, the nigral recipient zone of VA. Structural MRI of electrodes *in situ* verified recording sites.
Spiking activity of single neurons in lateral PFC showed selectivity for abstract rules, concrete rules, and goal target location. Unlike mouse MD, rule selectivity was also a prominent feature of primate MD spiking activity. VA neurons showed selectivity for the rewarded target location. Thus, thalamic neural activity closely resembled PFC neural activity.
Neural ensembles representing abstract rules, concrete rules, and rewarded target locations may be recruited and maintained through PFC-MD/VA interactions involving reciprocal loops. Information about these sequential trial events may be transformed and propagated across hierarchical PFC-subcortical circuits via the open loops, involving unreciprocated cortical projections issuing feedback to "lower" thalamo-cortical circuits. This may facilitate selection of appropriate action based on the combination of preceding rule cues.

Disclosures: **J.M. Phillips:** None. **N.A. Kambi:** None. **M.J. Redinbaugh:** None. **E.R. Johnson:** None. **S. Mohanta:** None. **Y.B. Saalmann:** None.

Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 420.05/Z31

Topic: G.03. Emotion

Title: Role of the paraventricular nucleus of the thalamus in regulating passive and active fear responses

Authors: *J. MA[1], M. A. PENZO[2];
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Abstract: The ability to form and retrieve fear memories is critical for animal survival. While significant progress has been made in identifying the neural circuit mechanisms that control the expression of passive (e.g. freezing) and active (e.g. avoidance) fear behavioral responses, little is known about those mechanisms driving the selection of one behavior over the other. The paraventricular nucleus of the thalamus (PVT) is a candidate structure for guiding behavioral selection given its prominent innervation of both the central amygdala (CeA) - which controls freezing behavior - and the nucleus accumbens (NAc) - which controls avoidance behavior. Indeed, we recently demonstrated that PVT projections to the CeA are critical for the expression of freezing behavior following fear memory retrieval (Penzo et a., 2015 *Nature*). However, whether or not the PVT also orchestrates avoidance behavior remains unclear. Here, we have

used a combination of fiber photometry and optogenetics in mice to uncover a PVT-NAc pathway that controls avoidance. Current efforts are being directed at investigating a potential role for the PVT in biasing the selection of freezing and avoidance behavior.

Disclosures: J. Ma: None. **M.A. Penzo:** None.

Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 420.06/Z32

Topic: H.01. Animal Cognition and Behavior

Support: NIMH IRP ZIAMH002950

Title: Identification of functional domains within the paraventricular nucleus of the thalamus

Authors: *C. GAO[1,2], Y. LENG[1], J. MA[1], S. RODRIGUES-GONZALEZ[1], C. RAMAKRISHNAN[3], K. DEISSEROTH[3], M. A. PENZO[1];
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Abstract: Classical models describe the midline together with the intralaminar thalamic nuclei as a non-specific relay node in an ascending reticular activating system involved in signaling arousal. One such nuclei within the midline thalamus is the paraventricular nucleus of the thalamus (PVT), which has been recently implicated in signaling cortical state and stimulus salience. However, how the neural circuits of the PVT are organized into functionally discrete domains and their precise contributions to these processes is unknown. Here we identify to classes of PVT cells, Type I and Type II, that encode different aspects of salient stimuli. Fiber photometry imaging of calcium transients from these two classes showed that while Type I neurons are sensitive to the valence of salient stimuli, Type II neurons are sensitive to stimulus salience and arousal. Because Type I and Type II neurons of the PVT are functionally distinct, we suspected that they target different downstream structures. Indeed, anterograde mapping of the projections of these two subpopulations showed segregated innervation of multiple brain regions including the amygdala, nucleus accumbens and medial prefrontal cortex (mPFC). Notably, Type II neurons of the PVT relay arousal-related information to the mPFC. Moreover, mPFC corticothalamic cells that send projections back to the PVT are similarly modulated by arousal and are involved in a cortical circuit signaling arousal. Overall, we have gathered the first evidence of distinct parallel streams of information arising from the same midline thalamic nuclei. By uncovering this functional diversity, our results challenge current conceptualizations of the PVT.

Disclosures: **C. Gao:** None. **Y. Leng:** None. **J. Ma:** None. **S. Rodrigues-Gonzalez:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** None. **M.A. Penzo:** None.

Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 420.07/Z33

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01MH112267

Title: Pupil dynamics track optimal adjustments of decision making process in rats performing tactile discrimination tasks

Authors: ***S. NARASIMHAN**, B. SCHRIVER, K. LOU, Y. LIU, Q. WANG;
Columbia Univ., New York, NY

Abstract: Under survival pressure, animals must adaptively change their behavior to optimize behavioral outcomes. Perceptual decision making is shaped by the statistical structure of cost, reward, and the sensory world. We investigated this topic by training head fixed rats to perform a Go / No-Go tactile discrimination task while systematically varying the statistics of the sensory environment. For each session, the probability of the presence of the Go stimulus (S+) was randomly set at 80%, 50% or 20%. Animals' behavioral output and pupil size were simultaneously recorded. We found that reaction time decreased as the probability of S+ increased. Impulsive licking did not change across these three paradigms. Interestingly, although Hit and false alarm (FA) rates decreased as the probability of S+ decreased, there was no significant change in perceptual sensitivity across the paradigms. However, the animals became more liberal as the probability of S+ increased, indexed by a decrease in the decision criterion, in line with the optimal adjustment of decision criterion predicted by the signal detection theory. We further used a hierarchical drift diffusion model (HDDM) to model the decision-making process in these paradigms. We found an increase in both decision bias towards Go and drift rate as well as a decrease in decision boundary for the paradigm with 80% probability of S+ presentation as compared with the other two paradigms. The task-evoked pupil responses were bigger when the probability of S+ was lower. Moreover, pupil dilation was positively correlated with the decision boundary and drift rate and negatively correlated with decision bias. Taken together, these results suggested that the animals adaptively changed their action for optimal behavioral yield in response to the changes in sensory environment, and that this adaptive adjustment in decision-making was indexed by their pupil dynamics. This project was supported by NIH R01MH112267.

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Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 420.08/Z34

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01MH112267

Title: The interplay between phasic pupil-linked arousal evoked by different cognitive processing components in perceptual decision-making

Authors: *B. J. SCHRIVER[1], Q. WANG[2];
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Abstract: Even within an unchanging, fully learned environment, skilled decision makers execute different actions upon encountering identical stimuli. This behavioral variability may be better accounted for by looking at fluctuating behavioral state, which influences sensory processing, perception, and behavior. In perceptual discrimination tasks, the central arousal system is activated in response to stimulus presentation, decision-making, and reward/punishment, evidenced by phasic pupil dilation associated with these processes. We have previously demonstrated that phasic pupil-linked arousal (i.e. phasic pupil dilation) was activated on trials with all four possible behavior outcomes (i.e. hit, false alarm, correct rejection, and miss) in a tactile Go/No-Go discrimination task. However, the individual contribution of the different components of cognitive processing in eliciting phasic arousal, and their functional consequence on behavior remains poorly understood. Here we used machine learning techniques to decompose phasic pupil-linked arousal associated with different components of perceptual decision-making. We found that phasic arousal evoked by stimulus presentation was larger for the Go stimulus than the No-Go stimulus despite the two stimuli being symmetric. For each session, the separation between distributions of phasic arousal evoked by the Go and by the No-Go stimulus was predictive of perceptual performance. Phasic arousal in response to sensory processing was negatively correlated with that in response to decision-making for withheld trials but not for responded trials, suggesting that phasic arousal evoked by both sensory processing and decision-making contributed to the No-Go action. When a Go stimulus was presented, the correct response was primarily determined by the phasic arousal related to sensory processing, while the correct response to the No-Go stimulus was determined by phasic arousal elicited by both sensory processing and decision-making. Drift diffusion modelling revealed significant correlations between phasic arousal and the different decision-making processes. Taken together, these preliminary results suggest that the interplay between phasic arousal evoked by different

cognitive processing components has important functional consequences on forming the choice in perceptual decision-making tasks. The authors would like to thank Sean Perkins and Mark Churchland for their technical assistance and comments. This project was supported by NIH R01MH112267.

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Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

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Program #/Poster #: 420.09/Z35

Topic: G.01. Appetitive and Aversive Learning

Title: Lateral parabrachial neurons expressing tachykinin 1 mediate anorexia, adipsia, and escape behaviors elicited by peripheral threats

Authors: *J. ARTHURS, R. D. PALMITER;
Biochem., Univ. of Washington-HHMI, Seattle, WA

Abstract: Foraging animals must continually weigh the benefit of obtaining food with the potential risk of becoming food themselves. The ability to rapidly switch between feeding or drinking to detecting and escaping from threats is critical to survival. We have identified tachykinin 1 (Tac1)-expressing neurons in the lateral parabrachial nucleus ($Tac1^{PBN}$ neurons) as critical for mediating anorexia, adipsia, and escape behaviors in response to peripheral threats. Chemogenetic activation of $Tac1^{PBN}$ neurons profoundly suppressed the intake of both food and water; it also increased jumping on a hotplate and locomotion in response to a foot shock. Optogenetic activation of $Tac1^{PBN}$ neurons elicited jumping in the absence of a threat. Conversely, functionally inactivating $Tac1^{PBN}$ neurons with tetanus toxin blunted behavioral responses to peripheral threats. Neurons expressing calcitonin gene-related peptide (CGRP) in the external lateral PBN ($CGRP^{PBN}$ neurons) are activated by a wide variety of threats (e.g., foot shock, visceral malaise) and their activation also leads to profound anorexia and adipsia. Fluorescent in situ hybridization and genetic expression of fluorescent proteins revealed substantial overlap in expression of Tac1 and CGRP in the PBN resulting in Tac1-only, Tac1/CGRP, and CGRP-only expressing populations. Chemogenetic or optogenetic activation of the $CGRP^{PBN}$ neurons is sufficient and necessary to establish a robust conditioned taste avoidance (CTA) of a novel food; however, activation of $Tac1^{PBN}$ neurons does not support establishment of CTA, despite the overlap in expression and the fact that Tac1 and CGRP neurons project their axons to most of the same targets in the forebrain. Our next step is to explore how $Tac1^{PBN}$ neurons suppress appetite without causing a learned avoidance of the associated food.

Disclosures: **J. Arthurs:** None. **R.D. Palmiter:** None.

Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 420.10/Z36

Topic: G.03. Emotion

Support: FAPESP 2014/05432-9
FAPESP 2018/00576-3

Title: Effects of the cuneiform nuclei on fear and hunting

Authors: ***R. P. BINDI**, N. S. CANTERAS, M. A. X. DE LIMA, C. C. GUIMARÃES DE SOUZA;
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Abstract: Fear is a highly conserved emotion emerging from adaptations to the environment across species. Studies have shown that the cuneiform nucleus is involved in defensive behavior and is a sympathoexcitatory area, controlling the defensive response as well as affecting the sympathetic response. Studies with c-fos have shown an increase in activity during nonnoxious, noxious stimuli and during cat exposure. More importantly, this region is deeply connected with central regions in the defensive circuitry, considering those evidences more investigation on this region is needed. To investigate the role of the cuneiform nuclei in encoding active and passive fear-coping strategies, we developed a series of behavioral paradigms. In one the paradigms male Wistar rats age 4 months (n=5) have the possibility to hunt crickets, and the hunting behavior can be readily shifted via optogenetic stimulation. In this case, we saw a markedly increase in defensive behavior and an immediate cessation of the hunting behavior. In the second one, we were able to see the effect of muscimol inhibition (n=4) on the hunting behavior in a new environment, decreasing the latency to the first attack by half of the control group (n=4). We also saw a marked decrease in the freezing behavior towards a cat in the group that received muscimol in the cuneiform during the cat exposure. When the inactivation occurred in the same place they were exposed to the cat (without having the cuneiform inactivated during the predator exposure) the rats exhibited a decrease in risk assessment. With this data it's possible to identify the cuneiform nuclei as a key region to an adaptive fear response and directly affecting the behavioral inhibition system, opening new frontiers to explore and expand the fear related circuitry.

Disclosures: **R.P. Bindi:** None. **N.S. Canteras:** None. **M.A.X. de Lima:** None. **C.C. Guimarães de Souza:** None.

Poster

420. Thalamic and Brainstem Circuits

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 420.11/Z37

Topic: G.01. Appetitive and Aversive Learning

Support: Australia Research Council (DP160100004)
UNSW Postgraduate Award

Title: Paraventricular thalamus controls behaviour during motivational conflict

Authors: *E. CHOI, P. JEAN-RICHARD-DIT-BRESSEL, C. CLIFFORD, G. MCNALLY; Psychology, Univ. of New South Wales - Kensington Campus, Sydney, Australia

Abstract: Decision-making often involves motivational conflict due to the competing demands of approach and avoidance for a common resource: behavior. This conflict must be resolved as a necessary precursor for adaptive behavior. Here we show a role for the paraventricular thalamus (PVT) in behavioral control during motivational conflict. We used Pavlovian counterconditioning in male rats to establish a conditioned stimulus (CS) as a signal for reward (or danger) and then transformed the same CS into a signal for danger (or reward). After such training, the CS controls conflicting appetitive and aversive behaviors. To assess PVT involvement in conflict, we injected an adeno-associated virus (AAV) expressing the genetically encoded Ca^{2+} indicator GCaMP and used fibre photometry to record population PVT Ca^{2+} signals. We show distinct profiles of responsivity across the anterior - posterior axis of PVT during conflict, including an ordinal relationship between posterior PVT CS responses and behavior strength. To study the causal role of PVT in behavioral control during conflict, we injected AAV expressing the inhibitory hM4Di DREADD and determined the effects of chemogenetic PVT inhibition on behavior. We show that chemogenetic inhibition across the anterior - posterior axis of the PVT, but not anterior or posterior PVT alone, disrupts arbitration between appetitive and aversive behaviors when they are in conflict but has no effect when these behaviors are assessed in isolation. Together, our findings identify PVT as central to behavioral control during motivational conflict.

Disclosures: E. Choi: None. **P. Jean-Richard-dit-Bressel:** None. **C. Clifford:** None. **G. McNally:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.01/Z38

Topic: H.02. Human Cognition and Behavior

Title: Dynamic factors in neuro-evolution

Authors: *D. B. ROSENFIELD[1], I. A. DUBOVOY[2];
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Abstract: We **hypothesize** that Emergent Properties in Complex Systems (EPCS) are a driving force in evolution. Salient evolutionary models must incorporate EPCS, including genetics and levels of motor performance.
We contend that neurophysiologic structures forerun the changes seen in evolution. Cerebral interactions in any task are associated with: anatomic loops (e.g., all possible anatomic connections); functional control loops (e.g., all possible anatomic loops that relate to performance); and performance loops (e.g., loops related to the overall problem posed). Modeling these depends upon the task being investigated (many routes can lead to the same solution) but can involve emergent interactions at all organizational levels.
A reductionist perspective can assess these multiple levels of organization, whether studying molecules, the genome, phenotypes, motor interactions or any functional property of the evolving organism. One can apply a reductionist approach top-down (e.g., from psychology to cells to chemistry to physics) or bottom-up (e.g., from physics to chemistry to cells to psychology).
Each approach has limitations: the top-down approach usually describes fewer variables, but it lacks a unique model of the observed behavior produced by the components at that level. Only the bottom-up approach provides such a model, by calculating the behavior of a level from its structure, function, connectivity and current conditions of its component parts. This calculation requires simultaneous measurements of current conditions and understanding input-output properties of the component parts, often not observable at most levels in biologic systems. Whether one measures a model from a top-down or bottom-up perspective, if the evolutionary outcome changes and a new function enhances survival, its connection to the brain must already exist so that the brain can integrate the phenotypic change into established loops.
Thus, when the thumb appears, we contend that the brain already has EPCS emanating from its associated anatomic and functional loops that incorporate how the animal can and will use apposition to make tools. Similarly, we contend that EPCS at a genetic level, interacting with a complex environment and multi-organism activities, produce changes that enhance survival: it is

not random changes in the genome/alleles that provide variation but, rather, the pre-existing EP waiting to manifest.

Disclosures: D.B. Rosenfield: None. **I.A. Dubovoy:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.02/Z39

Topic: H.02. Human Cognition and Behavior

Title: EEG correlates of reward and effort processing during reinforcement learning

Authors: *D. PALIDIS[1], P. L. GRIBBLE[2];
[1]Univ. of Western Ontario, London, ON, Canada; [2]Brain and Mind Inst., Western University, Canada, London, ON, Canada

Abstract: Human behavior tends to maximize reward and minimize physical effort or energy expenditure. We ask whether neural reinforcement learning processes can support adaptive effort minimization as well as reward maximization. A frontocentral event related potential called the feedback related negativity (FRN) encodes reward prediction error and is thought to be generated by a reinforcement learning process. We hypothesized that the FRN would be modulated not only by reward outcomes but also physical effort. We recorded EEG from human participants while they performed a task in which they were required to accurately produce specific levels of muscle activation to receive rewards. Participants performed isometric knee extensions while quadriceps muscle activation was recorded using EMG. Real-time feedback indicating the combined muscle activation for both legs as a proportion of the target activation was displayed on a computer monitor. On a given trial, the target muscle activation was either “low” (approx. 15% MVC) or “high” (85% MVC). The required effort was determined probabilistically according to a binary choice indicated by button press, such that the ‘correct’ and ‘incorrect’ responses were associated with 20% and 80% probability of high effort, respectively. Periodically the effort contingency was reversed. Participants were told that in order receive a small monetary reward, they must exceed a minimum level of muscle activation indicated by a visual target while remaining as close as possible to the target. Once muscle activation reached the required target level, feedback displaying the magnitude of muscle activation was withheld, so that participants could not see the size of their errors relative to the target. After each trial, binary reinforcement feedback was provided to indicate success or failure. Reward frequency was controlled at approximately 0.5 in both conditions. We found that participants switched responses more frequently after non-reward relative to reward, and more frequently after high effort relative to low effort trials. We observed an interaction between reward and effort; reward affected response switching in the high effort condition but not the low effort condition. Effort

feedback did not elicit an FRN potential. The FRN elicited by reinforcement feedback was not affected by effort, while a late positive potential was sensitive to reinforcement outcome in the high effort condition only. Our results suggest that while the FRN reflects processing only of reinforcement outcomes, a later ERP component reflects an integration between reward and effort that is mirrored in behavior.

Disclosures: D. Palidis: None. **P.L. Gribble:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.03/Z40

Topic: H.02. Human Cognition and Behavior

Support: CONICET
INECO Foundation
FONDECYT regular 1170010

Title: Social learning deficits in fronto-temporal dementia

Authors: *S. ABREVAYA, M. GONZÁLEZ GADEA, S. FITTIPALDI, S. ALARCO MARTÍ, A. GARCÍA, F. MANES, L. SEDEÑO, A. IBAÑEZ;
Lab. of Exptl. Psychology and Neurosci., Inst. of Cognitive and Translational Neurosci., Ciudad Autónoma de Buenos Aires, Argentina

Abstract: Previous studies have shown that social reinforcement is a potential facilitator of human learning. This integration between memories and social contextual information, named social learning, is critical to maintain and promote interpersonal interactions and bonds. A relevant lesion model to explore this phenomenon is afforded by the behavioral variant of the frontotemporal dementia (bvFTD), which is characterized by a relative spared episodic memory profile alongside insidious changes in behavior, decline in social and emotional conduct, and deficits in the integration of contextual information. Yet, there is scant evidence on how social cues modulate the behavioral and neural correlates of learning in this disease. To bridge this gap, we obtained high-density EEG measures from 14 bvFTD patients and 27 demographically matched healthy controls as they performed a task that tracks the effects of social and non-social reinforcers during the implicit learning of arbitrary associations between letters and numbers. We analyzed two relevant event-related potentials: the N170, which reflects early perceptual processes involving the structural encoding of faces; and the feedback error-related negativity (fERN), which is modulated after high-conflict responses during decision-making and encodes social rejection and explicit social expectancy violations. We also administered the mini-Social cognition & Emotional Assessment to control for the effects of basic emotional recognition.

Results showed that social reinforcement significantly improved learning and increased fERN modulation in controls, but no such patterns were observed in the bvFTD group. Also, the groups did not differ in their capacity to recognize basic emotions and N170 modulations showed no disparities between conditions or groups. Taken together, these results suggest that bvFTD may involve specific alterations in the integration of social information to reinforce learning, and that this impairment is not secondary to primary deficits in processing of facial emotional stimuli and basic emotional recognition.

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Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.04/Z41

Topic: H.02. Human Cognition and Behavior

Support: DFG Fellowship TE 1289/1-1
R01 DC 015426
Intramural Research Program at NIDA

Title: A pavlovian over-expectation task to investigate behavior and learning based on imagined outcomes

Authors: *J. TEGELBECKERS[1], G. SCHOENBAUM[3], T. KAHNT[1,2];
[1]Neurol., Northwestern Univ., Chicago, IL; [2]Psychology, Northwestern Univ., Evanston, IL;
[3]Natl. Inst. on Drug Abuse Intramural Res. Program, Baltimore, MD

Abstract: Expectations that guide decisions are typically based on direct experience with action- or stimulus-outcome associations. In novel situations, however, forming expectations requires imagination. The ability to mentally simulate future outcomes but also to learn from violations of these expectations is critical for outcome-guided behavior. In rats, these functions have been linked to the orbitofrontal cortex (OFC). Evidence for a specific role of the human OFC in behavior and learning based on imagined outcomes has, however, not yet been established. To close this gap, we developed a Pavlovian over-expectation task that closely matches tasks previously used in rats. Instead of food pellets, we used food odors as biologically meaningful outcomes in hungry subjects. During a *conditioning phase*, participants (N=9) first learned to associate visual and auditory cues with food odors (A, B, X) or no odor (C, D, Y). Then, during a *compound training phase*, some cues were presented simultaneously (AX and CY) while others continued to be presented in isolation (B, X, D, and Y). The outcome delivered for all cues remained the same as during conditioning. During a final *test phase* performed in extinction,

cues A, B, C, and D were again presented in isolation. On each trial subjects reported the expected outcome via button press. We measured accuracy of outcome predictions, response time, and breathing. Over-expectation as a form of mental simulation of future outcomes was evident in this initial work during the *compound phase*, when compound cue AX led to summation (i.e., significant stronger responding to AX compared to B). In addition, during the *probe test*, responding to A compared to B was nominally reduced, providing evidence for error-based learning (i.e., extinction) based on over-expectation during the compound phase. These results parallel findings in rats using the same task, indicating that we created a valid tool that will allow us to investigate OFC representations of simulated outcomes in humans using functional imaging (data collection ongoing).

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Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.05/Z42

Topic: H.02. Human Cognition and Behavior

Support: The project was funded by the federal state Saxony-Anhalt and the European Structural and Investment Funds (ESF, 2014-2020), project number ZS/2016/08/80645.

Title: Cognitive training based on EEG-neurofeedback to improve working memory in healthy volunteers

Authors: *B. BARBAZZENI[1], E. DÜZEL[3], O. SPECK[2];
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Abstract: Working memory (WM) processes have been associated with brain oscillatory activity, attention and motivation to learn while encoding new stimuli. We investigated whether providing individuals online feedback about their ongoing brain oscillatory activity over several sessions can improve working memory performance. We focused on brain oscillations that are associated with the anticipation of monetary reward for correct performance in a working memory trial. The anticipation of monetary reward has been correlated with increased alpha suppression. Thus, to improve WM in healthy volunteers, we combined working memory training with EEG-Neurofeedback (EEG-NF) to enhance alpha suppression in a monetary-rewarded delayed match-to-sample task (DMST). Individuals were trained over five-days in

double-blinded experiment. We investigated i) whether alpha suppression enhancement increases attention and whether monetary-reward increases motivation to learn while performing the DMST and ii) whether EEG-NF training facilitates the performance of different cognitive tasks, as well. In Reward and No-Reward conditions, participants were trained to suppress alpha receiving a real-time NF or a placebo control NF of their brain activity. Preliminary results show that while performing the DMST, as well as different cognitive tasks the accuracy level did not reach a significant difference when comparing training days, reward conditions and intervention type, although a trend for improvement appeared from the first to the last training day in both interventions and reward conditions. On the other hand, reaction times were significantly improved by real-time NF in Reward ($P = 0.006$) and No-Reward ($P = 0.001$) conditions. Moreover, real-time NF enhanced significantly alpha suppression when compared to placebo control NF.

Disclosures: **B. Barbazzeni:** None. **E. Düzel:** None. **O. Speck:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.06/AA1

Topic: H.02. Human Cognition and Behavior

Support: NIMH R01 MH063901

Title: The striatal feedback response reflects action updating

Authors: *I. C. BALLARD[1], M. DESPOSITO[2];
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Abstract: Decades of neuropsychology and neuroanatomical research has converged on the theory that the striatum is a *gate*: it selects between potential action or goal representations in cortex. In contrast, fMRI investigations often characterizes the striatal BOLD response as a reward prediction error signal arising from midbrain dopaminergic inputs. When a prediction error occurs, it is necessary to update one's goals and gate a new plan of action. We test whether apparent reward prediction error BOLD responses in the striatum are better described as *action updating* responses, and reflective of gating functions rather than the activity of dopaminergic inputs.
Each subject completed two different 2-arm bandit tasks: *learn* and *reversal*. The purpose of the *learn* task was to replicate the finding that striatal BOLD correlates with reward prediction error. However, in this task, *reward prediction error* is confounded with *action updating*. The purpose of the reversal task was to distinguish the predictions of *reward prediction error* and *action*

updating and measure which signal best tracks striatal BOLD.
In the *learn* task, subjects were told that the reward probability of each bandit changes over time and they must learn which arm is better. The *reward prediction error* hypothesis predicts that the striatal response is larger for positive than negative feedback. The *action updating* hypothesis predicts the same pattern: positive feedback tends reinforces the belief that the chosen arm is the good arm, whereas negative feedback reduces confidence in which arm is best.
In the *reversal* task, subjects were told that the good arm gives reward 100% of the time, but periodically the identity of the good arm reverses. Thus, when a subject earns negative feedback, she should switch arms. The *reward prediction error* hypothesis predicts a larger response to positive than negative feedback. In contrast, the *action updating* hypothesis predicts a larger response to negative feedback, because negative feedback indicates the need to gate a new goal.
In the *learn* task, the striatal signal tracked *reward prediction error,* consistent with both hypotheses. Critically, in the *reversal* task, the striatal signal was larger for negative than positive feedback, consistent with *action updating* and inconsistent with *reward prediction error*. That is, making negative feedback more informative increases the striatal response to negative feedback. This finding suggests that the striatal feedback response is related to changes in action policy, consistent with the gating theory of striatal function.

Disclosures: I.C. Ballard: None. **M. Desposito:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

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Program #/Poster #: 421.07/AA2

Topic: H.02. Human Cognition and Behavior

Support: Helse Nord Grant PFP1237-15

Title: Interacting effects of experimentally-induced helplessness and transcranial direct current stimulation on Pavlovian bias in action selection

Authors: *G. CSIFCSÁK[1], J. BJØRKØY[1], S. KUYATEH[1], H. REITHE[1], V. D. DALOS[2], L. RÓNAI[2], S. SZALÓKI[2], M. MITTNER[1];
[1]Dept. of Psychology, UiT The Arctic Univ. of Norway, Tromsø, Norway; [2]Dept. of Psychology, Univ. of Szeged, Szeged, Hungary

Abstract: Learned helplessness (LH) is a concept closely related to major depression and chronic pain. In our previous study (https://psyarxiv.com/jpq6f/), we induced a state resembling LH in healthy adults by manipulating control over action outcomes (“yoking”) in a reinforcement learning task. We found that yoking was associated with stronger Pavlovian influences over action selection (i.e., enhanced approach tendencies towards rewards and

behavioral inhibition when facing loss), accompanied by alterations in frontal EEG signals reflecting cognitive control and outcome evaluation. These results implicate the involvement of the medial prefrontal cortex (mPFC) in LH-induced modulations of task performance. In the current, pre-registered study (https://osf.io/h45ju/), we aimed at determining if transcranial direct current stimulation (tDCS) targeting the mPFC can reverse the behavioral manifestations of yoking in healthy adults. In a double-blind, sham-controlled study, we randomly assigned 154 healthy adults into one of our six experimental groups: 3 tDCS conditions (active tDCS, sham tDCS, natural history) x 2 LH conditions (yoked, control). Participants performed an orthogonalized Go/NoGo task whereby the yoked group faced the absence of control over outcomes in the first part of the task. Behavioral effects of tDCS and yoking were assessed by analyzing between-group differences in response accuracy and Pavlovian performance bias (PPB), a measure estimating the impact of the Pavlovian valuation system on instrumental choices. For response accuracy, we found worse performance for yoked participants during LH-induction ($F_{1,584}$=12.78, $p<0.001$), but this effect was not modulated by tDCS ($F<2.86$, $p>0.058$). For PPB, however, we found a significant interaction between tDCS type and LH ($F_{2,584}$=3.55, $p=0.029$), indicating weaker Pavlovian bias for LH induction only if yoking was combined with active tDCS ($p<0.001$). The magnitude of the effect was similar during and after the stimulation. Our finding suggests that tDCS above the mPFC enhances cognitive control over Pavlovian bias in action selection, but only in cases when mPFC activity is compromised by LH-induction. We interpret these effects by proposing an inverted U-shape relationship between mPFC activity and cognitive control over Pavlovian action tendencies.

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Poster

421. Human Learning: Feedback, Reinforcement, and Reward

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Topic: H.02. Human Cognition and Behavior

Support: Research Program, University Medical Center, University of Goettingen
Research Fellowship by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)–project number (392135994) awarded to ZT

Title: Expectation effects induced by transcranial electrical stimulation: Model-based vs. model-free components

Authors: *E. BJØRKEDAL[1], Z. TURI[2], C. J. JAKOBSEN[1], M. MITTNER[1];
[1]Psychology, UiT The Arctic Univ. of Norway, Tromsø, Norway; [2]Clin. Neurophysiol., Univ. Med. Ctr. Goettingen, Goettingen, Germany

Abstract: The effect of transcranial electrical stimulation (tES) on cognition show great variability between subjects and across studies. Some of the variability could be related to placebo effects of tES, but the placebo effect on cognition or of tES has not been well investigated. We have shown that sham tES induced placebo effects on objective performance and learning rate during reinforcement learning. In the present study, we sought to determine if the placebo effect on objective cognitive performance was associated with increases in model-based versus model-free control of learning. Healthy participants (n=100) did a two-step reinforcement learning task that allowed us to distinguish between model-free and model-based control. The goal of the task was to maximize rewards by choosing, at the first step, among pairs of kiosks that, at the second step, led to lottery tickets that had values determined according to a random walk. The value of the kiosks could be updated either through model-free or through model-based learning. We used computational modelling to model learning performance. Outcome measures were objective performance (amount earned), expectations, learning rate, exploration, and trade off between model-free vs model-based control. Subjects were randomized to three groups who performed the learning task 3 times. The placebo conditioning group received double-blind sham tACS before 2nd and 3rd task with information that it would increase performance. The 2nd task was surreptitiously made easier in order to reinforce the verbal information with experience. The placebo group received identical tACS protocol but no task manipulation. The natural history group simply performed the learning task 3 times. Our preliminary analyses showed that objective performance and model-based control increased in all groups over time, with no differences between the groups. Sham tACS increased expectations of cognitive performance, but we observed no placebo effect on objective cognitive performance or model-based control. However, compared to the natural history group, the placebo manipulation induced changes in the modelled parameters of learning performance. These results suggest that expectations can affect modelled parameters of reinforcement learning in the kind of task used here.

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Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.09/AA4

Topic: H.02. Human Cognition and Behavior

Support: Brain Research UK PhD Studentship
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MRC Clinician Scientist Fellowship

Title: Musical reinforcement learning paradigm in frontotemporal dementia: A model paradigm of abnormal reward prediction

Authors: *E. BENHAMOU, H. SIVASATHIASEELAN, C. HARDY, M.-C. REQUENA-KOMURO, J. JOHNSON, C. GREAVES, L. RUSSELL, K. MOORE, J. ROHRER, J. WARREN;
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Abstract: Accurate prediction of the emotional consequences of one's actions, seeking reward, avoiding punishment and learning from past experience are integral to normal goal-directed behaviour. These processes are targeted early and prominently in the behavioural variant presentation of frontotemporal dementia (bvFTD): these patients show misdirected reward valuation, inability to regulate goal-directed actions and failure to learn from aversive experience. bvFTD constitutes a unique human disease model of abnormal reward processing and impaired socio-emotional behaviour more generally. However, the deficits these patients develop are difficult to characterise experimentally.
Here we used music as a novel probe of reinforcement learning in bvFTD, capitalising on the frequent abnormalities of musical hedonic valuation exhibited by patients with bvFTD and the well codified potential of musical consonance and dissonance to change the pleasantness of musical stimuli. We hypothesised that, relative to healthy age-matched individuals (n=15), patients with bvFTD (n=15) would display impaired behavioural flexibility during learning in an uncertain musical environment. We designed a three-armed bandit reinforcement learning paradigm using musical stimuli based on: (Experiment 1) four-chord progressions in which the final chord delivered either an expected or unexpected/ rewarding (consonant) or aversive (dissonant) outcome; or (Experiment 2), string quartet samples previously rated as pleasant or unpleasant by healthy older British individuals (n=10). Pupillary responses were recorded simultaneously throughout.
Modelling the data computationally, we found that bvFTD was associated with impaired behavioural detection of the 'optimal' arm and attenuated pupil reactivity to both 'punishing' target chords (Experiment 1) and unpleasant naturalistic music excerpts (Experiment 2), on a trial-by-trial basis. In Experiment 2, the choice stochasticity parameter was significantly higher for the bvFTD group, signifying a failure to match one's choices to the stimuli value difference. Our findings reveal a specific impairment of reward prediction in uncertain environments in bvFTD that might shape the abnormal socio-emotional phenotype of this disorder.

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Poster

421. Human Learning: Feedback, Reinforcement, and Reward

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Program #/Poster #: 421.10/AA5

Topic: H.02. Human Cognition and Behavior

Support: Samuel F. Hulbert Chair endowment

Title: Selective attention and the effects of mismatched priming factors on the evoked response potentials of mental arithmetic processes

Authors: D. H. HUGHES, T. MILLARD, *A. W. CHIU;
Biomed. Engin., Rose-Hulman Inst. of Technol., Terre Haute, IN

Abstract: In this study, we investigated the human evoked-response potentials (ERPs) to incongruences during mental arithmetic processing (MAP), and mismatches in the expected sensory modes. Each human subject, between the age of 18-55, was given 360 simple math problems. During the presentation phase (PP), the problems were presented in one of two possible ways, selected randomly with 50% visually via a computer screen and 50% auditory through the speakers. The PP was followed by a brief pause during which the subjects were instructed to solve the math problems mentally and keep track of the correct solutions. Then, the subjects were presented with two numbers visually and auditorily, during the response phase (RP). The subjects were asked to press a clicker whenever they saw or heard an incorrect solution. The ERP would be labeled as the standard response (S) if the numbers presented both ways were correct. The target response (T) would be produced if one of the numbers was incorrect, and presented in the same mode as the PP stimulus. Finally, the distractor response (D) would be produced if one of the numbers was incorrect but presented in a mismatched mode from the PP stimulus. Consistent with our previous work, the preliminary results showed an increase in the latency and average peak amplitude for T when compared to S. Despite some subject-to-subject variability, the ERPs in the parietal cortical regions exhibited longer D-S delay ($p<<0.05$) when transitioning from a visual PP to an incongruent auditory RP, than from an auditory PP to an incongruent visual RP. In both situations, no significant increase in the peak ERP D/S ratio was found. Even though subjects were not explicitly instructed to focus on a particular sensory mode during the RP, delayed responses to the solution in the RP were observed when there was incongruence in the math solution, with a statistically significant longer D-T delay if they were auditory mismatched responses ($p<<0.01$). There was no statistical difference in the power of the theta and alpha rhythms between the distractor and target responses regardless of the mode of simulation, suggesting that fatigue was not a contributing factor to these observations.

Disclosures: D.H. Hughes: None. **T. Millard:** None. **A.W. Chiu:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.11/AA6

Topic: H.02. Human Cognition and Behavior

Title: The contribution of dopamine reward prediction errors to temporal representation and motor control

Authors: *W. H. ALEXANDER;
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Abstract: By far the most prominent interpretation of midbrain dopaminergic function suggests that dopamine neurons encode reward prediction errors (RPEs) that serve as the fundamental signal for learning in reinforcement learning frameworks. A vast body of literature has documented the relationship between activity in dopamine neurons and RPEs derived from computational models of learning, making it one of the earliest and strongest links between computational and empirical neuroscience. Despite the amount of evidence supporting dopaminergic signaling of RPEs, two critical deficits in this interpretation remain. First, while computational models of reinforcement learning draw a strong association between dopamine and learning, there is yet to be a similarly strong account for the established role of dopamine in motor control and temporal perception. Second, although dopamine activity is necessary in reinforcement learning frameworks to drive learning, evidence from Parkinson's patients suggests that dopamine signals themselves are not necessary to learn reward associations, but rather to express those associations behaviorally. In this poster I present a new computational model of dopamine function showing how RPEs signaled by dopamine neurons may be used to adjust the temporal receptive fields of neurons in target regions of striatum and cortex. Simulations of the model account for the development of spectral timing units in striatum, effects of temporal delay in choice behavior, and deficits in motor control and temporal estimation in Parkinson's disease. These results suggest that the primary role of dopamine RPEs is to adjust attention to features and time rather than to learn reward associations.

Disclosures: W.H. Alexander: None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.12/AA7

Topic: H.02. Human Cognition and Behavior

Support: CONACYT 894498

Title: Reinforcer and punishment during decision making: An electroencephalographic study

Authors: *F. A. IRIBE BURGOS, J. P. GARCÍA-HERNÁNDEZ, P. M. CORTES ESPARZA, C. SOTELO-TAPIA, M. A. GUEVARA, M. HERNÁNDEZ-GONZÁLEZ;
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Abstract: Decision-making (DM) is defined as the selection of an option from a range of existing alternatives by estimating the possible outcomes of the selection and its possible consequences for present and future behavior. These consequences (reinforcement, pleasant stimulus; or punishment, a non-attractive or aversive environmental object) are capable of modifying an individual's preferences and responses. The dorsolateral, prefrontal, parietal and temporal cortices have been shown to be involved in the processing and evaluating such stimuli, and the connectivity among them allows these cortical areas, with other brain circuits, to complete the perception-action process of DM. Hence, the objective of this study was to determine the electroencephalographic (EEG) differences under reinforcement (*i.e.*, decreased task execution time) and punishment (increased task execution time) on a DM task. Sixteen healthy, right-handed men aged 20-35 volunteered to participate. Electroencephalographic activity was recorded in the dorsolateral prefrontal (F3-F4), parietal (P3-P4), and temporal (T3-T4) cortices during a DM task delivered with reinforcement or punishment. Absolute power (AP) and interhemispheric (rINTER: F3-F4, P3-P4, and T3-T4) and intrahemispheric (rINTRA: F3-P3, F3-T3, P3-T3, F4-P4, F4-T4, and P4-T4) EEG correlations were analyzed for each EEG band: theta (4-7 Hz), alpha1 (8-10 Hz), alpha2 (11-13 Hz), beta1 (14-19 Hz), beta2 (20-30 Hz) and gamma (31-50 Hz). During the reinforcement condition, subjects had higher AP in theta at F3 and T3, and at P3 in gamma compared to the punishment condition. Also during reinforcement, a higher rINTRA between F3-T3 was observed in the theta, beta2 and gamma bands, between P3-T3 in alpha1, and between F4-P4 in gamma. A higher rINTER between P3-P4 was seen in beta1 during reinforcement, while under the punishment condition a higher rINTER was found for alpha1 between T3-T4. These data show that both the functionality and degree of cortical EEG coupling vary during DM according to the type of consequence received after each election. The high rINTER in alpha1 between temporal areas could be associated with greater emotional regulation during punishment, while the high rINTRA among F3-T3-P3 in the

fast bands could be related to the decoding and processing of the reinforcing stimulus, as well as to the processing and maintenance of the association between stimulus and response.

Disclosures: F.A. Iribe Burgos: None. **J.P. García-Hernández:** None. **P.M. Cortes Esparza:** None. **C. Sotelo-Tapia:** None. **M.A. Guevara:** None. **M. Hernández-González:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.13/AA8

Topic: H.02. Human Cognition and Behavior

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NSF Graduate Research Fellowship

Title: Bioinspired few-shot learning in robotic systems

Authors: *A. MARJANINEJAD[1], D. URBINA-MELÉNDEZ[1], B. A. COHN[2], F. J. VALERO-CUEVAS[1];
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Abstract: The vast majority of robotic systems in use today rely on hard-coded lookup tables and prescribed trajectories, amounting to little more than error correction. In the context of simulation, some robots are able to 'dream' of new tasks, and apply what they've learned from simulations in the real world. However, animals (or any physical system in general) do not have the convenience of excessive trial and error processes or unlimited simulations to learn how to move, manipulate, and navigate. As commonly used control algorithms sacrifice versatility, robustness, and generality across a wider set of tasks in favor of precision in just a few, what insights from biology can be brought to robotics? We present a bioinspired hierarchical learning approach [1] which, for the first time, demonstrates autonomous learning of different movement patterns in a bio-plausible tendon driven limb. In spite of the limb being an under- and over-determined control problem at the same time [2], it takes only a few minutes of interaction with the environment for our control system to achieve a challenging precision task. This bio-inspired learning approach (both in learning and physical implementation) enable our system to learn how to control its motor activation patterns to propel a passive treadmill in as short as about 10 minutes on average and led to the formation of 'movement habits/personalities" across

independent runs. This line of research (as well as a comparison to traditional methods such as genetic algorithms [3]) can revolutionize the utility of modern robots, as they could functionally resemble vertebrates in both anatomy and physiology (including learning and decision making). Our work sets forth a path for robots to interact with humans and other elements in the environment to learn to act properly in unforeseen scenarios [1]. The interplay of self-learning robotics and soft-body physics may ultimately uncover new insights in the fields of biomechanics and neuroscience and we discuss the implications of such research.
[1] marjaninejad et. al., 2019, nature machine intelligence; [2] marjaninejad and Valero-cuevas, 2019, Biomechanics of Anthropomorphic Systems; [3] marjaninejad et. al. IEEE EMBC 2018

Disclosures: A. Marjaninejad: None. **D. Urbina-Meléndez:** None. **B.A. Cohn:** None. **F.J. Valero-Cuevas:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.14/AA9

Topic: H.02. Human Cognition and Behavior

Support: Templeton Science of Virtue Award

Title: The development of curiosity across adolescence

Authors: *E. TEDESCHI, L. OUYANG, A. RATHE, C. VAN GEEN, C. MARVIN, N. L. TOTTENHAM, D. SHOHAMY;
Columbia Univ., New York, NY

Abstract: Curiosity is a fundamental aspect of learning, but there are open questions regarding the cognitive and neural mechanisms linking curiosity, learning and memory. Previous research has suggested that curiosity may leverage basic reward mechanisms, where information is the reward, and curiosity is the drive to obtain it. This framework makes predictions about how curiosity might change with age. Reward sensitivity is high in children and adolescents, and adolescents have been shown to be better at learning from reinforcement than adults. Anecdotally, curiosity is often linked with youth, and this framework would predict higher curiosity in younger individuals compared to older ones, along with higher curiosity-induced memory for information. We tested these predictions by measuring curiosity and curiosity-related memory in participants ranging from 10 to 30 years old (N=54). Participants completed three tasks measuring curiosity-related behavior. In the first task (Task 1), they read trivia questions and chose to wait for the answer or skip to the next question, with willingness-to-wait as the measure of curiosity. They then read a second set of trivia questions (Task 2) and self-reported their curiosity before receiving the answers. The final task (Task 3) was a visual free

exploration task, which used gaze-contingent eye tracking to allow them to slowly reveal hidden images. The amount each image was revealed was used as a measure of curiosity. Finally, they completed a surprise memory test for the answers to the trivia questions to test the effect of curiosity on memory. Contrary to the predictions, none of the curiosity tasks showed significant age-related declines. Instead, there were trends in the opposite direction: older subjects were more willing to wait for answers in Task 1, had higher average curiosity ratings in Task 2, and explored more in Task 3. On the memory test, older subjects remembered significantly more answers than younger subjects. For all ages, higher curiosity ratings predicted better memory, but there was a significant interaction with age where the effect of curiosity on memory was shallower for younger participants compared to older ones. Overall, curiosity was not higher for adolescents than for adults, as would be predicted by the information-as-reward framework. Furthermore, while memory increased for high-curiosity questions, this effect was blunted in the younger ages, and younger participants remembered fewer answers overall. Further investigation into the development of the relationship between curiosity and learning during adolescence may shed light on the mechanisms underlying curiosity across the lifespan.

Disclosures: **E. Tedeschi:** None. **L. Ouyang:** None. **A. Rathe:** None. **C. Van Geen:** None. **C. Marvin:** None. **N.L. Tottenham:** None. **D. Shohamy:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.15/AA10

Topic: H.02. Human Cognition and Behavior

Support: MOST 108-2634-F-002-022

Title: Towards a unified computational model of reinforcement learning

Authors: Y.-H. SU[1], C.-Y. CHANG[1], *T.-R. HUANG[1,2];
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Abstract: A computational model of reinforcement learning has its potential to quantitatively summarize and explain a large array of behavioral phenomena using only a few model parameters. Furthermore, computational modeling of behaviors has benefited model-based neuroimaging analysis to reveal specific neural substrates and their dynamics involved in reinforcement learning paradigms. However, diversities of model formulations and assumptions have led to conflicting results in attributions of model parameters to corresponding behavioral phenomena among prior literature, which urges a unified model to reconcile existing conflicts within/across paradigms and populations in a coherent framework. Without a model thoroughly

examined by its parsimony and generalizability, the inferred neural substrates from neuroimaging analysis based on arbitrary model candidates could also be misled. On the way towards a unified model, we propose that a systematic sampling approach is more favorable than conventional model-fitting techniques for directly examining parameter-dependent behaviors. Starting from a classic probabilistic selection task (Frank, Seeberger, & O'Reilly, 2004, *Science*) and a basic model having learning rates (for reward and punishment respectively: $0<LRr$, $LRp<1$) and behavioral consistency (formalized by temperature T: $0<T<2$) as model parameters, we demonstrate the power of systematic sampling by not only *replicating* impaired Go learning that have been observed in patient populations, but also *predicting* an unexpected region of impaired NoGo learning in the model parameter space that characterizes high behavioral consistency. In other words, human individuals with low behavioral consistency (i.e., high T) might perform better at avoiding punishments than human individuals with high behavioral consistency (i.e., low T). Moreover, our results imply that only by systematic sampling the qualitative, emergent behavioral changes due to quantitative changes of model parameters could be revealed, and those behavior changes could further serve as a model testifier in future studies. By extending systematic sampling to simulate more experimental paradigms and predicting more emergent behaviors that can be empirically tested, we are paving the way for a global model that not only could deepen and widen our understandings of reinforcement learning at a behavioral level, but also could benefit model-based neuroimaging analysis for investigating reinforcement learning in our brains.

Disclosures: **Y. Su:** None. **C. Chang:** None. **T. Huang:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.16/AA11

Topic: H.02. Human Cognition and Behavior

Title: Neural mechanisms underlying human reinforcement learning in a continuous choice space

Authors: *J. LEE, S. KIM;
Inst. of Basic Sci., Suwon, Korea, Republic of

Abstract: Computational, behavioral and neural correlates of human reinforcement learning are well understood in decision making with discrete choices, however, little has been known about more generalized decision making with continuous action space. Here, we designed an fMRI experiment in which subjects search a hidden target in a 2-dimensional space given binary feedbacks. Subjects received a monetary reward each time they selected a point close enough to a hidden target which was randomly set after 12 searches. A "reward zone" centered around a

hidden target continued to shrink only after each rewarded trial, guiding subjects to search a hidden target. We suggested two computational models accounting for individual subjects' search behavior: (1) Maximum a posterior model assuming the Bayesian update of expected reward probability with greedy selection (full expoitation) and (2) maximum information gain model suggesting a choice maximizing the reduction of uncertainty of the expected reward probability (full exploration). For the preliminary fMRI results, we found model-based reward prediction error modulated activities in the ventral striatum compatible with previous related studies and activities in the anterior hippocampus reflecting memory-guided search. We also discuss cortical substrates of arbitrating exploitation versus exploration, predicted by the suggested computational models.

Disclosures: J. Lee: None. **S. Kim:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.17/AA12

Topic: H.02. Human Cognition and Behavior

Support: Templeton Science of Virtue Award

Title: Rational predictions guide information-seeking behavior in temporally uncertain environments

Authors: *E. A. LANG, C. VAN GEEN, E. TEDESCHI, D. SHOHAMY;
Columbia Univ., New York, NY

Abstract: The motivation to acquire information is a fundamental driver of learning and memory. However, the mechanisms by which curiosity affects these constructs remain unclear. In particular, it remains unknown how and whether information-seeking behavior is sensitive to temporal features of the environment. In this experiment, we sought to determine if information seeking is modulated by estimation of uncertainty about the timing of information delivery. Prior work has shown that when seeking monetary rewards, people learn the approximate temporal statistics of their environment and use these statistics in order to maximize reward receipt (McGuire and Kable, 2012). We leveraged this same paradigm to test the extent to which the motivation to acquire information is modulated by temporal statistics of the environment. We tested 70 human participants on a behavioral paradigm in which participants could explore the answers to questions. Participants viewed trivia questions and could choose to wait for the answer, or to skip to a new question at any time. Their choices took place in two environments that differed only in the timing of when the answers were shown: in one environment the temporal distribution of answers was uniform (uniform distribution; UD), while the other utilized

a heavy-tailed distribution (HT). The UD environment was optimized by continuing to wait for the answers to appear, while the HT environment was optimized by skipping to a new question after a certain amount of time. We measured participants' choices (wait or skip), the timing of skip responses, and pupil dilation during choice and waiting epochs. Approximately 20 minutes after encoding, participants underwent a surprise subsequent memory test for the answers to the trivia questions they had been shown. Consistent with previous findings on monetary rewards, we found that participants demonstrated longer wait times in the UD than in the HT environment. However, indicating an overarching influence of curiosity regardless of environment, we also found that a question's curiosity level predicted willingness to wait in both environments. Furthermore, memory was increased for the answers to high-curiosity questions in both environments, with no difference between them. Finally, pupil size at encoding predicted later memory of the answers, potentially revealing a physiological marker of curiosity-modulated subsequent memory. Taken together, these results suggest that people encode and use temporal statistics of the environment in such a way as to maximize receipt of interesting information in temporally uncertain environments, even when that information may be functionally useless.

Disclosures: **E.A. Lang:** None. **C. Van Geen:** None. **E. Tedeschi:** None. **D. Shohamy:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.18/AA13

Topic: H.02. Human Cognition and Behavior

Support: NIH-MH081153

Title: Participants rely on both absolute and relative position when making inferences about ordered lists

Authors: *T. KAO[1,2], **C. E. MICHAELCHECK**[2], V. P. FERRERA[3], H. S. TERRACE[2], G. JENSEN[2];
[1]New York City Col. of Technology/City Univ. of New York, Brooklyn, NY; [2]Psychology, [3]Neurosci., Columbia Univ., New York, NY

Abstract: Human participants were trained to learn photographic images which belonged to 5 different 5-item ordered lists (e.g. $A_1B_1C_1D_1E_1$; $A_2B_2C_2D_2E_2$; etc) using a transitive inference (TI) paradigm in which only adjacent pairs of items in the ordered lists were presented. Participants were then tested with pairs from derived lists with maintained ordinal positions, in which only one item from one of the original 5 different 5-item ordered lists was selected to be presented with another item from another one of the original 5 different 5-item ordered lists, while maintaining their ordinal positions (e.g. $A_2B_5C_4D_1E_3$). At the start of testing, participants

exceeded chance in selecting the earlier item. This result cannot be explained by TI alone, since the pairs of items that were tested came from these lists that were not presented before. In a second experiment, a different group of participants were trained, as in the previous experiment, but were tested with derived lists with changed ordinal positions, in which only one item from one of the original 5 different 5-item ordered lists was selected to be presented with another item from another one of the original 5 different 5-item ordered lists (e.g. $A_1E_4C_5D_3B_2$), while systematically varying their ordinal positions pairs. At the start of testing, participants exceeded chance in selecting the earlier item, and did so with greater accuracy than the results from testing for first experiment. Our results demonstrate that participants make inferences using knowledge of both absolute, and relative, ordinal position when learning derived lists.

Disclosures: T. Kao: None. **C.E. Michaelcheck:** None. **V.P. Ferrera:** None. **H.S. Terrace:** None. **G. Jensen:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.19/AA14

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant 1533623

Title: Ventromedial prefrontal damage impairs model based learning

Authors: *L. Q. YU[1,2], A. FILIPOWICZ[2], M. R. NASSAR[1], J. W. KABLE[2];
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Abstract: The ventromedial prefrontal cortex and orbitofrontal cortex (vmPFC/OFC) have been thought to be important for learning the structure of the environment, especially when such structure is hidden and must be inferred from the available evidence. However, the current evidence linking the vmPFC/OFC to structure learning in humans has been mostly from correlational evidence from functional neuroimaging. Thus, it is unclear whether structure learning is critically dependent on the vmPFC/OFC in humans, and also whether the vmPFC/OFC is uniquely responsible for some forms of structure learning over other prefrontal regions. We use two tasks querying different types of structure learning on groups of individuals with ventromedial frontal (VMF) damage (N=5), individuals with other frontal damage sparing the VMF (N=7), and healthy, age and education-matched controls (N = 24). The first task asks the participants to find the hidden means of two Gaussian distributions, by integrating over the rewarded outcomes drawn from those distributions that are shown to them. The second task puts the participant in two contexts with different hazard rates, which is a higher order hidden

variable that governs the rate of switching between two reward sources. We find that in the first task, individuals with VMF damage produce responses that are significantly further away from the hidden means compared to both control groups, but their ability to respond appropriately to different hazard rate conditions in the second task is relatively less impaired. In contrast, the participants with frontal damage sparing the VMF are impaired on dissociating the two hazard rate contexts. Our data suggest that learning hidden structure is sensitive to VMF damage, and that structure learning becomes more sensitive to lateral frontal damage for higher order hidden variables.

Disclosures: L.Q. Yu: None. **A. Filipowicz:** None. **M.R. Nassar:** None. **J.W. Kable:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.20/AA15

Topic: H.02. Human Cognition and Behavior

Support: OU Vice President for Research

Title: Iron deficiency in college-aged women negatively affects neural measures of feedback and reward processing during procedural and declarative learning

Authors: *M. J. WENGER[1], S. E. RHOTEN[2], A. SAJID[2], T. L. LAWSON[2], L. A. DE STEFANO[1], L. BOOZARY[1];
[1]Psychology, Cell. & Behavioral Neurobio., [2]Psychology, The Univ. of Oklahoma, Norman, OK

Abstract: Of all the nutritional challenges in the world, iron deficiency (ID) is the most prevalent, and can be found at high rates in both developing and developed countries, particularly among infants, adolescents, and women of reproductive age. The negative impacts of ID on physical performance and work productivity are well documented, and there is accumulating evidence that ID has a range of effects on cognition and brain function, including evidence that ID in college-aged women negatively affects academic standing. Biologically, animal models of ID have indicated significant effects on dopamine, particularly D2 receptors and dopamine transporter, which are heavily involved in both learning and feedback/reward processing. The present study examined the extent to which ID affects brain activity and behavior during two types of learning. Two groups of women, one ID and not anemic (IDNA), and a matched group of iron sufficient (IS) participants learned two categorization tasks. The first was a rule-based task, which differentially relies on the medial-temporal/frontal circuits that support declarative memory. The second was an information integration task, which differentially relies on the corticobasoganglial circuits that support procedural memory. Participants performed both tasks while concurrent electroencephalographic (EEG) data was

collected, with our focus here being on three response-locked wave-forms: error-related negativity (ERN), error-related positivity (Pe), and feedback-related negativity (FRN). Stimuli were gabor patches that could vary on spatial frequency, orientation, and amplitude. Both groups of women learned both tasks successfully, with ID exerting negative effects on both reaction times and accuracy. In addition, ID women exhibited reduced amplitudes for the Pe and FRN, but not the ERN, and with differences in the FRN being attenuated with learning. In addition, amplitudes of these waveforms showed reliable relationships with both hemoglobin and serum ferritin. These results suggest that ID may negatively affect both neural signal quality (by way of effects on neurotransmitters) and brain energy expenditure (by way of oxygen transport) during cognitive work.

Disclosures: M.J. Wenger: None. **S.E. Rhoten:** None. **A. Sajid:** None. **T.L. Lawson:** None. **L.A. De Stefano:** None. **L. Boozary:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.21/AA16

Topic: H.02. Human Cognition and Behavior

Title: The effects of hypothesis generation and search patterns on curiosity

Authors: *A. HSIUNG[1], A. KHAN[2], B. NIEVES[2], K. C. DICKERSON[1], S. A. HUETTEL[1], R. ADCOCK[3];
[1]Ctr. for Cognitive Neurosci., [2]Dept. of Computer Sci., [3]Psychiatry and Behavioral Sci., Duke Univ., Durham, NC

Abstract: Curiosity, the intrinsic desire to seek new information, is a fundamental component of human cognition. Previous research has highlighted curiosity's role in facilitating memory, directing choice, and guiding exploration. Although this research has been integral in understanding how curiosity shapes behavior, the treatment of curiosity as a static state limits our ability to investigate if and how curiosity changes as the state of information accumulated also changes. Pulling from extensive research in decision neuroscience on evidence accumulation, the process of information gain can take a variety of shapes depending on the quality, clarity, and timing of incoming evidence. In order to investigate how these variables interacted with curiosity, we developed a novel paradigm that naturally and dynamically manipulates information gain as a function of time. In our task, participants (N = 262) watched videos of single continuous line drawings that slowly revealed an object over time. Participants were encouraged to report a guess about what the image was going to be as soon as they had formulated one. We found that our videos could be classified into two general information gathering shapes: one was characterized by a sigmoidal shape, where the majority of correct

identifications were concentrated in a short time window. The other was characterized by a linear shape, where correct identification slowly accumulated across the entire video duration. Importantly, our task was highly engaging (M = 8.80/10, sd = 1.67) and elicited curiosity (M = 86.9/100, sd= 7.94), confirming that our paradigm did in fact produce an intrinsic drive for information.Ongoing analyses use these stimuli to probe real-time curiosity ratings as a function of the shape of information gain, and relate these curiosity ratings to subsequent information-seeking behavior: allowing participants to control the speed of reveal for each video and the value of future information using a willingness-to-pay manipulation. Collectively these studies begin to elucidate how the process of curiosity dictates how and what we search for, lending insight into how we can foster its development to enhance learning in both educational and clinical settings for improved health and fulfillment of human potential.

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Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

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Program #/Poster #: 421.22/AA17

Topic: H.02. Human Cognition and Behavior

Support: MURI N00014-16-1-2832

Title: Overlap in stimulus-response rules is insufficient for retrieval of task set memories

Authors: *O. LOSITSKY, D. BADRE;
Brown Univ., Providence, RI

Abstract: How do we form abstract task representations that can be generalized to new contexts, and how do we update these representations when circumstances change? Recent models (Collins & Koechlin, 2012; Collins & Frank, 2016; Qian, Jaeger & Aslin, 2016) have proposed that people group together contexts into an abstract ‘task set’ when these contexts predict similar stimulus-response rules. Based on this work, we hypothesized that previously learned task sets should be retrieved in new contexts to the degree that the stimulus-response rules in the new context match those in the old context. In a series of five behavioral experiments (N=71 participants), we sought to manipulate whether two task sets would be stored within a single memory or two separate memories. In all experiments, participants learned to press one of four keys in response to fractal art images based on probabilistic feedback. Once they achieved a performance criterion, the associations between stimuli and responses changed and had to be relearned (task set B). In the last block, the S-R rules changed again either to the initial set (ABA games) or to a new set (ABC games). Importantly, all changes in task set were hidden and could

only be inferred from feedback. Experiments varied in the number of stimuli (2-4), feedback validity (80-90%), and the number of exposures to task set A prior to testing memory retrieval. In all cases, several minutes elapsed between the initial learning of task set A and its recurrence. Surprisingly, across all five experiments, we found no significant difference in learning speed (trials to criterion) or performance (accuracy or reaction times) between previously learned sets (A) and new sets (C). However, follow-up experiments revealed three conditions under which participants relearned old sets significantly better than new sets: 1) when participants noticed the temporal structure of the experiment and could predict the moment of task set A recurrence, 2) when participants were explicitly instructed about the temporal structure of the experiment and the presence of repeats, and 3) when a contextual cue (background color) uniquely identified which task set applied at a given time, i.e., when the context was visible. Our results suggest that overlap in stimulus-response rules may not be sufficient to trigger retrieval of task sets from long-term memory, and that either salient contextual cues or knowledge of the temporal sequence may be necessary for retrieval. Investigating these mechanisms further may shed light on the role of the medial temporal lobes in encoding and retrieving sets of stimulus-response associations, which has generally been attributed to fronto-striatal circuits.

Disclosures: O. Lositsky: None. **D. Badre:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.23/AA18

Topic: H.02. Human Cognition and Behavior

Support: Kavli Institute for Brain and Mind Innovative Research Grant

Title: A novel paradigm for social learning and decision-making: The social multi-armed bandit

Authors: *J. ADRIAN[1], S. SIDDHARTH[2], S. BAQUAR[2], T.-P. JUNG[2], G. DEAK[2];
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Abstract: Decision-making is a fundamental human skill. Its study involves usually only a single agent; however, most real-world decision-making is influenced by other agents acting in multifactorial environments. We validated a novel paradigm to study social decision-making: the social multi-armed bandit task. It requires two participants to alternate in choosing between different options that may or may not yield a reward. Each option is associated with a reward probability unknown to the players. Both players observe their own and their partner's choices and subsequent outcomes. They learn and over time make more informed decisions through exploration and integration of their outcomes. Reward probabilities for different options are constant throughout the game but different for each player, such that the options with the highest

reward probability for player 1 has the lowest probability for player 2, and vice versa. Participants also play a solo version of the game to assess how they use 'solipsistic' outcomes to update their decisions. Comparing performance in the solo and dyadic version reveals how people take into account not only their own outcomes but also their partner's. We tested 16 pairs of participants and recorded EEG (14 channels, at 128Hz) and pupillometry during both the solo and dyadic tasks. 50% (16/32) of participants learned which option yielded the most probably reward in the solo game, but did not learn as much in the dyadic version, receiving significantly fewer rewards ($F=47.76$, $p<0.001$). This suggests that these participants automatically integrated their partner's actions and outcomes, though it was detrimental to their overall outcomes. Event-related potentials time-locked to reward onset show qualitatively similar responses, but with attenuated amplitudes in the solo versus the dyadic game. This might indicate that arousal and attention after receiving reward are sustained when a second agent is present but not when playing alone. A similar pattern was observed in pupil response: diameter increased after receiving a reward but the magnitude of the increase was higher in the dyadic versus the solo task. Ongoing studies will disambiguate under which conditions own and other's information is integrated for decision-making.

Disclosures: **J. Adrian:** None. **S. Siddharth:** None. **S. Baquar:** None. **T. Jung:** None. **G. Deak:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.24/AA19

Topic: H.02. Human Cognition and Behavior

Support: NSERC Vanier Canadian Graduate Scholarship
NSERC Discovery Grant

Title: How the human nervous system defines the dimensionality of energy optimization in walking

Authors: *S. J. ABRAM[1], J. C. SELINGER[3], J. M. DONELAN[2];
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Abstract: People prefer to move in ways that minimize metabolic energy during walking. Our lab has demonstrated that this preference arises not just through evolution and development, but that people can optimize step frequency and step width, in real-time, to minimize energy. Here, we model this process as reinforcement learning and test our model predictions against human experiments to elucidate how the nervous system may represent the dimensionality of new

energetic cost landscapes in its energy optimization of walking. In this model, we define the cost landscape using either a low-dimensional representation, where control strategies are gait parameters whose energy optimum we have shifted, or a high-dimensional representation, where the amount of control strategies is much larger, such as with combinations of individual motor units. We simulate a protocol that tests for energy optimization in 1-dimensional cost landscapes that shift the energy optimal frequency or width, and a 2-dimensional cost landscape that shifts both the energy optimal frequency and width. We hypothesize that, to learn new energy optimal gaits in real-time, the nervous system reduces the dimensionality of energy optimization to be along relevant gait dimensions. We used our model to quantify our hypothesis and found that, in any cost landscape, the rate of adaptation was indeed faster with the low-dimensional representation compared to the high-dimensional representation. We also determined how each representation's rate of adaptation increased from 1 to 2-dimensional cost landscapes—with the low-dimensional representation that was the same as the respective cost landscapes, the rate of adaptation increased 20-fold, whereas it was roughly the same with any high-dimensional representation greater than 5. To test our hypothesis, we conducted human experiments using a custom-built system that alters the relationship between energetic cost and a given gait parameter. We found that, in 1-dimensional cost landscapes, participants adapted towards the new energy optimum with similar time constants for both the step width (n=8) and step frequency (n=5) dimensions (273 and 247 seconds, respectively). In a 2-dimensional cost landscape, participants (n=7) adapted towards—but did not converge on—the new energy optimal width and frequency with time constants of 303 and 297 seconds, respectively. The similar rates of adaptation in 1 and 2-dimensional cost landscapes suggests that the nervous system may use a high-dimensional representation, however, the smaller adaptations in a 2-dimensional cost landscape suggests that an increase in dimensionality indeed influences energy optimization.

Disclosures: S.J. Abram: None. **J.C. Selinger:** None. **J.M. Donelan:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 421.25/AA20

Topic: H.02. Human Cognition and Behavior

Title: Can I eat this? Event-related potentials are modulated by feedback regarding edibility

Authors: *R. GRAHAM, R. LOPAS, A. ZBOROWSKI, N. CEBALLOS, J. TREFFALLS; Psychology, Texas State Univ. San Marcos, San Marcos, TX

Abstract: Feedback-related negativity (FRN) is an event-related potential (ERP) component that has been shown to be sensitive to feedback during risk-taking, such that the FRN is larger for

negative outcomes. Another ERP wave, the P300, is known to play a role in attentional resource allocation and is typically larger for better outcomes. The present experiment was conducted to examine the sensitivity of the FRN and P300 to processes related to appetitive motivations. Twenty-five undergraduates (15 male, mean age = 21.5 years) viewed ambiguous close-ups of food/drinks or nonfood/drinks, and indicated whether they could consume the objects. Unambiguous feedback about stimulus type was then provided. Analyses focused on ERPs to feedback-related events, specifically ERPs associated with correct judgements (food vs. non-food) and incorrect judgements (food vs. non-food). In line with our expectations, a stimulus type by outcome interaction was observed for the FRN, such that amplitude was largest when participants incorrectly identified nonfoods as foods. The P300 was sensitive to positive feedback, but was highest when participants correctly identified foods. These results provide support for the hypothesis that the FRN is modulated by the magnitude of negative feedback. Additionally, the enhancement of the P300 can be interpreted to represent the salience and reinforcing properties of motivationally-relevant feedback to humans, especially information regarding edibility.

Disclosures: **R. Graham:** None. **R. Lopas:** None. **A. Zborowski:** None. **N. Ceballos:** None. **J. Treffalls:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

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Topic: H.02. Human Cognition and Behavior

Support: he Key Program for International S&T Cooperation Projects of China (MOST, 2016YFE0129100)
the National Natural Science Foundation of China (No. 31471068)
he Fundamental Research Funds for the Central Universities (2017EYT33)
the Thousand Young Talents Program of China

Title: Neural mechanisms of preserving alternative prediction errors

Authors: *N. YINMEI[1], S. WANG[1], J. SU[2], X. WAN[3], J. LI[4];
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Abstract: Adaptive optimal control in interaction with dynamic changing environments is a central feature of intelligent systems. Precise predictions of the dynamic environment changes are critical for adaptive optimal control. The prediction is trial-by-trial updated on the basis of

the prediction error. In many situations, we need to keep multiple prediction errors simultaneously in the brain and choose one to use and keep the other currently unused ones. How the brains preserve alternative prediction error information? In this presentation, we report this part of results to elucidate the neural mechanism of preserving alternative prediction errors. Thirty-five participants made trial-by-trial predictions in a classical conditioning task. They concurrently made predictions on gain and punishers loss, which were randomly interleaved and stochastically associated, respectively. Thereby, the participants updated the corresponding prediction when the cue was revealed at the beginning of the next trial, and they should also keep the unused prediction error information. The participants reported their prediction value associated with the presented cue by scrolling the cursor position with combination of several button presses in each trial. First, the neuroimaging results showed that during the prediction phase the neural system associated with currently used prediction error and unused prediction error were largely dissociated. The former was in the orienting network, including the middle DLPFC (mDLPFC), parietal cortex, while the latter was in the executive function network, including dorsal ACC (dACC) and frontopolar cortex (FPC). However, there were also some overlapped regions, including primary motor cortex (PMC) and VTA. Secondly, our accompanying study showed that during the feedback phase the current prediction error information was separately encoded in the PMC, caudal ACC (cACC) and rostral ACC (rACC), while the unused prediction error information was still encoded in the dACC and FPC regions. Thirdly, when the coming-out cue was different from the previous cue, the used and unused prediction error information should be switched (switching trials). In the switching trials, the prediction error information generated from the previous trial became unused information, and was encoded in dACC and FPC regions, while the unused prediction error information encoded in dACC and FPC regions in the previous trials switched to Putamen and PMC, driving the current motor action. The overlapped regions of PMC and VTA, however, conveyed the unused prediction error information to the current trial, confounding the current prediction change.

Disclosures: **N. Yinmei:** None. **S. Wang:** None. **J. Su:** None. **X. Wan:** None. **J. Li:** None.

Poster

421. Human Learning: Feedback, Reinforcement, and Reward

Location: Hall A

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Support: Key Program for International S&T Cooperation Projects of China 2016YFE0129100 (most)
National Natural Science Foundation of China No. 31471068
Fundamental Research Funds for the Central Universities 2017EYT33 (partially)
Thousand Young Talents Program of China

Title: Computational and neural mechanisms of error-based learning in a dynamic environment

Authors: *S. WANG[1], Y. NI[1], J. SU[1], J. LI[3], X. WAN[1,2];
[1]State Key Lab. of Cognitive Neurosci. and Learning, [2]IDG/McGovern Inst. for Brain Res., Beijing Normal Univ., Beijing, China; [3]Sch. of Psychological and Cognitive Sci., Peking Univ., Beijing, China

Abstract: Precise predictions of the dynamic environment changes are critical for adaptive optimal controls. The prediction is trial-by-trial updated on the basis of the prediction error. The environment is often stochastically and unexpectedly changed, thereby, the prediction error brings uncertain information about the environment change. The model-based Bayesian learning is theoretically optimal. However, its computation and implementation are usually burdened, and the underlying neural mechanisms remain elusive. Here we elucidate an neural mechanism to approximate the optimal Bayesian learning as a function of prediction errors. Thirty-five participants made trial-by-trial predictions in a classical conditioning task. To clearly separate the feedback and prediction phases, two different types of cues were randomly interleaved and stochastically associated with rewards (gain) and punishers (loss), respectively. Thereby, the participants updated the corresponding prediction when the cue was revealed at the beginning of the next trial. The participants reported their prediction value by scrolling the cursor position with combination of several button presses in each trial. The right button presses would increase the magnitude and the left ones would decrease the magnitude. The initial position of the cursor was always at the prediction value reported in the previous trial with the same cue type. The optimal Bayesian model revealed that the participants' trial-by-trial learning rates held a linear relationship with the prediction errors. That is, the prediction change could be approximated by a combination of a linear and quadratic function of the prediction error. During the feedback phase, we found that the linear and quadratic components were associated with the primary motor cortex (PMC) and caudal anterior cingulate cortex (cACC), respectively, while the regression beta values of each participant were proportional to the corresponding coefficients. During the prediction phase, the VTA and Putamen activities were associated with the magnitudes of prediction changes, and independently correlated with the above two components in the feedback phase, indicating that VTA and Putamen integrated the learning processes and formed the motivational signals, to drive the motor actions, which were associated with PMC activities again. Taken together, these findings elucidate the neural and computational mechanisms of error-based learning in a dynamic changing environment.

Disclosures: **S. Wang:** None. **Y. Ni:** None. **J. Su:** None. **J. Li:** None. **X. Wan:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

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Program #/Poster #: 422.01/AA23

Topic: H.02. Human Cognition and Behavior

Support: PSI2017-84933-P
PSI2017-91955-EXP
Agencia Canaria de Investigación, Innovación y Sociedad de la Información de la Consejería de Economía, Industria, Comercio y Conocimiento.
Fondo Social Europeo (FSE) Programa Operativo Integrado de Canarias 2014-2020, Eje 3 Tema Prioritario 74 (85%).

Title: Spontaneous rs-fMRI activity within the classic hippocampal subfields

Authors: *L. M. EZAMA-FORONDA[1], E. PEREDA[2], N. JANSSEN[3];
[1]Psychology, Univ. of La Laguna, University of La Laguna, Spain; [3]Inst. for Biomed. Technologies, [2]Univ. of La Laguna, San Cristóbal de La Laguna, Spain

Abstract: The hippocampus (HP) is a brain structure implicated in Alzheimer's Disease and is thought to play a role in memory formation. The HP contains a number of subfields among which the Subiculum, CA1, CA2/3, CA4 and Dentate Gyrus. However, the functional characteristics of these substructures remain poorly understood. We used resting-state functional magnetic resonance imaging (rsfMRI) to explore whether spontaneous activity within the HP respects the classic hippocampal subdivisions. RS networks have been found to reflect different cognitive states and to depend on the interactions between different brain regions. We collected high-spatial resolution fMRI data and examined whether RS networks could be detected within the HP using a method called Spatially Restricted group-Independent Component Analysis (SR-gICA). If hippocampal substructures serve a functional role, one would expect RS networks detected within the HP to differentiate between hippocampal substructures. In this study, we recruited 33 healthy participants, 16 male and 17 female (mean age 22). The MRI protocol involved a 6 min partial volume GRE-EPI RS scan, and T1w and T2w acquisitions. Preprocessing of rsfMRI data involved the standard FSL FEAT pipeline followed by manual cleaning of ICA components. We obtained subject-specific ROIs for the location of the HP and its subfields from Freesurfer v6.0. A SR-gICA was performed on the 4D rsfMRI data restricted to each participant's entire HP. Obtained components within the HP were evaluated as signal or noise, and their functional connectivity between the hippocampal subfields and between the rest of the brain was explored using Dual Regression (DR). The degree to which a given detected RS network activated a given area of the brain was evaluated using linear regression (LR). The RS-gICA revealed 5 spatially independent RS networks. Careful consideration of their corresponding timecourses and periodograms indicated that IC3 and IC4 were likely of non-neural origin and therefore not considered. Results from DR of IC0-2 revealed they differentially depended on the substructures of the HP. This was confirmed by the LR results that showed that, for example, the subiculum was activated in RS networks IC0 and IC2 but not in IC1. In addition, these three networks differed in their connectivity with the rest of the brain. Here we have provided evidence that spontaneous rsfMRI activity detected within the HP yields networks that differentially depend on the classic divisions of the HP. In future research we will evaluate how spontaneous and task-related network activity within the HP is affected by aging and pathology.

Disclosures: **L.M. Ezama-Foronda:** None. **E. Pereda:** None. **N. Janssen:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

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Program #/Poster #: 422.02/AA24

Topic: H.02. Human Cognition and Behavior

Support: US NIH R01MH1023921
US NIH R01AG053555

Title: A memory computational basis for the other-race effect

Authors: ***J. L. YAROS**, D. A. SALAMA, D. DELISLE, M. S. LARSON, B. A. MIRANDA, M. A. YASSA;
Ctr. for the Neurobio. of Learning and Memory, Univ. of California Irvine, Irvine, CA

Abstract: People often recognize and remember faces of individuals within their own race more easily than those of other races. While behavioral research has long suggested that the Other-Race Effect (ORE) is due to extensive experience with one's own race group, the neural mechanisms underlying the effect have remained elusive. Predominant theories of the ORE have argued that the effect is mainly caused by processing disparities between same and other-race faces during early stages of perceptual encoding. Our findings support the alternative view that the ORE is further shaped by downstream mnemonic processing mechanisms beyond perception. Using a "pattern separation" paradigm based on computational models of episodic memory, we report evidence that the ORE may be driven by differences in successful memory discrimination across races as a function of degree of interference or overlap between face stimuli. In contrast, there were no ORE-related differences on an analogous working memory task, suggesting that the effect is not simply attributable to perceptual encoding. These findings suggest that the ORE may emerge in part due to "tuned" memory mechanisms that may enhance same-race, at the expense of other-race, face detection.
Our findings highlight the importance of a memory-based account in characterizing the ORE, suggesting future investigations of neural loci of the effect not only include perceptual but also traditional memory processing regions. Until now, neuroimaging approaches have focused primarily on the ventral visual stream's fusiform face area (FFA). However, while the FFA demonstrates significantly higher activity to same-race than other-race faces, these signals are not reliably predictive of ultimate memory for these faces - the hallmark behavior of the ORE. For this reason, we predict activity in medial temporal areas - in particular the perirhinal cortex (PrC) will better predict performance in an ORE task. Previous research has established a role for the PrC in mnemonic and perceptual discrimination of objects with high interference and feature ambiguity. In addition, PrC signal intensity predicts accuracy in both perception of and memory

for face stimuli, while the FFA does not. Taken together, these findings suggest the PrC is vital for interference resolution and ultimate accuracy for facial recognition- both functions that if compromised for other-race faces, would theoretically give rise to the ORE. In order to test the role of PrC in the emergence of the ORE we are adapting our behavioral task design and collecting data from subjects while their brain activity is imaged in the scanner.
addtl tags: face race mnemonic discrimination perirhinal

Disclosures: J.L. Yaros: None. **D.A. Salama:** None. **D. Delisle:** None. **M.S. Larson:** None. **B.A. Miranda:** None. **M.A. Yassa:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.03/AA25

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 5P50AG016573-20

Title: Ultrahigh resolution diffusion imaging reveals abnormal medial temporal lobe integrity predicts poor performance on RAVLT delayed recall in the oldest old

Authors: *S. J. GRANGER[1], M. S. LARSON[2], M. T. SATHISHKUMAR[2], A. P. SMITH[2], L. MCMILLAN[2], D. GREENIA[2], M. M. CORRADA[3], C. H. KAWAS[3], M. A. YASSA[2]; [1]Neurobio. and Behavior, [3]Dept. of Neurol., [2]Univ. of California, Irvine, Irvine, CA

Abstract: The number of persons over 90 years of age is expected to grow roughly 4 times by the year 2050 and the resources needed to care for them will only increase as well. Non-invasive imaging methods such as volumetry and functional brain activity are commonly used to study endophenotypes of cognitive decline and neurodegenerative diseases, however, they alone cannot distinguish specific white-matter circuits involved in decline. A more sensitive biological marker that precedes gross gray-matter volumetric change is needed. Using ultrahigh resolution diffusion-weighted MRI, in this cross-sectional analysis, we investigated if microstructural differences within the medial temporal lobe (MTL) were associated with poorer memory performance in people over 90 years old from The 90+ Study. We segmented subfields of the MTL using machine learning techniques and assessed MTL integrity using voxel-wise diffusion-tensor imaging and “model-free” high-angular deterministic tractography methods to create MTL connectomes. Cognitive status was measured as memory performance on the Rey Auditory Verbal Learning Test (RAVLT) delayed recall. Participants were a small cohort of 16 persons (13 females, 3 males), age range 90 to 98 years old, 11 of which had normal cognition, and 5 of which had cognitive impairment with no dementia. Using one-way ANOVA, we provide evidence that tensor-derived measures dissociate MTL subfields and accurately represent tissue

vs. non-tissue samples. Further, we show that distinct MTL structural abnormalities in both fractional anisotropy ($r = -0.6$, $p < 0.01$) as well as mean diffusivity ($r= -0.4$, $p = 0.06$) are negatively correlated with delayed recall ability. Finally, as an exploratory approach, we provide evidence that greater structural connectivity of the entorhinal cortex with the rest of the MTL is associated with poorer delayed recall ability ($r = -0.5$, $p = 0.052$). Together, these results indicate that novel and specific MTL structural abnormalities are associated with poor memory capacity in the oldest old. With the addition of subjects, covariates, and multimodal neuroimaging methods we believe these techniques and results could possibly yield novel insights into the transition to cognitive decline and even Alzheimer's related pathology.

Disclosures: S.J. Granger: None. **M.S. Larson:** None. **M.T. Sathishkumar:** None. **A.P. Smith:** None. **L. McMillan:** None. **D. Greenia:** None. **M.M. Corrada:** None. **C.H. Kawas:** None. **M.A. Yassa:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.04/AA26

Topic: H.02. Human Cognition and Behavior

Support: NICHD R01 HD065160

Title: Reduced long-range default mode network connectivity predicts conversion to Alzheimer's disease in older individuals with Down syndrome

Authors: *N. D. DIPROSPERO[1], D. B. KEATOR[2], T. G. VAN ERP[2], E. DORAN[3], I. T. LOTT[3], M. A. YASSA[1];
[1]Dept. of Neurobio. and Behavior, [2]Dept. of Psychiatry and Human Behavior, Univ. of California, Irvine, Irvine, CA; [3]Dept. of Pediatrics, Univ. of California, Irvine Med. Ctr., Orange, CA

Abstract: Individuals with Down syndrome (DS) are at increased risk for developing Alzheimer's disease (AD) and have earlier symptom onset at an average age of 55, compared to age 80 in the general population. Determining preclinical biomarkers of AD is critically important for early diagnosis and effective intervention within this high-risk population and more broadly in the general population, but little is known about the biomarkers that may predict clinical onset in DS. Previous studies in non-DS individuals have shown weaker functional connectivity within the default mode network (DMN) and stronger functional connectivity within the medial temporal lobe (MTL) in cognitively normal older adults in the preclinical stage of AD. However, few studies have investigated functional connectivity as a potential biomarker of AD in individuals with DS ages 40+. 15 non-demented older adults with DS (mean age

51.7±5.3, 47% women) underwent MRI, (including a 1mm MPRAGE scan and a 3mm resting state fMRI scan), PET, and clinical evaluation at their first visit. Participants were followed for two years, returning to the clinic roughly every 12 months for neuropsychological testing and clinician diagnosis for AD. 4 participants transitioned to AD during follow-up. We hypothesized that older adults with DS who subsequently developed dementia would have weaker functional connectivity within the DMN and stronger functional connectivity within the MTL compared to those who remained cognitively normal during follow-up. We also predicted that aberrant functional connectivity would precede cognitive decline, and regions with aberrant functional connectivity would exhibit greater amyloid burden in individuals with DS who transition to AD compared to those who do not transition to AD. Older individuals with DS who later developed AD had weaker antero-posterior DMN functional connectivity one year prior to conversion. Lower levels of functional connectivity between distal DMN hubs is associated with faster cognitive decline. Functional connectivity between proximal DMN hubs and between MTL hubs does not predict AD conversion or precede cognitive decline. Individuals who transitioned to AD had greater amyloid accumulation in DMN hubs relative to those who did not develop dementia during follow-up. Antecedent hypoconnectivity between distant DMN hubs may predict future conversion to AD and faster cognitive decline in older adults with DS. Those who convert to AD exhibit greater amyloid burden in the same DMN hubs with aberrant functional connectivity.

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Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.05/AA27

Topic: H.02. Human Cognition and Behavior

Support: U19 NS107609-01 (PI: Buffalo EA ; Site PI: Lin JJ)

Title: Pattern separation beyond the hippocampus: Neocortico-hippocampal mechanisms of pattern separation in humans

Authors: *S. GATTAS[1], J. J. LIN[2], M. A. YASSA[3];
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Abstract: Pattern separation is a cognitive process whereby two inputs sharing overlapping content are transformed into two orthogonal, non-overlapping outputs. In the context of memory processing, there is evidence pointing to the involvement of the hippocampus (HC), specifically

DG and CA3, in pattern separation. Reported hippocampal mechanisms of pattern separation include changes in firing rate, recruitment of new coding units, and increases in theta and gamma oscillations. In this study, we aim to further investigate oscillatory mechanisms supporting pattern separation both within HC and between HC and neocortex (NC). In order to do so, we recorded local field potential (LFP) in humans implanted with electrodes for clinical monitoring. Recordings were conducted while patients performed a task in which they indicated whether presented images of objects were identical replicas of previously shown images (repeats) or new ones (similar but not identical, or completely different foils). During successful discrimination between previously encoded images and their similar newly presented counterparts, we observed early (~200ms) theta power and late (preceding subject response) gamma power increases in the HC. Early theta power increases were also observed in NC. Moreover, during the same condition type denoting pattern separation, we observed neocortico-hippocampal theta-gamma phase-amplitude coupling. These reported effects were not observed in three remainder conditions: repeats, new foils, or similar but incorrectly discriminated images. These findings suggest that NC and HC theta power, HC gamma power, as well as neocortico-hippocampal theta-gamma coupling support pattern separation in humans.

Disclosures: S. Gattas: None. **J.J. Lin:** None. **M.A. Yassa:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.06/AA28

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 5P50AG016573-20

Title: Mnemonic discrimination in the oldest old and relationship with volumes of medial temporal lobe and striatal regions

Authors: *M. S. LARSON[1], M. T. SATHISHKUMAR[1], N. DIPROSPERO[1], A. SMITH[2], L. MCMILLAN[2], D. GREENIA[3], M. WITBRACHT[3], M. M. CORRADA[3], C. H. KAWAS[3], J. GRILL[3], M. A. YASSA[2];
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Abstract: Individuals over the age of 90 are the fastest growing sector of the U.S. population and make up the group at the highest risk for dementia. However, comparatively little is known about how cognitive changes in the oldest old may be linked to structural and functional decline in brain networks. Participants in this study included two groups: older adults - ages 65-85 ($n=40$, $mean_{age}=77.7$, 26 female), and oldest old - ages 90-104 ($n=23$, $mean_{age}=94$, 18 female),

with participants from both groups without dementia diagnosis. Dementia diagnosis was assessed within 6 months of their MRI study visit in clinical interviews at their annual visit with the UCI ADRC Longitudinal Study or their semi-annual visits with the 90+ Study. At their MRI study visit, the Mini Mental State Exam (MMSE), a measure of gross cognition, was administered ($mean_{65\text{-}85}$ =27.07 $mean_{90+}$ =27.37) found to be without significant group differences. We then compared their performance on a mnemonic discrimination task that is sensitive to hippocampal function, and in particular a hippocampal computation known as pattern separation - the ability to discriminate among similar memories and store new experiences with high fidelity. We have previously shown that this ability is compromised with age but have not examined this in the oldest old in detail. Using Welch's unpaired t-test, we found that High and Low Similarity Lure Discrimination Index scores were not significantly different between the groups (LDI High: $mean_{65\text{-}85}$ =0.12, $mean_{90+}$ = 0.13 LDI Low: $mean_{65\text{-}85}$ =0.26, $mean_{90+}$ =0.19). We collected high resolution T1-weighted structural MPRAGE scans on a 3T scanner (90+: 0.80mm isometric, 65-85: 0.65mm isometric) and used FreeSurfer to calculate regional volumes in medial temporal lobe regions, as well as other default mode network regions. All regional volumes were corrected with total brain volume (TBV). In a Welch's unpaired t-test we found significant cohort differences in TBV corrected volumes of the caudate and posterior cingulate (Caudate $mean_{65\text{-}85}$ = 0.002968 $mean_{90+}$ =0.002709, PCC $mean_{65\text{-}85}$ = 0.000719 $mean_{90+}$ =0.0006433). Surprisingly we did not find differences in hippocampal volumes by cohort (HPC $mean_{65\text{-}85}$=0.003053 $mean_{90+}$ =0.002897). Further study is needed with a larger sample size to evaluate sex differences, and to determine if the lack of difference in hippocampal volume is the result of decreased rate of change or simply a greater starting volume in the oldest old. Study is also needed to evaluate the volume of hippocampal subfield differences between the two groups, and if performance on the mnemonic discrimination task is predictive of longitudinal volume change.

Disclosures: **M.S. Larson:** None. **M.T. Sathishkumar:** None. **N. DiProspero:** None. **D. Greenia:** None. **M.M. Corrada:** None. **L. McMillan:** None. **M.A. Yassa:** None. **A. Smith:** None. **M. Witbracht:** None. **J. Grill:** None. **C.H. Kawas:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.07/AA29

Topic: H.02. Human Cognition and Behavior

Title: The neural circuit bases of prolonged maternal grief for the loss of her child

Authors: *M. T. SATHISHKUMAR[1], ***J. ADAMS**[1], T. Z. BARAM[2], M. A. YASSA[1]; [1]Neurobio. and Behavior, [2]Pediatrics, Univ. of California, Irvine, CA

Abstract: Rationale: Grief is considered an appropriate and time-constrained response to a loss. Yet, mothers who lose their children often experience overwhelming sense of grief that may last for years and decade. The profound grief is often triggered by memories of the lost child or by cues that provoke these memories. Loss of a child upon trauma, war, suicide or drug overdose is common, and the population of grieving mothers is large. Yet, we know little about the neurobiological basis of chronic persistent maternal grief. **Methods:** Here, we explore the impact of prolonged grief on memory and brain circuitry using structural, functional, diffusion and resting state brain magnetic resonance imaging (MRI). We recruited 9 mothers who have experienced a recent loss of a child, (mean age = 61.3 years); and 8 age and demographically matched controls, (mean age = 57.8 years). We assessed cognitive functions, mood and symptoms of grief using Neuropsychological tests. We employed functional MRI and measured grief and control subjects' brain responses to pictures of their own children (dead for the subjects, alive for the controls), deceased celebrities or unfamiliar people. Subjects also rated the level of emotionality they felt about the images.
Results: Comparing measures of brain activation to their own children vs celebrities or unfamiliar people distinguished grieving mothers from the controls. Specifically, increased activation of components of the prefrontal cortex, and especially both left and right inferior frontal gyrus (IFG) and superior frontal gyrus (SFG) was observed in the grief group. These regions contribute to a network involved in emotional regulation, control, and motivational processing.
Conclusion: The disrupted operation of an executive networks balancing motivation (motherhood) and emotional regulation may begin to uncover a potential basis for the overwhelming and persistent grief of mothers losing their children.

Disclosures: M.T. Sathishkumar: None. **J. Adams:** None. **T.Z. Baram:** None. **M.A. Yassa:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.08/AA30

Topic: H.02. Human Cognition and Behavior

Support: NIA P50 AG16573
NSF Bridge to the Doctorate
NSF Graduate Research Fellowship Program

Title: Functional connectivity of the medial temporal lobe contributes to spatial discrimination impairments in non-demented older adults with and without memory impairments

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[1]Univ. of California Irvine, Irvine, CA; [2]Neurobio. and Behavior, Univ. of California Irvine Dept. of Neurobio. and Behavior, Irvine, CA

Abstract: Studies suggest that two neocortical systems interact with subcortical areas (including the hippocampus) to support memory for objects and memory for spatial navigation. The hippocampus is connected to other brain regions via two major limbic tracts: the fornix and the cingulum bundle, which includes the hippocampal cingulum and superior cingulum. In healthy adults (51.8 ± 18.9 years; MMSE: 29.02; RAVLT Delay: 11.52), significant relationships between chronological age and integrity of the hippocampal cingulum and fornix tracts have been described previously, and behavioral assays have shown a relationship between object discrimination and fornix integrity. While asymptomatic older adults have major deficits on object discrimination, they only show subtle deficits on the comparable spatial discrimination task when compared to young adults. If spatial discrimination is supported by hippocampal connections to a broader neocortical network, then deficits in spatial discrimination may be mediated by the integrity of limbic tracts. In this study, older adults (74.6 ± 7.5 years; MMSE: 27.94 ± 1.39 RAVLT Delay: 9.94 ± 4.46) underwent diffusion tensor imaging (2.2 mm nominal isotropic resolution) and completed the object and spatial mnemonic discrimination tasks. Behaviorally, we found a positive correlation between performance on RAVLT Delay and spatial discrimination (r^2=0.271, p=0.0054), but no relationship between performance on RAVLT Delay and object discrimination (r^2=0.002, p=0.80). Targeted tractography analyses revealed that hippocampal cingulum integrity (fractional anisotropy, mean diffusivity, and restricted diffusion) was significantly related to mnemonic discrimination for spatial memory, but not for object memory. These findings support the idea that deficits in spatial memory manifest due to hippocampal disconnection to neocortical systems via the hippocampal cingulum. The results additionally suggest that the integrity of the hippocampal cingulum may be a biomarker that is associated with cognitive changes particularly related to spatial memory.

Disclosures: F. Marquez: None. **M.A. Yassa:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.09/AA31

Topic: H.02. Human Cognition and Behavior

Support: Famille Charron
NSERC Grant no. 77848

Title: Spatial memory is protective against Alzheimer's disease

Authors: *S. FOO[1], D. SODUMS[3], V. D. BOHBOT[2];
[1]Psychiatry, [2]Dept. of Psychiatry, McGill Univ., Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Aim: The hippocampus (HPC) is critical for supporting memory functions, such as spatial memory, and a volume reduction in this structure is associated with future clinical diagnosis of Alzheimer's disease (AD). Previous research in our laboratory demonstrated that spatial memory correlates to grey matter in the HPC. Moreover, MRI SNIPE grading measures in the HPC were previously shown to predict ensuing diagnosis of AD with a 73% accuracy. As such, the aim of the current study was to investigate whether spatial memory is protective against MRI AD pathology.
Methods: Seventy-four healthy older adults (43 female, 31 male; mean age: 65.5 ± 4.2 years) received MRI scans and SNIPE was used to compare their hippocampi to patients with AD versus controls. The MRI SNIPE measure involves computing the similarity of every hippocampal voxel of each participant to a library of manually segmented MRI datasets from the ADNI database consisting of equal numbers both healthy cognitively intact older adults and AD patients. Participants were tested on the Concurrent Spatial Discrimination Learning Task (CSDLT) and the Wayfinding task, both of which are sensitive to spatial memory and hippocampal grey matter. The CSDLT is a 12-arm radial maze in which participants have to learn the location of objects within pairs of arms, after which the presentation of arms is recombined but the reward contingency remains the same (stage 2). Stage 2 errors indicated whether the position of objects on the radial maze was learned in relation to the landmarks in the environment. The wayfinding task measures the ability to build and use a cognitive map of a virtual environment containing landmarks (e.g. pool, shop, etc.). Probe errors measure the participant's ability to find target locations in a straight path in the virtual town, hence measuring spatial memory.
Results General linear modelling was used to regress spatial memory and age on SNIPE scores in men and women separately. Results showed that performance on the wayfinding task significantly predicted hippocampal SNIPE grading scores in men (β = 0.493, $p < 0.01$). The CSDLT task positively predicted hippocampal SNIPE grading scores in women (β = -0.318, $p < 0.05$).
Conclusion: The current study shows that participants with a good spatial memory have higher SNIPE scores, which are predictive of healthy cognition in normal aging. On the other hand, poor spatial memory is associated with lower SNIPE grading in the HPC, which is predictive of increased risk of future AD diagnoses. Sex differences in navigation may help explain differences in the sensitivity of navigation tests to the MRI measures for men and women.

Disclosures: S. Foo: None. **D. Sodums:** None. **V.D. Bohbot:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.10/AA32

Topic: H.02. Human Cognition and Behavior

Support: Famille Charron
NSERC Grant no. 77848

Title: Negative correlation between grey matter in the hippocampus and caudate nucleus in healthy aging

Authors: *V. D. BOHBOT;
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Abstract: Aim: Spatial memory impairments, as well as decreases in fMRI activity and grey matter in the hippocampus have been documented in healthy older adults engaged in a navigation task. However, navigation in a virtual maze relies on spatial or response strategies known to depend on the hippocampus and caudate nucleus of the striatum respectively. Furthermore, the proportion of people using spatial strategies decreases across the lifespan in favor of response strategies. As such, the aim of the current study was to investigate whether the decreases in grey matter in the hippocampus were associated with corresponding increases in grey matter in the caudate nucleus.
Methods: Thirty-nine healthy older adults (21 women and 18 men; mean age:64.69 ± 4.11 years) were scanned on a 1.5T scanner and were tested on the Concurrent Spatial Discrimination Learning Task (CSDLT), a radial task that dissociates between spatial and response strategies. The CSDLT is a 12-arm radial maze in which participants have to learn the location of objects within pairs of arms, after which the presentation of arms are recombined but the reward contingency remains the same (stage 2). Stage 2 errors indicated whether the position of objects on the radial maze was learned in relation to the landmarks in the environment (use of spatial strategies).
Results: A regression of strategies against structural MRIs showed for the first time in older adults that the response strategy was associated with an increase in grey matter in the caudate nucleus. As expected, the spatial strategy was associated with increased grey matter in the hippocampus, which in turn, was negatively correlated with grey matter in the caudate nucleus. Interestingly, a sex difference emerged showing that among older adult response learners, women response learners have the least amount of grey matter in the hippocampus. This difference was absent among spatial learners showing that the use of spatial memory is protective against grey matter loss in the hippocampus, especially in women.
Conclusion: The current study reports a negative correlation between grey matter in the

hippocampus and caudate nucleus during normal aging. There results are consistent with a negative correlation between these structures previously shown in mice, young adults and in patients with Alzheimer's disease. Since the use of the caudate nucleus has previously been shown to actively inhibit activity and grey matter in the hippocampus, our results suggest that the caudate nucleus may play an active role in hippocampus-dependent memory deficits observed in normal aging.

Disclosures: V.D. Bohbot: None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.11/AA33

Topic: H.02. Human Cognition and Behavior

Title: Landmark-dependent navigation strategy use within a mobile video game declines with age and is associated with better spatial memory performance

Authors: *G. WEST[1], E. PATAI[2], A. COUTROT[3], M. HORNBERGER[4], H. J. SPIERS[5], V. D. BOHBOT[6];
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Abstract: Sea Hero Quest (SHQ) is a mobile video game designed to measure human spatial navigation ability through gameplay. The game involves navigating a boat in search of sea creatures in order to photograph them. Previous studies have focused on the game's wayfinding and path integration levels. The focus of the current research was to examine performance within the radial arm maze optional bonus section, completed by more then 40,000 people globally. The radial maze levels contain six pathways surrounded by proximal and distal landmarks (e.g., rock, hut, volcano) and consists of two parts: in Part 1, three of the six pathways are blocked and the player is required to navigate the boat to visit the three open pathways to collect a star fish that pops out of the water. In Part 2, all six pathways are made available and the player is required to visit the pathways that were unavailable during Part 1 to collect the remaining three star fish. Errors, as defined as visiting a previously entered pathway were recorded. When the radial arm maze level is completed, the player is asked a multiple-choice question about how they found the star fish (i.e., a counting strategy vs. a landmark-based strategy). The player's navigation strategy was established based on the second trial (in the second experimental level) because the first one was considered practice. We also obtained demographic data from each player (i.e., age, sex, country, & growing up in a city vs rural environment). Results showed that the use of landmarks

declined with age. This was predicted by the large body of research that has demonstrated reduced spatial memory, structure and function of the hippocampus with age. In addition, people who used landmark-based strategies in the radial arm maze displayed moderately better spatial memory performance within the game's wayfinding levels. These results confirm previous observations from smaller studies showing a decreased use of spatial strategies with age, as well as the relationship between landmark-dependent navigation strategy use and spatial memory performance using a substantially larger and representative sample size from various regions world-wide.

Disclosures: G. West: None. **E. Patai:** None. **A. Coutrot:** None. **M. Hornberger:** None. **H.J. Spiers:** None. **V.D. Bohbot:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.12/AA34

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01-MH104606

Title: Human ripples in spatial memory

Authors: *T. GEDANKIEN, J. MILLER, J. JACOBS;
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Abstract: The ability to remember trajectories between locations is central to human experience. However, the precise neural mechanisms underlying spatial memory in humans remain elusive. In rodents, previous research has linked the occurrence of sharp-wave ripples (SWRs) during sleep and awake rest to good spatial memory. Recent findings in rodents and humans have also linked good memory to coupling between ripples in the cortex and medial temporal lobe (MTL) during sleep. These findings support the theory of systems consolidation, thereby ripples facilitate memory transfer between the hippocampus and cortex. Here, we tested the hypothesis that awake ripple-like events in humans, like SWRs in animals, play a role in spatial memory. We also investigated how ripples spread within and across the human MTL and cortex. We analyzed intracranial recordings from epilepsy neurosurgical patients as they performed a hybrid spatial-memory virtual task. We detected ripples in the 80-120-Hz band, using previously published methods. Within the MTL, we found three classes of ripple events based on their spatial spread: those seen in a single channel in time (single events), those seen simultaneously across nearby channels (cluster events), and those seen simultaneously in distant channels (coupled events). Across sixteen subjects, we found that ripples were most prominent in hippocampal CA1 region and during the feedback phase of the spatial task. These results suggest

a role of hippocampal ripples in facilitation of human learning and memory consolidation related to feedback.

Disclosures: **T. Gedankien:** None. **J. Miller:** None. **J. Jacobs:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.13/AA35

Topic: H.02. Human Cognition and Behavior

Support: NIH R21 MH117682

Title: Direct electrical stimulation of the human cortex modulates narrowband low frequency oscillations

Authors: *U. R. MOHAN[1], J. JACOBS[2];
[2]Dept. of Biomed. Engin., [1]Columbia Univ., New York, NY

Abstract: Low frequency oscillations across the cortex underly coordination of brain networks in humans and play critical roles in cognition. Researchers have used direct electrical brain stimulation to treat a range of neurological and psychiatric disorders in which these oscillations are impaired. Direct electrical stimulation holds the potential to modulate these cortical oscillations; however, we do not yet have a detailed understanding of how stimulation alters narrowband low frequency oscillations. Therefore, to fill this gap in our understanding, we investigated the effects of different types stimulation on narrowband low frequency oscillations. We collected human electrocorticographic recordings from neurosurgical epilepsy patients while systematically delivering stimulation at different combinations of frequency and amplitude at specific locations. In order to understand how stimulation affects oscillations, we measured the amplitude and frequency of narrowband oscillations before and after stimulation events. Overall, 10Hz direct electrical stimulation can enhance or diminish the amplitude of a narrowband low frequency oscillation or altogether change the frequency of that oscillation. Low frequency stimulation often induces or enhances ongoing low frequency narrowband oscillations while decreasing oscillation frequency. Whereas our previous work shows that low frequency stimulation primarily inhibits local neuronal activity, these results show that low frequency stimulation modulates specific features of narrowband oscillations. This implies that the impact of brain stimulation on cortical activity is much more nuanced than excitatory and inhibitory effects. By characterizing the link between brain stimulation and low frequency oscillations, our results provide insight into how brain stimulation protocols may be designed to modulate specific features of endogenous network oscillations.

Disclosures: **U.R. Mohan:** None. **J. Jacobs:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.14/AA36

Topic: H.02. Human Cognition and Behavior

Support: MH104606

Title: Predictive cognitive map representations by human single neurons

Authors: I. MOMENNEJAD[1], A. PATEL[1], C. S. INMAN[2], R. E. GROSS[3], B. C. LEGA[4], L. ROBINSON[5], C. A. SCHEVON[1], G. MCKHANN[6], A. WATROUS[7], R. J. BUCHANAN[8], ***J. JACOBS**[1];
[1]Columbia Univ., New York, NY; [2]Neurosurg., Emory Univ., Decatur, GA; [3]Dept Neurosurg., Emory Univ. Sch. Med., Atlanta, GA; [4]Neurosurg., [5]UT Southwestern Med. Ctr., Dallas, TX; [6]Dept. of Neurol., Columbia Univ. Med. Ctr., New York, NY; [7]Neurol., Univ. of Texas at Austin, Austin, TX; [8]Neurosurg., Seton Brain and Spine Inst., Austin, TX

Abstract: In previous work we show that multi-step predictive maps, known as the successor representation (SR), can best explain flexible human decision making. We showed this for reward and transition revaluation, when structures of rewards and transition change (Momennejad et al. 2017). Here, we test the hypothesis that single cells in human MTL learn and update such predictive map representations. We used a virtual navigation study in which the predictive maps would be used for making value-based decision-making. We measured single neuron recordings (Behnke-Fried electrodes) in epileptic patients while they engaged in navigation and reward-based decision making in virtual environments, following the design of our past experiment. Each virtual environment has two sequential tracks, each with three connected rooms that are linear tracks (seq 1: rooms 1-3-5, seq 2: rooms 2-4-6). The eventual reward value of each sequence is seen once the patient opens the briefcase in the third room (room 5: $20, room 6: $5). During relearning, participants experienced partial changes to the environment that reflected 'reward revaluation' (final briefcase cash value changes) or 'transition revaluation' (suddenly room 3's door opens to room 6, room 4's door opens to room 5: the architecture is restructured). Patients needed to integrate this new information to make optimal decisions between rooms 1 and 2 at test. SR predicted that rooms that are connected, one-step or multi-step, would show more similarity to each other, and this multi-cell similarity pattern would reflect changes in the world. To test the SR hypothesis, we computed pairwise correlations between mean firing rates of hippocampal cells for each pair of rooms to construct a 6X6 matrix of neural similarity among the 6 rooms. We compared the resulting matrix with SR's predictive representation. Preliminary results comparing changes before and after transition revaluation

show that the 6-roomX6-room matrix of multi-cell correlations reflects the change in the structure of the environment and revaluation behavior: Multi-cell correlations increased for rooms, 1-step and 2-step away, that were not connected before but are now connected.

Disclosures: I. Momennejad: None. **A. Patel:** None. **C.S. Inman:** None. **R.E. Gross:** None. **B.C. Lega:** None. **L. Robinson:** None. **C.A. Schevon:** None. **G. McKhann:** None. **A. Watrous:** None. **R.J. Buchanan:** None. **J. Jacobs:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.15/AA37

Topic: H.02. Human Cognition and Behavior

Support: MH104606

Title: The spatiotemporal structure of human cortical traveling waves correlates with cognitive processes

Authors: *H. ZHANG[1], J. JACOBS[2];
[2]Dept. of Biomed. Engin., [1]Columbia Univ., New York, NY

Abstract: A broad range of research has found correlations between brain oscillations and features of cognitive processes. However, we still do not understand the physiological role of these oscillations. One hint of a functional role for brain oscillations came from our recent discovery that many oscillations in the human cortex behaved as traveling waves, propagating across the surface of the brain, much like a wave moving across the ocean. This suggested a role for brain oscillations in coordinating the activity of large groups of neurons across space and time in the cortex. Here we further test this idea by examining the timing and directional features of traveling waves in relation to behavior during a memory task. We examined direct recordings of traveling waves from neurosurgical patients who had electrodes implanted in their brains for clinical needs. The patients performed verbal working and episodic memory tasks and we used a novel analytic framework to measure the properties of individual traveling waves and compare their features to task events. Using this framework, we identified two novel functional roles of traveling waves in human cognition. In both tasks, traveling waves generally propagated in a posterior-to-anterior direction, although some patients showed the opposite propagation direction. When a probe stimulus was presented in the working memory task, subject's reaction times correlated with the position on the cortex of of the traveling wave (i.e., the traveling wave's spatial phase). This indicates that the location of individual traveling waves on the surface of the cortex correlate with how rapidly a subject will respond to a new stimulus, consistent with the idea from previous studies that brain oscillations could reflect a "cortical

scanning" process. We also examined the phase of traveling waves during the recall phase of our free recall task. Traveling waves in many subjects exhibited particular spatial phase patterns ~1 s prior to recall. Among the waves propagating in an anterior-to-posterior direction, more waves showed phase resets before recall implicating these waves in the top-down flow of information during memory retrieval, suggesting that a role for traveling waves in supporting memory recall by spatially organizing cortical activity. More broadly, our work demonstrates a new role for brain oscillations in cognition, by organizing the large-scale spatio temporal structure of neural activity in relation to the timing of behavioral events.

Disclosures: H. Zhang: None. **J. Jacobs:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.16/AA38

Topic: H.02. Human Cognition and Behavior

Support: NSF GRFP DGE - 1644869
DARPA RAM N66001-14-2-4032

Title: Low theta oscillations in the anterior hippocampus predict successful encoding of spatial and verbal memories in humans

Authors: *S. E. QASIM, J. MILLER, J. JACOBS;
Biomed. Engin., Columbia Univ., New York, NY

Abstract: A preponderance of research has demonstrated that theta oscillations in the human hippocampus vary during memory encoding, depending on whether the encoded cue is subsequently recalled or not. This "subsequent memory effect" (SME) has been observed across a range of low frequencies throughout the hippocampus, suggesting that hippocampal theta oscillations play a role in the successful encoding of memory. However, recent work has identified functional and regional distinctions in theta oscillations at different frequencies in humans, suggesting that low theta (1-5 Hz) and high theta (5-10 Hz) may play distinct roles in memory circuits. Furthermore, numerous studies have now shown that connectivity, function, and genomic anatomy significantly vary along the longitudinal axis of the human hippocampus, suggesting that the different poles of the hippocampus may feature distinct memory circuits. This led us to hypothesize that that successful memory encoding would engage distinct theta oscillations along the longitudinal axis of the hippocampus in humans. We analyzed data from depth electrodes implanted in neurosurgical patients who performed a verbal memory task (n=405 hippocampal electrodes) and/or a spatial memory task (n=266 hippocampal electrodes). We found that increases in low theta oscillations (1-5 Hz), specifically, predicted successful

memory encoding, primarily in the anterior hippocampus, during both verbal and spatial memory encoding. Furthermore, successful verbal memory encoding was best predicted by low theta power in the right-anterior hippocampus, while successful spatial memory encoding was best predicted by low theta power in the left-anterior hippocampus. High theta power did not predict spatial memory encoding, whereas decreases in high theta power (5-10 Hz) predicted subsequent memory across the entire left hippocampus, but not the right, during verbal memory encoding. These results suggest that variations in theta oscillations across the anterior-posterior axis, as well as across hemispheres, may play an important role in the strength of memory encoding and the type of memory encoded. These results also further demonstrate the distinct functional and anatomical characteristics of low and high theta oscillations in the human hippocampus.

Disclosures: S.E. Qasim: None. **J. Miller:** None. **J. Jacobs:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.17/AA39

Topic: H.02. Human Cognition and Behavior

Support: NINDS Grant 1U19NS107609-01

Title: Dimensionality reduction in human hippocampal dentate/CA3, but not CA1 support pattern separation

Authors: *H. ZHANG[1,2], I. SKELIN[2], M. A. YASSA[3], J. J. LIN[1,2];
[1]Biomed. Engin., [2]Neurol., [3]Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: The ability to discriminate between the similar experiences (i.e. pattern separation) is essential for episodic memory, but the underlying neural mechanisms are unclear. Hebb and Marr proposed that the dentate gyrus (DG) separates neural activity patterns by expanding dimensionality of neural ensembles, enabling downstream decoder neurons in CA3 to orthogonalize signals. However, the neurophysiological signature of ensemble code expansion and reduction at hippocampal subfield resolution in humans is absent. Recent work in primates suggests that local field potentials (LFP) gamma bursts reflect the convergence of neural ensembles to transient attractor states (Lundqvist et al., 2016). To test the Hebb and Marr hypothesis, we analyzed the dynamics of gamma bursts recorded from hippocampal subregions (DG/CA3 and CA1) of presurgical patients undergoing seizure localization.
Five patients performed a pattern separation task in which they were asked to discriminate similar (lure), novel and repeat images from previously encoded stimuli. We reconstructed the high-frequency component (45-300 Hz) of the LFP by implementing Ensemble Empirical Mode Decomposition (EEMD) and calculating the time-frequency response during the stimulus

presentation at the retrieval period (2000 msec). The spectrum dimensionality was calculated for each of the 500 msec moving window by participation ratio (PR) from its spectral principal component space (Gao et al., 2017; Vítor Lopes-dos-Santos et al., 2018&2011). In DG/CA3, we found an initially increased dimensionality, followed by rapid dimensionality reduction for correct lure rejections compared to lure false alarms ($p<0.05$, Wilcoxon test). Critically, this conditional difference in dimensionality was not present in the downstream CA1 subfield. At single trial level, correct lure rejection trials are characterized by the transient dimensionality reduction in the DG/CA3, lasting ~500 msec. In contrast, the lure false alarm trials were characterized by both increased and decreased dimensionality periods of variable durations. In summary, these results provide neurophysiological evidence for the Hebb and Marr model of code expansion and reduction in DG/CA3 for pattern separation.

Disclosures: H. Zhang: None. **I. Skelin:** None. **M.A. Yassa:** None. **J.J. Lin:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.18/DP12/AA40

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: H.02. Human Cognition and Behavior

Support: U19 NS107609-01 (PI: Buffalo EA ; Site PI: Lin JJ)
NSF Grant 1631465 to BLM
DARPA Grant HR0011-18-2-0021 to BLM

Title: Distinct functional connectivity patterns predict the peri-ripple population activity in human amygdala and temporal cortex

Authors: *J. J. LIN[1,2], H. ZHANG[2], B. L. MCNAUGHTON[3,5], I. SKELIN[4];
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Abstract: Sharp wave/ripples (SWRs) are the windows of memory reactivation and hippocampal-cortical/subcortical communication during offline states. Various memory consolidation models propose the initial storage of memory traces in distributed cortical/subcortical networks, followed by their gradual integration and loss of hippocampal-dependence through the establishment of horizontal connectivity. We hypothesized that the local field potential (LFP) synchrony between the cortical/subcortical populations activated around the time of SWRs (peri-SWR; +/- 250 ms relative to SWR peak) is necessary for enabling this process, but the functional connectivity mechanisms synchronizing these populations are

unknown.
We analyzed the overnight sleep (nREM phases 2-4) LFP from hippocampus, amygdala and temporal cortex, from 12 human subjects undergoing presurgical localization of epileptic foci. The peri-SWR active populations were defined at the single electrode level, based on significant modulation of high gamma activity (HGA; 70-200 Hz) - a proxy measure of spiking activity. Functional connectivity was quantified for 3 frequency bands (delta 0.5 – 4 Hz; theta 4-8 Hz; alpha 8-12 Hz) using the baseline-corrected phase locking value (PLV), an index of phase offset consistence between the LFP oscillations. PLV was averaged at the region-of-interest (ROI) pair level, the ROI-pair grand average was computed over all subjects and compared to distribution based on 200 trial-shuffling permutations. Significant increases or decreases were defined as >97.5% or <2.5% of shuffled distribution, respectively.
Within temporal cortex, the peri-SWR decreased in theta PLV between the active populations (electrodes with significant HGA modulation). In contrast, there were no PLV changes between the inactive populations (electrodes with no significant HGA modulation). Inter-regional theta PLV between the amygdala and temporal cortex increased between active populations and decreased between non-active populations.
This functional connectivity pattern could allow selective recruitment of anatomically distributed neuronal populations during peri-SWR windows, which could facilitate the formation of horizontal connections, resulting in gradual independence of distributed memory traces from hippocampus.

Disclosures: **J.J. Lin:** None. **H. Zhang:** None. **B.L. McNaughton:** None. **I. Skelin:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.19/AA41

Topic: H.02. Human Cognition and Behavior

Title: Changes in frequency power during anesthesia induction with propofol: A comparison between frontal cortex and the mesial temporal lobe

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Abstract: The neural mechanisms by which anesthetic agents induce loss of consciousness (LOC) are poorly understood despite their common use for sedation and general anesthesia. Propofol-induced anesthesia has been associated with the increased power of slow delta and alpha oscillations on scalp EEG and ECoG after LOC, however, how Propofol influences the

oscillatory activities of deep structures, such as the mesial temporal lobe (MTL) is unclear. To test if Propofol has a similar effect on the oscillatory activity of MTL compared to cortex, we performed intracranial recordings in four patients implanted with multiple depth electrodes for invasive epilepsy monitoring during anesthesia induction with Propofol. The targets of the depth electrodes included the amygdala, hippocampus, orbitofrontal cortex, and anterior cingulate cortex. The recording was carried out from ten minutes prior to induction to fifteen minutes following LOC. Contacts residing within the gray matter of the target structures were referenced to a contact in the white matter. We compared the spectrograms of the LFP of the target structures from 5 minutes before the administration of Propofol to 5 minutes following LOC. Z-scored power changes within individual frequency bands were examined, including the slow oscillation (0.1-1 Hz), delta (1-3 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (13-25 Hz), low gamma (25-40 Hz), and high gamma (40-100 Hz). In the cortical area (including the orbital frontal cortex and anterior cingulate cortex), a significant broadband (0.1Hz-40Hz) power increase is observed in all patients (all $p < 0.05$, paired t-test). However, in the MTL (including hippocampus and amygdala), the power change varies from patient to patient; both increases and decreases compared to baseline prior to induction of anesthesia. which renders the grand average of power change in MTL not statistically significant. In summary, these uncorrelated spectral power changes between the cortex and the MTL suggest functional dissociations in these two structures, which may link to a putative mechanism of LOC and the retrograde amnesia caused by anesthesia.

Disclosures: **S. Ma:** None. **M.R. Paff:** None. **I. Skelin:** None. **H. Zhang:** None. **J. Lin:** None.

Poster

422. Human Long-Term Memory: Medial Temporal Lobe III

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 422.20/AA42

Topic: H.02. Human Cognition and Behavior

Support: NINDS Grant R37NS21135 to Knight RT
NINDS Grant U19 NS107609-01 (PI: Buffalo EA ; Site PIs: Knight RT, Lin JJ)

Title: Feedback-tuning of single neurons: Evidence for distributed learning signals in the human brain

Authors: I. SKELIN[1], *H. ZHANG[2], A. DEDE[4], S. VADERA[3], M. PAFF[3], I. SEN-GUPTA[1], L. MNATSAKANYAN[1], F. P. K. HSU[3], E. A. BUFFALO[5], R. T. KNIGHT[6], J. J. LIN[1]; [1]Neurol., [3]Neurosurg., [2]Univ. of California Irvine, Irvine, CA; [4]Physiol. and Biophysics, [5]Dept. of Physiolgy and Biophysics, Univ. of Washington, Seattle, WA; [6]Psychology, Univ. of California Berkeley, Berkeley, CA

Abstract: During learning, humans utilize action outcome feedback, but the details of implementation at the individual neuron and network levels are largely unknown. The Wisconsin Card Sorting Task (WCST) consists of 12 possible rules, classified in 3 different dimensions (color, shape and texture), with the current rule unpredictably switching intra- or extra-dimensionally. This experimental schema provides a suitable model for investigating the neural and behavioral dynamics underlying feedback assessment during rapid rule acquisition and switching. We recorded ~250 isolated single units from the hippocampus (HIPP), amygdala (AMY), orbitofrontal (OFC) and anterior cingulate cortices (ACC), over 6 behavioral sessions in 4 patients with pharmacoresistant epilepsy undergoing presurgical evaluation. Single unit feedback tuning was assessed by contrasting the activity during the feedback phase (1500 msec) when the 'correct' or 'incorrect' message was displayed on the screen. Peri-stimulus trial histograms (PSTH) were constructed by averaging the individual unit activity separately on correct and incorrect trials (200 msec windows, 2 msec step size). Statistical significance of feedback-tuning was assessed for each time bin, using a two-tailed t-test with 100 permutations of trial labels. We identified feedback-tuned neurons in all four structures (ACC 37.3%; OFC 30.2%; AMY 24.3% and HIPP 8.1 %), with a significantly lower proportion in the hippocampus (chi-square test, p's < 0.005). Individual unit feedback-tuning modulations included both increased or decreased activity during both correct or incorrect trial feedback. The presence of anatomically-distributed feedback signals at the single neuron level provides evidence for a network-level integrative mechanism that utilizes this information to update the task representation and guide decision-making. Future work focuses on network-level interactions including directionality of information flow and task-state dependence.

Disclosures: I. Skelin: None. **H. Zhang:** None. **A. Dede:** None. **S. Vadera:** None. **M. Paff:** None. **I. Sen-Gupta:** None. **L. Mnatsakanyan:** None. **F.P.K. Hsu:** None. **E.A. Buffalo:** None. **R.T. Knight:** None. **J.J. Lin:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.01/AA43

Topic: H.02. Human Cognition and Behavior

Support: Marie Curie Individual Fellowship, EU Horizon2020 Framework (704506)

Title: Integrating memories: How congruency and reactivation aid integration of old and new memories

Authors: *M. T. VAN KESTEREN[1], P. RIGNANESE[2], P. GIANFERRARA[3], L. KRABBENDAM[1], M. MEETER[1];

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Abstract: In everyday life we continuously build up knowledge, which is important for forming consistent knowledge schemas that organize information and guide future learning. Successful knowledge building has been suggested to occur through reactivation of prior knowledge during new learning in item-specific perceptual brain areas. This reactivation is proposed to yield integration of new with old memories, supported by the medial prefrontal cortex (mPFC) and medial temporal lobe (MTL). Possibly as a consequence, congruency of new information with prior knowledge is known to enhance subsequent memory. Yet, it is unknown how reactivation and congruency interact to influence memory integration, and how these factors affect educational learning. To investigate this question, we used an AB-AC inference paradigm. University students first studied an AB-association followed by an AC-association, so B (a scene) and C (an object) were indirectly linked through their common association with A (an unknown pseudoword). Moreover, BC-associations were either congruent or incongruent with prior knowledge (e.g. a bathduck in a bathroom, or a hammer in a bathroom), and participants were asked to report subjective reactivation strength for B while learning AC. Behaviourally, both the subjective congruency and reactivation measures enhanced subsequent associative memory for the inferred BC-association. In the brain, these analyses yielded parametric effects of congruency and reactivation on activity patterns in perceptual areas (such as the Parahippocampal Place Area; PPA), the MTL, and the mPFC. Moreover, mPFC exhibited larger connectivity to the PPA for more congruent associations. Currently, we are running additional Representational Similarity Analyses (RSA) to investigate how these factors may affect the similarity of patterns throughout the brain. These outcomes show beneficial effects of both congruency and reactivation strength on memory formation, and provide insights into the neural mechanisms underlying these processes. With these findings, we aim to better understand and improve everyday knowledge building techniques, such as regularly practiced in e.g. educational situations.

Disclosures: M.T. Van Kesteren: None. **P. Rignanese:** None. **P. Gianferrara:** None. **L. Krabbendam:** None. **M. Meeter:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.02/AA44

Topic: H.02. Human Cognition and Behavior

Support: DARPA Contract No. N66001-17-2-4011

Title: Anterograde interference of reactivated memories only during the first hour after training: Evidence of an early reconsolidation loop

Authors: *A. E. CLAIN[1], B. A. WRIGHT[1,2,3];
[1]Dept. of Communication Sci. and Disorders, [2]Inst. for Neurosci., [3]Knowles Hearing Ctr., Northwestern Univ., Evanston, IL

Abstract: During learning, memories are transformed from a fragile to a stable state through a process of consolidation. There is growing evidence that when consolidated memories are accessed they re-enter a state of instability, during which they can be modified, and then must be consolidated again. This cycle of reactivation and reconsolidation has been demonstrated by showing that if a reminder of a consolidated memory is given days to months after an initial training session, that memory becomes vulnerable to retrograde interference. We previously reported behavioral evidence in humans of a mechanism similar to reconsolidation, but in which reactivated memories are susceptible to anterograde interference only within 24 h of the initial training. In that investigation, we documented anterograde interference of a reactivated memory 30 min, but not 24 h, after the initial training. Here we investigated the susceptibility of reactivated memories to anterograde interference at two intermediate time points: 1 h and 6 h. As previously, we used a perceptual-learning paradigm for which we had identified a case in which learning on a target task--interaural-level-difference discrimination (L)--was disrupted by training on a non-target task--interaural-time-difference discrimination (T)--in the anterograde (TL), but not retrograde (LT), direction. Then, also as previously, we used the task order that elicited anterograde interference (TL) in a paradigm akin to that typically used to test for reconsolidation. Listeners practiced a bout of L in isolation and sometime later practiced TL. This regimen yielded no improvement on L the day after training if the TL pair was trained 30 min after the initial training (previous data), suggesting that the initial bout of L was reactivated by the reminder and that both bouts of L were disrupted by the anterograde interferer. However, when the TL pair was trained 1 h (n=9), 6 h (n=7), or 24 h (previous data) after the initial training, listeners improved on L from the initial bout to the test bout the next day, suggesting that after 1 h the initial bout of L was no longer vulnerable to anterograde disruption during reactivation. Furthermore, the >1-h groups already showed improvement at the reminder bout of L, while the 30-min group did not (though learning on L normally emerges by 30 min), suggesting that resistance to anterograde interference at the reminder bout itself is associated with subsequent protection of the initial memory. These data imply that the first hour after training is a distinct stage of memory formation during which recently acquired memories can be reactivated and then disrupted by anterograde interference.

Disclosures: A.E. Clain: None. **B.A. Wright:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.03/BB1

Topic: H.02. Human Cognition and Behavior

Support: NSF GRFP Grant DGE-1650044

Title: Sleep age and race linked to neural pattern similarity of associative memory

Authors: *E. HOKETT, S. MIRJALILI, A. L. DUARTE;
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Abstract: Compared to young adults, older adults tend to have both worse sleep quality and episodic memory. It has been suggested that sleep fragmentation may interfere with memory consolidation and in turn, memory accuracy. Because these findings have been established in racially homogeneous samples, it is unclear how these effects extend to racial/ethnic minority groups. Racial/ethnic minorities have demonstrated worse self-reported sleep quality than non-minorities, which could interfere with sleep-based memory consolidation. Individual differences in the degree of reinstatement of neural activity present during learning at the time of retrieval has been shown to support episodic memory accuracy. Thus, we hypothesized that individual differences in sleep quality would decrease pattern similarity between neural oscillations at encoding and retrieval. To test this hypothesis, we recruited a diverse sample of young and older adults and measured their sleep quality for one week using accelerometry. Participants' EEG was monitored as they performed an associative memory test to determine relationships between memory-related neural oscillations, behavioral memory performance, and sleep quality. We found that greater neural pattern similarity between encoding and retrieval was related to better memory performance. Furthermore, Black participants, across age, demonstrated greater sleep fragmentation, which was associated with reduced pattern similarity and reduced memory performance. Thus, poor sleep quality is associated with episodic memory at both the behavioral and neural level. This research illuminates important relationships among habitual sleep quality, episodic memory, race, and age. Future research should include both age and race as variables of interest in order to further investigate the detrimental effects of poor sleep on memory.

Disclosures: E. Hokett: None. **S. Mirjalili:** None. **A.L. Duarte:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

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Program #/Poster #: 423.04/BB2

Topic: H.02. Human Cognition and Behavior

Support: NSF grant BCS-1461088
Human Frontier Science Program
Zuckerman STEM Leadership Program

Title: Multiple memories can be simultaneously reactivated during sleep as effectively as a single memory

Authors: *E. SCHECHTMAN[1], J. W. ANTONY[4], A. LAMPE[2], B. WILSON[1], K. A. NORMAN[5], K. A. PALLER[3];
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Abstract: Memory consolidation during sleep involves reactivation of memory traces. Targeting specific memories by presenting learning-related cues during sleep selectively enhances memory, but the mechanism behind this benefit is not fully understood. To better characterize the process of memory consolidation in humans, we tested whether multiple memories can be reactivated in parallel using a spatial-memory task. Results showed no differences between memory benefits for items in cued sets of one, two, or six items, which implies that reactivation resources are not divided between different memories. A compelling explanation for these findings is that a generalized context - and not individual memory traces - is reactivated during sleep. Intriguingly, sleep spindles and delta power modulations were sensitive to set-size and encode the extent of previous learning. Taken together, our results refute the notion that resource availability restricts the capacity of simultaneous sleep-reactivation and bring forward alternative and testable models for sleep-related consolidation.

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Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

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Program #/Poster #: 423.05/BB3

Topic: H.02. Human Cognition and Behavior

Support: Agencia Nacional de Promoción Científica y Tecnológica, PICT 2016-0229
Agencia Nacional de Promoción Científica y Tecnológica, PICT 2016-0243

Title: Updating the meaning of a word: Memory reactivation boosts integration into an existing memory trace

Authors: *L. KACZER[1], J. LAURINO[1], F. CHAVES[1], C. FORCATO[2], M. PEDREIRA[1]; [1]IFIBYNE CONICET, Buenos Aires, Argentina; [2]Unidad Ejecutora de Estudios de Neurociencias y Sistemas Complejos, CONICET, Univ. Nacional Arturo Jauretche, Hosp. de Alta Complejidad en Red El Cruce "Néstor Kirchner", Florencio Varela, Argentina., Buenos Aires, Argentina

Abstract: Words are extremely malleable memories, subject to updating and modification. However, the mechanisms that allow this plasticity remain unclear. While memory reconsolidation has been implied in memory updating, its role has not been addressed in the context of lexical-semantic learning. In this study we analyzed the contribution of memory reactivation to the process of a word's meaning updating. In four different experiments (N=128), native speakers of Spanish (19-35 years) learned either familiar or low-frequency words within their native language (Spanish), with their corresponding definitions (List 1). The following day, Reactivation groups were exposed to a reminder, consisting of the list of words they learnt the previous day, but with no chance of giving a response (thus increasing the prediction error). No Reactivation groups, on the other hand, did not receive a reminder. After this, all participants learned a new list of unrelated meanings for the words learned the previous day (List 2). Finally, memory retention was evaluated 48 hours or 7 days after training, using two procedures: a cued-recall test, and a semantic judgement task. Results were analyzed using a linear mixed-model. In the explicit recall task, our data show that memory for updated meanings is marginally enhanced by the reminder presentation one week after training. In the priming task, a significant enhancement of semantic recognition speed is revealed in the Reactivation group. Thus, memory reactivation might be one of the mechanisms that allow the rebuilding of the mental lexicon.

Disclosures: L. Kaczer: None. **J. Laurino:** None. **F. Chaves:** None. **C. Forcato:** None. **M. Pedreira:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.06/BB4

Topic: H.02. Human Cognition and Behavior

Support: DARPA Contract No. N66001-17-2-4011

Title: Evidence that anterograde learning interference arises from an interaction of memories in different learning stages

Authors: *R. NING[1], B. A. WRIGHT[1,2,3];
[1]Communication Sci. and Disorders, [2]Knowles Hearing Ctr., [3]Northwestern Univ. Inst. for Neurosci., Northwestern Univ., Evanston, IL

Abstract: Training induces learning on many tasks. However, learning on one task can be disrupted by training on another. Retrograde learning interference, in which learning on the first task is prevented by practicing the second task, is typically attributed to a disruption of consolidation. In contrast, the nature of anterograde learning interference, in which learning on the second task is disrupted by practicing the first task, is unknown. One possibility is that anterograde interference occurs because an influence of training on the first task lingers in the encoding stage, altering the encoding of the second task. If so, alternating training between the two tasks, while both are still being encoded, should also disrupt the learning. Here we tested this prediction using a perceptual-learning paradigm in order to take advantage of a previous observation that learning on an interaural-level-difference (ILD) discrimination task (L) was disrupted by training on an interaural-time-difference (ITD) discrimination task (T) in the anterograde [T-L], but not retrograde [L-T], direction. Given this case of isolated anterograde interference, we assessed whether interference also occurs when T and L are interleaved. Specifically, we examined learning on L between a training session (300-360 trials per task) and a testing session the next day (300 trials), following one of four training regimens: L alone, T before L, and T alternating with L at two different rates. Results showed that practicing only L (two-interval-forced choice; standard: 4 kHz, 0 dB ILD) generated across-day learning on L (n=13). This learning was disrupted when practice on T (standard: 0.5 kHz, 0 microsecond ITD) was completed before practicing L (anterograde interference) (n= 11), but not when practice alternated between T and L every 60 trials (n=10). Decreasing the alternation rate to every 180 trials led to a partial release from the interference (n=10). Similar patterns were observed in within-session learning and off-line gain. Thus, contrary to the prediction, the anterograde interference induced by blocked training on two tasks was alleviated by interleaved training. This outcome suggests that anterograde interference does not occur simply because the influence of the training on the first task lingers in the encoding stage. Rather, it appears that the training on

the first task must enter the consolidation stage, by sufficient training, to be disruptive to the encoding of the second task. The implication is that the initially encoded form of learning on the first task is modified during consolidation into another form and it is this consolidated form that is disruptive to learning on the second task.

Disclosures: R. Ning: None. **B.A. Wright:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

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Program #/Poster #: 423.07/BB5

Topic: H.02. Human Cognition and Behavior

Support: I-CORE program of the Planning and Budgeting Committee Grant 51/11
ISF Grant 526/17
Swedish Research Council (2017-00957)

Title: Memory reactivation: A mechanism underlying enhanced persistence of emotional memories

Authors: *N. HERZ[1], Y. BAR-HAIM[1], H. SHARON[2], E. HOLMES[3], N. CENSOR[1];
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Abstract: Memories of negative emotional events tend to persist over time relative to memories for neutral information. Such memory persistence has been mainly attributed to heightened encoding and consolidation processes. However, reactivation of the encoded information may also lead to memory enhancement through reconsolidation. We first tested the hypothesis that spontaneous reactivations of stressful events function as part of a mechanism enhancing persistence of visual emotional memory. 18 participants watched a film containing eight scenes of aversive material and their visual recognition memory of the film content was then immediately tested. In the following five days, participants recorded any spontaneously reactivated intrusive memories of the film using a digitized online diary, and the corresponding scene from which the content of their intrusive memories emerged was noted. At the end of the 5-day period, participants' visual recognition memory for the film content was retested. Results indicated that persistence of memory for scenes that intruded was enhanced relative to memory for scenes that did not intrude, suggesting that spontaneously reactivated intrusive memories reduce normative visual emotional memory decay. We then tested whether synchronizing lab-induced memory reactivations with inhibitory 1 Hz repetitive Transcranial Magnetic Stimulation (rTMS) would impair the intensity of intrusive memories. 40 participants watched a trauma film

and were requested to record their intrusive memories in a digital diary in the following 5 days. One day following film watching participants' memory of the film was reactivated by brief reminders, followed by rTMS over primary visual cortex (V1, in light of previous results showing that the vividness of mental images is coupled with early visual cortex activity, n=19) or over a control site (Vertex, n=21). Preliminary results indicate that V1-rTMS alleviated the distress of memory intrusions. Our findings point to a reactivation mechanism through which negative emotional memories gain their stabilization over time, and to potential interventions to downregulate their intensity.

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Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.08/BB6

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 MH069456

Title: The impact of predictability on memory representations

Authors: *M. KUMAR[1], N. B. TURK-BROWNE[2], K. A. NORMAN[1];
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Abstract: Our memory representations are shaped by our interactions with the world. What is not well understood is the exact nature of the mechanisms that cause memory representations to integrate (become more similar) or differentiate (become more distinct) over time. Recent work from our group has explored the implications of the non-monotonic plasticity hypothesis (NMPH) for integration and differentiation. According to the NMPH, moderate activation of memories causes synaptic weakening and higher activation cause synaptic strengthening. Applied to distributed patterns, the NMPH predicts a U-shaped function relating memory activation to changes in neural representations: If a memory does not activate, it is not changed;

if a memory activates moderately, it is differentiated from other, highly-active memories (due to weakening of connections to those memories); and if a memory activates strongly, it is integrated with other, highly-activated memories (due to strengthening of connections to those memories). To test these predictions, we ran an fMRI study using a statistical learning paradigm in which scene pairs (A-B) were embedded in a continuous stream of scenes; transition probability (i.e., the probability of B appearing after A) was varied in a parametric fashion across pairs, at eight levels ranging from 12.5% to 100%, in order to manipulate predictive activation strength. Extending prior work by Schapiro et al. (2012), we expected that intermediate transition probabilities would be sufficient to trigger prediction and moderate activation of B upon presentation of A, but this prediction would frequently be violated, leaving the activation moderate and leading to differentiation of B from A (which was presented and therefore strongly activated). Furthermore, we expected that higher transition probabilities would trigger stronger activation of B (including because the prediction was less likely to be violated), leading to integration of B with (also strongly activated) A. The advantage of this design is that it allows us to parametrically track levels of memory activation and the resulting changes in neural similarity between B and A, with the goal of showing a smooth transition from no learning to differentiation to integration as a function of memory activation. Preliminary results indicate prediction effects in the hippocampus, which we are now relating to changes in representational similarity.

Disclosures: **M. Kumar:** None. **N.B. Turk-Browne:** None. **K.A. Norman:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

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Program #/Poster #: 423.09/BB7

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 MH112357-01

Title: Twisting your memory: How the brain rewrites memories as the understanding of the past changes

Authors: *A. ZADBOOD[1], S. A. NASTASE[1], J. CHEN[2], K. NORMAN[1], U. HASSON[1]; [1]Princeton Univ., Princeton, NJ; [2]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: The brain actively re-shapes its past memories in light of new incoming information. In the current study, we used fMRI to track how neural representations are updated during naturalistic recall. Participants watched a movie (“The Sixth Sense”) in the scanner and -- at the end of the movie -- were exposed to a dramatic “twist” that changed the interpretation of the

previously encoded movie. Next, participants were asked to verbally recall the events in the scanner, while taking the new information into account. Most participants updated their recall to incorporate the twist. Two control groups recalled the movie without the need to update their memories during recall: one group never saw the twist, and thus recalled the original movie as-is; the second group was exposed to the twist prior to the beginning of the movie, which enforced encoding and recall of the same "twist" version of the movie. Our results show that providing participants with information about the twist beforehand (spoiled condition) altered the neural response patterns during the encoding phase (movie-viewing). Moreover, providing participants with information about the twist after the end of the encoding phase (post-viewing) transformed the neural representation of the encoded information during recall. The similarity between activation patterns during encoding and recall was attenuated in participants who better updated their recall to accommodate the new information. This suggests that neural representations of our past can be dynamically molded and integrated with new information that reshapes our memories.

Disclosures: A. Zadbood: None. **S.A. Nastase:** None. **J. Chen:** None. **K. Norman:** None. **U. Hasson:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.10/BB8

Topic: H.02. Human Cognition and Behavior

Title: Anticipation of temporally structured events in the brain

Authors: *C. LEE, M. ALY, C. BALDASSANO;
Psychology, Columbia Univ., New York, NY

Abstract: Higher-order regions in the brain play a role in organizing perceptual input into semantically-meaningful schematic representations, and they do so at differing timescales up the cortical hierarchy (Baldassano et al., 2017, 2018). Such integration in higher-order regions, such as the posterior medial network, critically depends on the availability of temporal structure over long timescales (Aly, Chen, Turk-Browne, & Hasson, 2018; Hasson, Chen, & Honey, 2015). The ability to extract temporal structure is particularly adaptive because it enables the generation of predictions about upcoming events. How does the brain use repeated experience in structured environments to anticipate what is likely to come next? In the present work, we examine how the brain can anticipate event boundaries in familiar sequences of actions.
Critically, we used a naturalistic stimulus (a movie), in which regularities are present at multiple timescales, enabling us to identify multiple timescales of prediction in the brain. We hypothesized that the timescale of neural prediction would vary continuously, with progressively

higher-order regions (e.g., prefrontal cortex) predicting further in the future than lower-order regions (e.g., visual cortex). To test this, we examined brain activity with fMRI while individuals watched a 90-second clip from the movie *The Grand Budapest Hotel* , six times. To uncover neural anticipation, we used a searchlight approach in which, for each region of interest, we fit a Hidden Markov Model to identify temporal shifts between multivariate activity patterns evoked by the first viewing of the movie clip compared to repeated viewings. We found temporal shifts in event patterns throughout the brain, up to 12 seconds prior to event onset. The scale of prediction varied hierarchically in an anterior (more prediction) to posterior (less prediction) fashion. Together, these results demonstrate that prediction is ubiquitous in the brain, but the amount of prediction varies across the cortical hierarchy.

Disclosures: C. Lee: None. **M. Aly:** None. **C. Baldassano:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.11/BB9

Topic: H.02. Human Cognition and Behavior

Title: A neural network model of naturalistic schema learning with computer-generated poetry

Authors: *M. E. SIEGELMAN, N. KRIEGESKORTE, C. BALDASSANO;
Psychology, Columbia Univ., New York, NY

Abstract: Naturalistic narrative stimuli such as movies and stories have proven to be a valuable tool for investigating how perception and memory are shaped by semantic and temporal structure in the environment. Studying and modeling the process of learning naturalistic structure is challenging, since it requires incorporating artificial experimental associations into naturalistic stimuli. We propose a novel text corpus generation method that can automatically construct a virtually unlimited number of natural-text stimuli with a pre-specified semantic structure. Specifically, we have designed a rhyming iambic pentameter poetry generator that writes poems progressing through ten distinct themes, with each four-line stanza in each poem embodying a single theme (e.g. *nature*, *music*, *warfare*). We have also developed a novel trial-and-error-based learning paradigm in which subjects must continuously select the correct next stanza (theme) from several competing options.
A pilot study of N=17 human subjects demonstrated that 76% subjects could achieve complete learning of the topic sequence (defined as the ability to correctly select the next stanza on twenty consecutive trials) after a short learning session ranging from 15-60 minutes. All subjects who performed at ceiling were able to correctly recall the full thematic sequence from memory following a short debriefing session in lab. These results demonstrate the feasibility of using computer-generated natural text stimuli for studying how abstract schematic sequences are

learned. Planned fMRI experiments will assess how responses in the language and semantic systems vary before and after learning when listening to the same set of poetry.
Since an arbitrarily large corpus of poetry can be generated using this approach, complex machine-learning models such as recurrent neural networks (RNNs) can also be fit to this same learning task. We found that a family of LSTM (Long short-term memory) models were able to achieve a word-level perplexity of 12.50 on this corpus, indicating that the model successfully captured iambic pentameter, rhyme-scheme, and critically, the thematic sequence. Temporal features of the model such as the timecourse of internal state changes (e.g. representing topic shifts) and the predictability of upcoming words can then be compared directly to fMRI responses for the same set of poetry.

Disclosures: M.E. Siegelman: None. **N. Kriegeskorte:** None. **C. Baldassano:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.12/BB10

Topic: H.02. Human Cognition and Behavior

Support: James S McDonnell Foundation Scholar Award to MDB
NSERC USRA to AHS

Title: Prediction errors at event boundaries drive episodic memory reconsolidation

Authors: *A. H. SINCLAIR[1], G. M. MANALILI[2], M. D. BARENSE[2];
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Abstract: Through the process of *reconsolidation*, memories can be destabilized and distorted. Recent research has proposed that *prediction error*, or surprise, is a critical prerequisite for reconsolidation. Yet, the neural mechanisms of this process remain elusive, particularly with regards to naturalistic episodic memories. In our novel fMRI paradigm, we demonstrated that prediction error drives episodic memory reconsolidation. On Day 1, the Experimental group (N=24) viewed 70 narrative videos, each depicting an action-outcome event. During the Day 2 fMRI session, we reactivated memories by presenting the videos again. Critically, we elicited surprise by interrupting half of the videos to violate the action-outcome contingency. On Day 3, we assessed memory for the videos. Control group participants (N=24) completed the memory test on Day 2, preventing the hours-long reconsolidation process. Behaviorally, we found that interrupting videos during reactivation significantly increased false memories in the Experimental group. The Control group exhibited fewer false memories and was not influenced by prediction error. Using a text-based similarity analysis, we demonstrated that semantic

interference among the videos was associated with false memories. In an event-related design, we examined neural activity after video offset, and found distributed post-event activity in the hippocampus, striatum, cingulate, and lateral parietal cortex. Relative to full-length videos, interrupted videos elicited less hippocampal activity, and greater activity in the angular gyrus, precuneus, and TPJ. Lastly, we related trial-wise hippocampal and striatal activity to subsequent false memories. Surprising interruptions may have disrupted episodic binding by attenuating the hippocampal response after an event boundary. For the first time, we demonstrate that prediction error allows naturalistic episodic memories to be altered, and implicate the neural correlates of memory destabilization.

Disclosures: A.H. Sinclair: None. **G.M. Manalili:** None. **M.D. Barense:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.13/BB11

Topic: H.02. Human Cognition and Behavior

Support: DARPA Contract No. N66001-17-2-4011

Title: Transient memory loss in humans 1-3 hours after training

Authors: *B. A. WRIGHT, A. E. CLAIN, R. NING;
Northwestern Univ., Evanston, IL

Abstract: During learning, memories are transformed from a fragile to a stable state through a process of consolidation. It has been proposed that this process involves multiple memory phases that each have different molecular requirements and distinct time courses. One behavioral pattern that is consistent with this proposal is the ‘Kamin effect,’ in which learning that is demonstrated soon after training is temporarily lost and then returns; the idea is that this transient memory lapse reflects a transition period between two memory phases. There have been numerous demonstrations of these memory lapses in animal models, but only a few in humans. Here we report two examples of a memory lapse in humans occurring 1-3 hours after training on a perceptual-discrimination task. We trained human young adults on an auditory interaural-level-difference (ILD) discrimination task (two-interval-forced choice; standard: 4 kHz, 0 dB ILD) for 300 trials and then tested performance on the trained task (learning), or on an untrained task--interaural-time-difference (ITD) discrimination (standard: 0.5 kHz, 0 μs ITD) (generalization)--at a variety of time points 30 minutes to 10 hours after training. Different groups completed each tested-task/time-point combination. We also assessed naïve performance on each task. Discrimination performance was non-monotonic over the post-training period, with a memory lapse at 1-3 hours revealed by a statistical trend toward a learning decrement for the trained task

(ILD discrimination) and a significant decrement for the untrained task (ITD discrimination) during that time period. After training on ILD discrimination, thresholds on that task were ~1.4 dB lower (better) than the starting thresholds at 30 minutes (n=11) and 6-10 hours (n=20) post-training, but only ~0.9 dB lower at 1-3 hours (n=17) post-training. Similarly, after training on ILD discrimination, thresholds on the untrained ITD-discrimination task were ~28 μs lower (better) than for a naïve control group (n=60) at 0 minutes (n=16), 30 minutes (n=38) and 10 hours (n=16) post-training, but were ~6 μs higher than naive performance at 1 hour (n=8) post-training. These results thus illustrate memory lapses in human perceptual learning 1-3 hours after training and demonstrate that these lapses can occur both in learning and generalization. To the extent that these lapses reflect transitions between different memory phases, these data suggest that the form of perceptual memories undergoes a marked shift in the first few post-training hours.

Disclosures: B.A. Wright: None. **A.E. Clain:** None. **R. Ning:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.14/BB12

Topic: H.02. Human Cognition and Behavior

Support: NRF-2017M3C7A1031333

Title: A neural marker of declarative memory maintenance

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Abstract: The hippocampus has been thought to be critical for the formation of declarative memory. Prior memory studies suggest that the changes in neural activation and synaptic strength in the hippocampus underlie the consolidation and maintenance of declarative memory. However, it remains unclear whether the change of neural response patterns in the hippocampus during learning determines the maintenance of declarative memory in human subjects. Here, we performed an event-related functional magnetic resonance imaging (fMRI) experiment to investigate whether subsequent memory can be predicted from changes in neural response patterns during learning. In the experiment, participants were asked to conduct a memory task, consisting of separate learning, 1-day retrieval and 4-week retrieval sessions. During the learning session, the participants were trained to memorize pairs of images (object or building images) and person's names inside the scanner while the cortical activity was monitored. While each image-name association was learned individually, an object image and a building image were

associated with a common name. During the 1-day or 4-week retrieval session, the participants were asked to indicate whether the presented two images were associated. Using trial-by-trial similarity analysis, we found that memory performance at the 4-week retrieval session could be predicted from the change of the between-stimulus pattern similarity for the associated object-building pairs in the CA3/DG region of the hippocampus. This prediction was not available from the change of the neural pattern similarity for the non-associated pairs. These results showed that changes of neural pattern similarity in the hippocampus during learning can be used as a neural marker for the maintenance of declarative memory.
This work was supported by the Brain Research Program (NRF-2017M3C7A1031333) through the National Research Foundation (NRF) of Korea.

Disclosures: M. Kwon: None. **S. Lee:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.15/BB13

Topic: H.02. Human Cognition and Behavior

Support: SNF Grant 172761

Title: How memory-based decisions evolve over time

Authors: *P. M. KRAEMER, S. GLUTH;
Dept. of Psychology, Univ. of Basel, Basel, Switzerland

Abstract: Human decision making often relies on information which is retrieved from episodic memory. Those memory-based decisions are composed of at least two cognitive processes: a) memory retrieval which gathers information from an episodic memory store, and b) preferential choice which weighs choice options against each other, contributing to action selection. Despite growing interest in the interaction of these processes (e.g. Shadlen & Shohamy, 2016, Neuron) it is still unclear how they interact on the neural level. In particular, the temporal characteristics of the interaction are unknown. To investigate the temporal emergence of these processes, we developed an EEG experiment in which participants performed a remember-and-decide task. During an encoding period, they associated peripherally presented food snack items with centrally presented abstract symbols. During a decision period, only the symbol was presented. The participants had to retrieve the associated snack and make a preferential choice between the snack and a fixed money amount by pressing a button with the left vs. right index finger. The experiment was designed to find hemisphere-specific neural events which can be attributed to a) the peripheral encoding of snack items or b) the lateralization of motor responses. Adopting contralateral-control methods (Gratton, 1998, Psychophysiology) allowed us to identify neural

signatures for memory and decision-making processes. We performed a time-frequency analysis on the obtained data. During decision periods, we found early stimulus-locked alpha- and beta activity in central and parietal electrode clusters. Furthermore, our analysis indicated that a cluster of centro-parietal electrodes shows signs of memory and decision-making processes in the beta-band activity, prior to response initiation. Our findings contribute to the understanding of the neural underpinnings of memory-based decision making. Furthermore, based on the results, we discuss the development of a cognitive process model that accounts for the temporal relationship of memory retrieval and decision making.

Disclosures: P.M. Kraemer: None. **S. Gluth:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.16/BB14

Topic: H.02. Human Cognition and Behavior

Support: NSF EPSCoR Grant 1632738

Title: Exploring the evolving geometric structure of experiences and memories

Authors: *P. C. FITZPATRICK, A. C. HEUSSER, J. R. MANNING;
Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Each event we experience in our day-to-day lives undergoes a series of transformations as it is encoded and maintained in memory. Our brains' distributed representations of memories by their contextual features gives rise to a framework in which temporally distant events may be recognized as related, and into which subsequent memories may be incorporated. In turn, existing memories within this framework influence how we process and remember future experiences. We recently developed an approach that models experiences and their corresponding recollections as "thought trajectories"—geometric shapes that unfold in a high-dimensional space defined by the experience's content (Heusser et al., 2018). Here, we build upon that approach to characterize how these thought trajectories are altered by the passage of time and the contextual congruence of existing memories. Participants viewed the pilot episode of a popular TV show (*Atlanta*), verbally recalled its content, and predicted how the next episode would unfold. A week later, they again recalled the pilot before viewing and recounting either the show's second episode or the pilot of a different show (*Arrested Development*). We examine how participants' memories are morphed over the intervening week, and how successful recall of the second week's episode is influenced by that of the first. We then draw upon a model of distributed neural semantic representations (Huth et al., 2016) and a massive online database of neuroimaging studies (Yarkoni et al., 2011) to map

these trajectories and their distortions onto the human cerebral cortex. This enables us to examine how the neural representation of an experience's content influences its inherent memorability.

Disclosures: P.C. Fitzpatrick: None. **A.C. Heusser:** None. **J.R. Manning:** None.

Poster

423. Human Long-Term Memory: Modulation

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Topic: H.02. Human Cognition and Behavior

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Institute of Information & Communications Technology Planning & Evaluation(IITP) grant funded by the Korea government (MSIT) (No.2019-0-01371, Development of brain-inspired AI with human-like intelligence)

Title: Predicting subjective and objective memory recollection from prefrontal cortex activations using high-density fNIRS

Authors: *Y. RAH[1], J. SHIN[2], S. LEE[1];
[1]Dept. of Bio and Brain Engin., [2]Program of Brain and Cognitive Engin., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Meta-memory is the subjective judgement and monitoring of one’s own memory. Although it has been associated with the prefrontal cortex (PFC), it is unclear whether meta-memory and actual memory accuracy have dissociable neural signatures. In this study, we began addressing this issue by identifying PFC activity related to meta-memory judgments using a portable, versatile high-density functional near infrared spectroscopy(fNIRS) system.
Healthy adults(n=8) watched various animations of objects moving one-by-one into baskets. After a delay, subjects were shown the target video or a lure video and were asked whether they recognized the video as one that they had seen before. To measure meta-memory, subjects were asked to rate the confidence of their recognition judgment and the amount of details that they remembered. To assess memory accuracy, subjects were prompted to recreate the original video by clicking with the mouse the objects and baskets in the correct sequence.
Overall, subjects had 81% accuracy in active recollection and 69% accuracy in recognition. Subjects reported higher confidence on correctly recognized trials than on incorrect trials ($t(82)=-2.89$, $p=.005$). Analysis of fNIRS activity showed that, consistent with previous studies, right lateral PFC activity was associated with subjective uncertainty of memory during recall (r=-

0.72, p=.067 for confidence of recognition, r=-0.76, p=.048 for reported amount of detail) while left lateral PFC activity was associated with the higher meta-memory (r=0.71, p=.076). Moreover, different neural signals during encoding were predictive of subjective and objective recollection. Higher activation of the right PFC during encoding predicted later reporting of more details in subjective recollection. In contrast, higher frontopolar cortex activation during encoding predicted higher accuracy during recollection (right: r=0.79, p=.033, left: r=0.87, p=.010). Though it had been previously shown that the rPFC is associated with subjective recollection and that the frontopolar cortex is associated with episodic memory retrieval, their involvement in encoding has not been widely reported. One possible explanation is that the rPFC's monitoring of new episodic information during encoding contributes to better performance during recollection.
In conclusion, we observed multiple signals from the PFC that correlate with subjective/objective memory and revealed that these predictive activations may be specific to the encoding or retrieval. Such measurements provided by a portable fNIRS system could have wide applications for assessing meta-memory in various clinical and educational settings.

Disclosures: **Y. Rah:** None. **J. Shin:** None. **S. Lee:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.18/BB16

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant BCS 1539361

Title: REM sleep enhances emotion-relevant recapitulation

Authors: ***R. M. BOTTARY**[1], S. M. KARK[1], R. T. DALEY[1], J. D. PAYNE[2], E. A. KENSINGER[1];
[1]Psychology, Boston Col., Chestnut Hill, MA; [2]Psychology, Univ. of Notre Dame Dept. of Psychology, Notre Dame, IN

Abstract: Episodic memory retrieval is enhanced when brain regions engaged during memory encoding become reactivated at the time of retrieval, a phenomenon termed recapitulation. Additionally, slow wave sleep (SWS) is implicated in memory consolidation and rapid eye-movement (REM) sleep in emotional memory processing. Here, we investigate the links between individual differences in the spatial extent of emotionally-enhanced recapitulation and REM and SWS. Twenty-two healthy adults (11F, 11M; age: 19-29 years) underwent fMRI scanning during an incidental encoding task and a surprise recognition memory task following a 24-hr delay that included overnight, polysomnographically-recorded sleep. During encoding, participants viewed

line drawings of negative, neutral and positive images, each followed by their full-colored photo. At recognition, participants distinguished new from encoded line drawings, and rated the vividness of their memories. For each valence (negative, neutral, positive), recapitulation was defined as the whole-brain encoding-to-retrieval spatial overlap (i.e., conjunction of Encoding Hits > Misses ∩ Recognition Hits > Misses). We then calculated the spatial extent of valence-specific recapitulation processes (recap%) for each participant by dividing the total number of voxels observed in the Encoding ∩ Recognition maps by the total number of voxels observed in their corresponding encoding maps. To calculate emotion-specific recapitulation, we subtracted neutral recap% from negative and positive recap% separately, creating two summary values (neg-neu recap%; pos-neu recap%). Correlations between recap%, sleep metrics, and memory performance were conducted using Pearson coefficients or Spearman's Rho. REM sleep percentage was positively associated with neg-neu recap% ($r=.440$, $p=.041$) and pos-neu recap% ($r=.444$, $p=.039$). SWS percentage did not correlate with these metrics (ps > 0.5). Neutral recap% was positively associated with neutral memory ($r=.526$, $p=0.012$) and positive recap% was positively associated with positive memory at a trend level ($r=.397$, $p=.067$). The relation between negative recap% and negative memory did not reach significance. Individuals who spent a greater proportion of time in REM sleep were better at re-engaging encoding-related brain regions during the retrieval of emotional vs. neutral memories. This effect of REM sleep may provide a mechanistic explanation for why REM sleep is linked to enhanced emotional memory performance.

Disclosures: R.M. Bottary: None. **S.M. Kark:** None. **R.T. Daley:** None. **J.D. Payne:** None. **E.A. Kensinger:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.19/BB17

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01AG049002

Title: Cortical thickness predicts recall improvement due to noninvasive stimulation targeting the hippocampal-cortical network in older adults

Authors: *Y. LAGOUTINA[1], A. S. NILAKANTAN[2], M. A. NIASARI[4], L. WANG[5], J. L. VOSS[3];
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Abstract: Aging is associated with cortical atrophy, as well as with long-term episodic memory decline. Previous findings have suggested that it may be possible to alter function of the hippocampal-cortical network using transcranial magnetic stimulation (TMS), leading to memory improvement. The present experiment aimed to evaluate whether age-related cortical atrophy may predict the amount of memory improvement caused by TMS targeting the hippocampal-cortical network. A sample of 16 participants, aged 60-81, and 31 participants, aged 18-35, received TMS targeting the hippocampal-cortical network for 5 consecutive daily sessions. Effects of stimulation on memory were tested using an object-location paired-associates task administered before and after stimulation, as well as before and after a sham stimulation condition. Cortical thickness regions of interest (ROIs) were identified and evaluated using FreeSurfer software. We identified a canonical pattern of cortical thinning by comparing thickness values for younger versus older adults in our sample. Regions with thinner cortex for older versus younger adults included the default mode network (DMN), as well as several other regions that have shown aging-related atrophy in previous studies. Reduced cortical thickness in these regions predicted greater improvement in object-location recall memory ($R^2 = .25$) in the older participants, such that older adults with thinner cortex had relatively greater recall improvements. These findings suggest that memory gains due to the manipulation of hippocampal function via TMS are more salient for older adults with greater levels of cortical atrophy. This may be due to reduced compensatory mechanisms that are normally supported by the cortex in these individuals, causing them to be more dependent on hippocampal contributions to memory.

Disclosures: Y. Lagoutina: None. **A.S. Nilakantan:** A. Employment/Salary (full or part-time):; Northwestern University. **M.A. Niasari:** None. **L. Wang:** A. Employment/Salary (full or part-time):; Northwestern University. **J.L. Voss:** A. Employment/Salary (full or part-time):; Northwestern University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH Grant no. R01AG049002.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.20/BB18

Topic: H.02. Human Cognition and Behavior

Support: NSERC Discovery Grant 03637
Canada Foundation for Innovation - John R. Evans Leaders Fund (Infrastructure)
Queen's University Research Initiation Grant (Infrastructure)
NIH Grant 1U54MH091657

Title: Conformance of mental events to a group timeline during movie-viewing reflects cognitive ability

Authors: *J. TSENG[1], J. POPPENK[2];
[1]Ctr. for Neurosci. Studies, [2]Dept. of Psychology, Queen's Univ., Kingston, ON, Canada

Abstract: Movies induce similar brain activity across individuals. This organization can be found not only in perceptual cortices, but also higher-order brain areas, perhaps driven by semantic content. We recently validated procedures for deriving mental events from whole brain activity and found that these events, too, were organized by movies. However, the degree to which mental events conformed to the group timeline (i.e., conformity) differed across individuals. Do these individual differences reflect noise or something meaningful about those individuals?

To answer this question, we developed and validated a method to quantify the degree of shared structure, then investigated its neuroanatomical and behavioural correlates. Behavioural studies have shown that the tendency to identify the same conceptual boundaries in movie timelines as others is predictive of memory and general cognitive ability; thus, we hypothesized a positive correlation between conformity and cognitive ability. Also, because previous studies have found hippocampal activation at conceptual boundaries during movies, we hypothesized that properties of the hippocampus would predict conformity.

We conducted our analysis on 184 healthy participants (age: 22-35 years) from the Human Connectome Project dataset. Each participant had four 15-min movie-viewing functional MRI runs. Previously, we developed a method to obtain a timeline of network meta-state changes, which we determined to correspond to mental events, for each participant and movie run (*step distance vector*; Tseng & Poppenk, 2019). After applying this method, we calculated each participant's conformity by correlating the log of the participant's step distance vector to the log of the corresponding median group step distance vector. Because we found conformity to be internally stable across runs gathered on different days (mean $r = 0.50$), we averaged conformity values within participant to obtain a maximally stable trait estimate.

Our final step was to assess the relationship between conformity and recollection ability, cognitive function scores, and hippocampal volume. Higher conformity was correlated to higher Total Cognition Composite scores, driven by performance on the Picture Vocabulary subtest. Conformity was also correlated to better recollection memory and larger anterior/smaller posterior hippocampal volume. We conclude that conformity does not just reflect random statistical variation, but arises due to cognitive and neural characteristics of individuals. Our results further suggest that semantic knowledge helps to parse and encode streams of new information.

Disclosures: J. Tseng: None. **J. Poppenk:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

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Program #/Poster #: 423.21/BB19

Topic: H.02. Human Cognition and Behavior

Support: Natural Sciences & Engineering Research Council Discovery Grant 03637
Canada Foundation for Innovation - John R. Evans Leaders Fund (Infrastructure)
Queen's University Research Initiation Grant (Infrastructure)
NIH Grant 1U54MH091657

Title: Familiar movies are less effective at aligning viewers' cognitions

Authors: *Y. LU[1], J. TSENG[2], J. POPPENK[1];
[1]Psychology, [2]Neurosci., Queen's Univ., Kingston, ON, Canada

Abstract: What is different when we watch a movie for the second time? It is well established that novel stimuli better grab our attention, and that familiar stimuli are processed more efficiently. But what does this mean for our mental experience of movie-viewing? To address this question, we examined the impact of novelty on the tendency of movies to align participants' mental events. We have previously established (Tseng & Poppenk, 2019) that movie stimuli exert a powerful organizing quality over the timing of large-scale network transitions, and linked these transition points to mental events (apparently reflecting a shift to a new thought). Here we examined the possibility that novel movies are particularly effective at affecting such alignment due to greater stimulus control over cognition, and examined neural correlates of this phenomenon. As the anterior temporal lobe is known to play an important role in novelty processing, we focused our neural analysis on this region.
To this end, we analyzed fMRI data from 184 participants in the 7T Human Connectome Project dataset. Participants watched the same 82-second movie clip during four different scanning sessions. We distinguished between the first viewing (novel stimulus) and subsequent viewings (familiar stimulus). To identify mental events, we used procedures developed by Tseng and Poppenk to isolate large-scale brain network transitions. We then computed the group alignment representing how each individual's thought trajectory was in synchrony with that of the group as a whole, and the rate of underlying transitions. Finally, we regressed transition rate and group alignment with the hippocampus and amygdala volumes.
We found that group alignment for familiar runs was significantly lower than the alignment for the initial run and the transition rate for familiar runs was lower than the transition rate for the initial run. With respect to hippocampal and amygdala predictors, we found that a larger left and right hippocampal head, as well as a larger left and right amygdala, predicted more group alignment and fewer transitions for the initial run. However, these effects were weakened in the

familiar runs. These findings suggest that familiar stimuli are less effective at organizing our thoughts, likely because they become more idiosyncratic and less stimulus-driven. The implication of the hippocampus and amygdala is also consistent with their known role in novelty processing. We conclude that watching a movie for the second time really is different - not only do viewers know what's coming, but a director's ability to command their viewers' thoughts is diminished.

Disclosures: **Y. Lu:** None. **J. Tseng:** None. **J. Poppenk:** None.

Poster

423. Human Long-Term Memory: Modulation

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Topic: H.02. Human Cognition and Behavior

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Queen's University Research Initiation Grant to J.P. (supported by the Canada Research Chairs program)

Title: Relatively large posterior hippocampal volumes predict greater memory precision in movie timeline task

Authors: *S. W. GORLICK, J. POPPENK;
Psychology, Queen's Univ., Kingston, ON, Canada

Abstract: Observations of anatomical and functional differences along the long axis of the hippocampus (HPC) have drawn attention to the nature of specializations of its anterior and posterior segments. More specifically, it has been proposed that gist-like episodic memory representations reside in the anterior portion, whereas more detailed episodic memory representations lie in the posterior portion. Here we sought to test this idea by evaluating memory for a movie narrative using tasks designed to measure both accuracy and precision. We conducted our analysis on data from 66 healthy participants between the ages of 22 and 35 years. Each participant first completed an ultra-high-resolution T2w image of their medial temporal lobe (0.5 mm isotropic) and a lower-resolution T1w whole-brain scan (0.7mm isotropic) to calculate hippocampal volume and the volumes of proximal structures. We corrected these values for intracranial volume. Participants then viewed a 20-minute movie, *Bang! You're Dead*, prior to completing memory tests assessing detail and gist memory for the

film. These included a timeline test, in which participants moved still movie frames to their location within the movie timeline; and a movie sequence task, in which participants rearranged three still movie frames into the order in which they occurred. Finally, the movie exam contained multiple choice and fill-in-the-blank questions about the film. Scores from each test were correlated with results from structural scans to assess whether individual differences in hippocampal volume predicted memory performance.
We found that a larger right anterior, but not posterior hippocampus predicted significantly more errors and lower precision on the timeline task. By contrast, in both hemispheres, a larger posterior hippocampus predicted smaller numeric errors in quantitative exam questions. Finally, larger right posterior hippocampus volumes predicted better relative ordering of stimuli in the sequence task.
These results support the notion that the anterior hippocampus plays a role in more approximate memory, whereas the posterior hippocampus features more precise ones. More broadly, it reinforces findings that distinguish between the volumetric properties of anterior and posterior hippocampal segments as predictors of memory.

Disclosures: S.W. Gorlick: None. **J. Poppenk:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.23/BB21

Topic: H.02. Human Cognition and Behavior

Support: SSHRC Insight Grant 435-2018-0895
NSERC Discovery Grant 03637
CFI – John R. Evans Leaders Fund
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Canada Research Chairs Program

Title: Emdr is not "special": De-arousal effects reflect domain-general memory-weakening phenomena

Authors: *J. POPPENK, D. WILSON;
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Abstract: Arousal associated with aversive memories can be attenuated using the eye-movement desensitization and reprocessing (EMDR) technique, which typically requires participants to visualize the target memory while tracking a stimulus bilaterally. Such "de-arousal" can, however, also be obtained using substitute tasks for stimulus tracking, which is interpreted as evidence that it is mental visualization that lies at the core of EMDR's effects. In the current

study, we explored the possibility that visualization, too, is immaterial, as would be predicted by proposals that it is partial memory reactivation in general that underlies memory weakening, rather than a particular procedure for memory retrieval. We further explored whether neural characteristics might offer further clues into the specificity of the underlying mechanism. 65 participants completed a word-scene encoding task in which words described crimes that putatively occurred in the scenes. In an emotional appraisal task, participants then rated the scenes as more arousing than others. They next tracked a moving dot while scenes were presented behind the viewing area. In another emotional appraisal after dot-tracking, arousal for reactivated scenes returned to baseline, whereas arousal for non-reactivated scenes did not. We found this de-arousal could be predicted by patterns of structural and functional connectivity involving the anterior hippocampus, posterior hippocampus and corpus callosum. This pattern was most consistent with the idea that it was incomplete memory reactivation that contributed to weakening effects. Because neither stimulus oscillation nor mental visualization appear necessary to obtain EMDR-like effects, we argue EMDR is not distinctive among paradigms that induce memory-weakening, and that its effects can be parsimoniously explained using the non-monotonic plasticity hypothesis. Our findings also offer the first evidence that theoretically meaningful biological predictors can be used to predict which individuals will find memory weakening procedures most effective. In a health context, memory-weakening mental health treatments could one day be calibrated based upon neural inputs; in other contexts (such as eyewitness memory), the findings could one day reveal which individuals are most susceptible to memory-weakening.

Disclosures: **J. Poppenk:** None. **D. Wilson:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.24/BB22

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust/Royal Society Sir Henry Dale Fellowship

Title: Sequential reactivation during offline periods in humans

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Abstract: Our ability to remember past events relies on the re-emergence of learning patterns during sleep, a process referred to as memory reactivation. Memory reactivation has been linked to sleep spindles (10-16Hz), an oscillatory pattern thought to mediate the re-emergence and,

hence, strengthening of previously learned associations. In humans, previous studies focused on simple paired-associate learning (e.g. learning of word-picture associations), although episodic memories tend to contain multiple, sequentially experienced elements. Indeed, animal studies have provided evidence for reactivation of learning sequences ('replay') and suggest that sequential reactivation occurs in a compressed and forward manner. However, little is known about the temporal dynamics of sequential reactivation during offline periods in humans. Here, we applied targeted memory reactivation (TMR) to cue previously learned sequences of object-face-scene triplets during a post-learning nap (N = 19) or a matched post-learning wake period (N = 21) using high-density Electroencephalography (EEG). Memory cueing was performed during NREM sleep, presenting previously learned sounds (semantically linked with 50% of the objects) and control sounds (without any semantic content). Behavioural results confirm that triplets were indeed encoded as a sequence, as the conditional probability to correctly retrieve a face without remembering the following scene was higher than correctly retrieving a scene without remembering the preceding face ($p < .001$). Importantly, memory performance for sequences that were cued during the nap was higher compared to sequences not cued ($p = .014$), establishing that TMR for sequences was successful. On an electrophysiological level, both target and control cues elicited an evoked response (k-complex) and a significant increase relative to a pre-cue baseline in a broad frequency range (5-20Hz) roughly 1 second after cue onset ($p < .05$). Most interestingly, target cues elicited a stronger response compared to control cues in the sleep spindle frequency range between 1 - 2.5 seconds post cue ($p < .05$). Together, our data propose that sequential memories benefit from TMR and that cue-triggered spindles are likely to ignite the sequential reactivation of a memory trace.

Disclosures: **M. Petzka:** None. **G. Balanos:** None. **B.P. Staresina:** None.

Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

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Program #/Poster #: 423.25/BB23

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust/Royal Society Sir Henry Dale Fellowship to B.P.S. (107672/Z/15/Z)

Title: Post-learning sleep oscillations track learning networks as revealed via human electrocorticography

Authors: *H.-V. V. NGO[1], Y. Y. CHEN[2], D. YOSHOR[3], B. L. FOSTER[2], B. STARESINA[1]; [1]Univ. of Birmingham, Birmingham, United Kingdom; [2]Dept. of Neurosurg., Baylor Col. of Med., Houston, TX; [3]Baylor Col. Med., Houston, TX

Abstract: Memory consolidation, the transformation of labile memory traces into stable long-term representations, is facilitated by post-learning sleep. Computational and biophysical models suggest that cortical slow waves and thalamo-cortical sleep spindles play a key mechanistic role in the service of consolidation. In particular, by gating molecular signal cascades, these oscillations are thought to ignite structural changes at cortical learning sites. Here we tested the prediction that slow wave/spindle patterns are enhanced specifically over learning-related cortical areas. Six patients implanted with intracranial grid/strip arrays performed an associative word-image learning task in the evening prior to sleep. For half of the recording sessions, words were paired with images of objects and for the other half, paired with images of scenes. Memory was tested prior to sleep (PM) and again next morning (AM). Intracranial data was recorded during memory tests and intervening sleep. On the PM test, participants recalled the correct target image for 51% trials (SEM=5%), indicated the wrong target image on 20% (SEM=3%) and forgot the target on 25% (SEM=5%). On the AM test, the corresponding proportions were 41% (SEM=4%), 23% (SEM=3%) and 31 % (SEM=6%), pointing to moderate overnight forgetting rates. To identify cortical sites tuned to the learned image category, a separate visual localizer session was employed. Here, participants processed various image categories (objects, scenes, faces, body parts, numbers and words) and category-specific tuning was defined as a selective gamma band (50-150 Hz) increase for the target category (objects or scenes) relative to all other categories. Across participants, we recorded from 720 cortical sites, with 95 contacts (13%) showing object- or scene-specific tuning. Slow wave and spindle activity was algorithmically detected for non-rapid-eye-movement (NREM) sleep during the first half of the night. Intriguingly, both slow wave and spindle amplitudes were significantly increased at cortical sites selectively tuned to the learned image category as compared to non-target sites ($P < .01$). These results are consistent with the notion that slow wave and spindle activity track learning networks and thereby facilitate memory consolidation.

Disclosures: **H.V. Ngo:** None. **Y.Y. Chen:** None. **B.L. Foster:** None. **B. Staresina:** None. **D. Yoshor:** None.

Poster

423. Human Long-Term Memory: Modulation

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Program #/Poster #: 423.26/BB24

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Support: Wellcome Trust/Royal Society Sir Henry Dale Fellowship to B.P.S. (107672/Z/15/Z)
Swiss National Science Foundation (P300P1_174450) to TS

Title: Differential contributions of sleep oscillations to item- and associative memory consolidation

Authors: *T. SCHREINER, B. P. STARESINA;
Sch. of Psychology and Ctr. for Human Brain Hlth., Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Post-learning sleep bolsters the stabilization of newly formed episodic memories. At the scalp level, the two oscillatory sleep signatures most consistently linked to memory processes are neocortical slow oscillations (SOs, < 1 Hz) and thalamocortical spindles (10-16 Hz). In particular, SOs are thought to orchestrate the intricate interplay of spindles with hippocampal ripples, the latter presumably reflecting reactivation of recent memory traces. A key prediction emerging from this framework is that SOs specifically mediate the consolidation of hippocampus-dependent memories, e.g. associative memories as opposed to non-associative item memories.Here we set out to elucidate the precise contributions of SOs to the consolidation of different expressions of declarative memory. Prior to sleep, participants (n = 17) encoded word-picture associations. During subsequent retrieval, participants first performed old/new judgments on previously presented ('old') and novel ('lures') words (item memory), followed by recall of the associated image (associative memory). Importantly, half of the stimuli were tested immediately after learning and half after an afternoon nap, ~2h later. The difference between post-sleep and pre-sleep performance indexed the extent of memory consolidation. Throughout the experiment, brain activity was recorded using electroencephalography (EEG). To elucidate the impact of SOs on the consolidation of item and associative memory, we detected SOs during non-rapid eye movement (NREM) sleep and correlated their density (number/minute) at fronto-central sensors with memory performance (difference in retrieval performance before and after sleep).Behavioural results revealed that participants recognized 73.6 ± 2.7% of the presented old words (HITs) during the initial memory test, out of which 65.2 ± 3.7% of the associated images were retrieved. After the sleep interval participants still recognized 64.1 ± 2.7% of the items and remembered 56.9 ± 4.5% of the targets. On a neuronal level, SO density positively correlated with memory consolidation across participants. Critically though, this was only the case for associative memory performance ($r = 0.38$, $P = 0.02$), while no such correlation was found for item memory ($r = 0.11$, $P = 0.51$). These results suggest that SOs are preferentially engaged in the consolidation of associative memories, consistent with a role in orchestrating a hippocampal-neocortical dialogue in service of episodic memory formation.

Disclosures: T. Schreiner: None. **B.P. Staresina:** None.

Poster

423. Human Long-Term Memory: Modulation

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 423.27/BB25

Topic: H.02. Human Cognition and Behavior

Support: NRF 2018M3C7A1022317
NRF 2018R1A6A3A01011079

Title: Structural brain integrity of patients with medial temporal lobe resection in relation with episodic memory

Authors: *W. JEONG[1,2], J. KIM[3], C. CHUNG[2,4];
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Abstract: Purpose: The neural mechanisms of episodic memory in the absence of one medial temporal lobe (MTL) structures has recently started to be investigated. It has been suggested that increased functional connectivity of distributed brain areas, including the hippocampus contralateral to the resection, is the supporting mechanism for normal range of episodic memory function in patients with MTL resection (MTLR). However, regardless of its close relationship between function, behavior, and structure, structural substrates of memory reserve after MTLR has not been well characterized. In the present study, we aim to understand structural characteristics of MTLR brain and its relationship with episodic memory function. **Methods**: We studied 35 adult patients who underwent unilateral MTLR for the treatment of medically intractable epilepsy (17 left, 18 right) and 21 healthy controls (median age 32 years). All participants underwent diffusion-weighted imaging (3T). Group differences in white matter integrity, which were assessed via fractional anisotropy (FA), was investigated by applying tract-based spatial statistics. Finally, FA values were compared with memory capacity which was assessed by a standardized neuropsychological test. Additionally, we assessed the axial diffusivity (AD), the radial diffusivity (RD), and the mean diffusivity (MD). **Results**: All subject groups exhibited normal range of memory capacity. As for the white matter integrity, while HCs showed higher FA values in the tracts near the resection cavity, both patient groups showed higher FA values within ipsi-lesional superior corona radiata ($p<0.05$, corrected). The mean values for FA, AD, RD, and MD were extracted by using the cluster showing higher FA values in patients as a mask. Compared to the HC, both patient groups showed lower RD and higher AD values ($p<0.05$). For the relationship with memory function, all these values showed no significant correlation with any memory scores. **Conclusions**: Both reduced and increased microstructural integrity in similar tracts with our observation have also been reported in previous postoperative studies, which were often interpreted as reflecting cognitive impairment of patients. Given that our patients had a normal range of memory capacity and white matter integrity had no correlation with memory scores, altered integrity in our patients may reflect pathological condition rather than memory function. In the future, more sophisticated analysis methods (e.g., tractography started from hippocampus contralateral to the resection) should be adopted in order to reveal the structural substrates of normal episodic memory function without MTL.

Disclosures: W. Jeong: None. **J. Kim:** None. **C. Chung:** None.

Poster

423. Human Long-Term Memory: Modulation

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Program #/Poster #: 423.28/BB26

Topic: H.02. Human Cognition and Behavior

Support: National Centre for Research and Development grant POIR 01.01.01-00-178/15

Title: Training related suppression of EEG signals complexity

Authors: *U. MALINOWSKA[1], J. WOJCIECHOWSKI[2], M. WALIGÓRA[1], J. ROGALA[1]; [1]Nencki Inst. of Exptl. Biol., Warsaw, Poland; [2]World Hearing Ctr. of Inst. of Physiol. and Pathology of Hearing, Warsaw/Kajetany, Poland

Abstract: Learning processes associated with multiple task repetitions are deemed to affect brain plasticity via postsynaptic potentiation leading to long term changes in functional connectivity. These changes may cause increased or reduced activation of existing neural architecture. In this study, we examined the effect of three repetitions of delayed match-to-sample task on changes in the EEG signal complexity and spectral components. We modified the original task by adding control trials that did not require engagement of attention and memory. These additional trials allowed us to compare impact of attended versus passive conditions. We exanimated 14 subjects with the two types of trials in three sessions performed within 10-20 days. Scalp EEG was collected with 21 electrodes in the 10-20 system. For each electrode channel and trial of the correct response, we selected three 1-second long windows in different time points across a trial. For the selected signals we analyzed power in consecutive frequency bands <40 Hz and signals complexity measures such as sample entropy, Shannon entropy and Higuchi fractal dimension (HFD). Measures of signal entropy, as well as HFD, revealed gradual suppression of signal complexity in the consecutive sessions. The effect was significant in all examined windows extracted across a trial. Moreover, the changes were observed for both groups of tasks - for attentional and passive trials. We found also that the signal complexity remains always higher for the attentional trials. Comparison of spectral analysis for the group of subjects after the three sessions showed an increase in alpha power in frontal and parietal electrodes and in low beta activity (13-20 Hz) in central channels during the control trials. For the attentional trials, the alpha increase was observed only in a few frontal electrodes and in one parietal channel for a low beta activity. No significant changes were found for theta and high beta power in both conditions. However, we did not observe significant improvement of the test performance during the three sessions, the results of EEG analysis indicated changes in neuronal network manifested by the modified EEG signals. While in the case of passive trials, the suppression of the signal complexity may reflect increase of power in lower frequencies associated with inhibition of unnecessary attention and memory processes, confirmed by increase of the alpha power, the

changes observed in attentional trials, not accompanied by broad spectral changes, suggest rather learning related synchronization and reconfiguration of the network activity, potentially reflecting optimization of the underlying cortical processing networks.

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Poster

423. Human Long-Term Memory: Modulation

Location: Hall A

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Program #/Poster #: 423.29/BB27

Topic: H.02. Human Cognition and Behavior

Title: Evolutionary conserved role of neural cell adhesion molecule-1 (NCAM1) in memory

Authors: *P. MASTRANDREAS[1], V. VUKOJEVIC[1], F. PETER[1], I. KOLASSA[2], T. ELBERT[3], D. J. DE QUERVAIN[1], A. PAPASSOTIROPOULOS[1], A. STETAK[1];
[1]Univ. of Basel, Basel, Switzerland; [2]Univ. of Ulm, Ulm, Germany; [3]Univ. of Konstanz, Konstanz, Germany

Abstract: The neural cell adhesion molecule 1 (NCAM1) is involved in several brain-related biological processes, including neuronal migration, axonal branching, fasciculation, and synaptogenesis, with a pivotal role in synaptic plasticity. Here, we investigated the evolutionary conserved role of NCAM1 in learning and memory. Olfactory conditioning induced sustained changes in *ncam-1* expression in up to 24 hours after training. Loss of *ncam-1* function selectively impaired associative long-term memory, without causing acquisition, sensory, motor or short-term memory deficits. Expression of *C. elegans* or human NCAM1 fully reversed the memory loss of *ncam-1(lf)* worms, suggesting a conserved role of NCAM1 for learning and memory. In two independent healthy Swiss cohorts, DNA methylation of the NCAM1 promoter was associated with memory performance. In two independent Sub-Saharan populations of conflict zone survivors who had faced severe trauma, DNA methylation at an alternative promoter of the NCAM1 gene was associated with traumatic memories. Taken together, our results support a role of NCAM1 in associative memory in nematodes and humans.

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Poster

424. Cognition and Connectivity

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: H.02. Human Cognition and Behavior

Support: Office of Naval Research MURI N00014-16-1-2832
Office of Naval Research DURIP N00014-17-1-2304

Title: An fMRI investigation of medial prefrontal network dynamics during a context-dependent rule learning task

Authors: *W. MA[1,2], T. M. MORIN[3,2,1], A. E. CHANG[2,1], C. E. STERN[2,1];
[1]Cognitive Neuroimaging Ctr., [2]Ctr. for Systems Neurosci., [3]Grad. Program for Neurosci., Boston Univ., Boston, MA

Abstract: The purpose of this study was to investigate how functional interactions between brain networks support human context-dependent rule learning. In the task, healthy naive participants (N = 30) learned a series of context-dependent rules through trial and error during an fMRI scan. Our analysis examined changes in functional connectivity across the transition from learning to learned rule behavior. We first applied a community detection algorithm to examine changes in functional connectivity between brain regions across time (Bassett et al. 2011, PNAS). Flexibility was defined to be the number of times a node changed its community allegiance normalized by the number of possible changes. We found that flexibility was higher in anterior cingulate cortex and orbitofrontal cortex during learning compared to after learning. We used these same regions to perform a difference of correlation analysis. Results indicated that both anterior cingulate cortex and orbitofrontal cortex were more strongly connected with regions in the ventral attention network during learning compared to after learning. These results suggest that anterior cingulate cortex and orbitofrontal cortex are affiliated with different networks during learning compared to after learning.

Disclosures: W. Ma: None. **T.M. Morin:** None. **A.E. Chang:** None. **C.E. Stern:** None.

Poster

424. Cognition and Connectivity

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Topic: H.02. Human Cognition and Behavior

Support: Office of Naval Research MURI N00014-16-1-2832
Office of Naval Research DURIP N00014-17-1-2304
NSF Major Research Instrumentation 1625552

Title: Cortical contributions to perceptual and symbolic reasoning using a one-dimensional raven's progressive matrices task

Authors: *T. M. MORIN[1,2,3], A. E. CHANG[2,3], C. E. STERN[2,3];
[1]Grad. Program for Neurosci., [2]Ctr. for Systems Neurosci., [3]Cognitive Neuroimaging Ctr., Boston Univ., Boston, MA

Abstract: The goal of this study was to investigate brain activity that underlies abstract reasoning of discrete symbolic and continuous texture stimuli. We developed a simplified, one-dimensional version of the Raven's Progressive Matrices task suitable for testing during an fMRI scan. During the task, participants (N = 27) viewed patterns composed of either discrete symbols or detailed continuous textures. Symbols or textures either varied from left to right according to a sequential rule or remained uniform throughout. Subjects were required to deduce and apply a sequence-rule to determine which of two answer choices correctly fit into a blank white square covering part of the stimulus. Participants were instructed on a paper version of the task outside of the scanner and completed 384 trials of the task during fMRI scanning. Participants exhibited high levels of accuracy and similar response times for all conditions. For both discrete symbolic and continuous texture stimuli, stimuli that required correctly deducing and applying a sequence rule resulted in increased BOLD signal in the frontoparietal control network (FPCN). Notably, regions of the FPCN strongly connected with the Default Network exhibited stronger activity when subjects were reasoning about a symbolic sequence, whereas regions of FPCN that were strongly connected with the Dorsal Attention Network showed greater activity when subjects were reasoning about a texture sequence. Increased BOLD signal in ventral visual regions was also associated with reasoning about a texture sequence. Our preliminary results suggest differential frontoparietal, dorsal attention, and default network contributions to reasoning about discrete symbolic and continuous texture stimuli.

Disclosures: T.M. Morin: None. **A.E. Chang:** None. **C.E. Stern:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.03/BB30

Topic: H.02. Human Cognition and Behavior

Title: Overlapping frontal network mediates task-related connectivity changes between intrinsic cognitive control networks

Authors: *S. L. COOKSON, M. D'ESPOSITO;
Univ. of California, Berkeley, Berkeley, CA

Abstract: Regions of the lateral frontal cortex (LFC) are part of two separate intrinsic networks - the fronto-parietal (FP) and the cingulo-opercular (CO) - that are proposed to support distinct cognitive control processes (Dosenbach 2008). The FP and CO networks become more integrated during tasks versus rest, thought to indicate increased between-network communication (Cohen & D'Esposito 2014); however, it is yet unclear what drives this integration. Recently, using a mixed membership algorithm to parcellate resting fMRI data into overlapping intrinsic networks, we found a previously unidentified network that spans the majority of frontal cortex, overlapping with and containing LFC regions from the otherwise distinct FP and CO networks. The LFC regions in this intrinsic frontal network also form a hierarchically connected, task-related network proposed to implement cognitive control via top-down selection processes (Badre 2008). We have hypothesized that FP-CO integration may be facilitated by the frontal network via these overlapping connections. To test this hypothesis, we analyzed previously published fMRI data from our lab during which subjects performed a task that orthogonally manipulated two factors, each thought to engage the processing of distinct regions of the frontal network (Nee & D'Esposito 2016). In the original study, rostral LFC regions showed increased top-down influence as they were recruited by the task demands in each condition. In this analysis, we extended these results by assessing how integration between the frontal network and the FP and CO networks changed with different cognitive control demands. We assessed integration between the FP and CO networks and the frontal network by measuring beta series correlations between the regions unique to each network for each combination of task factors. We found that integration between the frontal and FP/CO networks depended simultaneously on both task factors; that is, the frontal network became more integrated with both the FP and CO networks when the task required processes supported by both networks. These results suggest that integration between the FP and CO networks is mediated by a third overlapping network, and may suggest ways to relate regional hierarchical interactions to network integration.

Disclosures: S.L. Cookson: None. **M. D'Esposito:** None.

Poster

424. Cognition and Connectivity

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant MH096801
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Title: Cognitive control networks balance domain generality and specificity in representing task rule information across multiple cognitive domains

Authors: *D. H. SCHULTZ[1], T. ITO[2], M. W. COLE[2];
[1]Ctr. for Brain, Biol. & Behavior, Univ. of Nebraska, Lincoln, Lincoln, NE; [2]Rutgers The State Univ. of New Jersey, Newark, NJ

Abstract: Cognitive control is a set of processes that allow for flexible information processing and behavior based on current goals. Some evidence suggests that cognitive control processes are domain general, reflecting general processes that are engaged regardless of context or modality. Other results have suggested that cognitive control can be better explained by a number of domain specific processes that are engaged in specific contexts or modalities (e.g. working memory, attention, visual stimuli, auditory stimuli, etc.). We hypothesized that activity patterns within regions of the brain would contain information regarding task rules, and more specifically, that cognitive control networks would contain information across a number of different contexts and modalities reflecting domain general processes consistent with the concept of "mixed selectivity" information coding. This would reflect the ability of these networks to integrate domain specific representations so they can be coordinated for task performance when different rules are encountered in novel situations. We also hypothesized that some brain regions would exhibit more specific information content (pertaining to a specific context or modality), reflecting the need for the brain to balance domain general, and domain specific representation. We tested this hypothesis in a large dataset (100 young adults) in which participants completed a flexible control task called the Concrete Permuted Rule Operations (C-PRO) paradigm in the scanner (fMRI). The C-PRO paradigm consists of three rule domains (Logic, Sensory, Motor) with four variants within each domain. When all of the rules are permuted it results in 64 unique rule combinations. This task design allowed us to compare task rule information content across three different modalities (Logic, Sensory, Motor) while controlling for attention, arousal, sensory input, and motor output across tasks. We examined multivariate patterns of activity across 360 cortical parcels. Information estimates were measured using representational similarity analysis, quantified as similarity of the activation pattern elicited by tasks with a common rule relative to tasks not sharing a rule. Consistent with our hypothesis, we found that cingulo-opercular, frontoparietal, and dorsal attention cognitive control networks contained domain general information, while portions of these networks also showed a preference for specific domains. These results suggest that cognitive control networks contain domain general information, but portions of these networks still maintain some level of domain specificity.

Disclosures: D.H. Schultz: None. **M.W. Cole:** None. **T. Ito:** None.

Poster

424. Cognition and Connectivity

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.05/BB32

Topic: H.02. Human Cognition and Behavior

Title: Impulsivity and cognitive control in human intracranial and extracranial EEG

Authors: *E. H. SMITH, B. KUNDU, B. PHILIP, T. DAVIS, J. D. ROLSTON;
Neurosurg., Univ. of Utah, Salt Lake City, UT

Abstract: Impulsive choice refers to the tendency to favor immediate, small rewards over delayed, large rewards, is a component of many psychiatric disorders, particularly substance use disorder. Impulsive choice may initially predispose individuals to substance abuse, and has been shown to be essential in relapse. Neuroimaging studies of impulsive choice broadly highlight brain areas involved in decision making, suggesting that no single anatomical area is implicated as causing impulsivity. Here we seek to assess the extent to which impulsivity reflects a failure of cognitive control over decision making or a failure to incorporate a model of the task into decision making circuitry. In order to address these questions, we examined intracranial LFP and scalp EEG from neurosurgical patients undergoing monitoring for refractory epilepsy who performed a task that induced impulsivity for anticipated rewards: the balloon analog risk task (BART). Overall seven patients carried out 222.9 ± 10.8 trials of BART with an accuracy of 84.9 ± 5.8%. There was a bimodal distribution of overall impulsive behavior across patients, determined by the Kullback-Leibler divergence between successful active trials, in which subjects successfully ceased balloon inflation, and passive trials, in which balloons were inflated to their maximum size while subjects watched. Three subjects (43%) exhibited overall impulsivity using this metric. In order to gauge neural representations of an internalized task model, we examined broadband high frequency LFP (70-200 Hz; BHF) responses to increasing risk, reward, and uncertainty during balloon inflation (linear mixed effects model in Wilkinson notation: balloon size ~ 1 + BHF + (1 + BHF | trial)). We found that electrodes in the dorsolateral prefrontal and anterior lateral temporal cortices significantly tracked these variables (family-wise error corrected $p < 0.05$ for each electrode). In order to gauge intracranial correlates of canonical cognitive control signals, we examined differences in BHF in response to BART outcomes (wins, losses; point acquisition, balloon pops) using cluster corrected permutation tests, accounting for adjacent intracranial electrodes. We found significant differences following outcomes in the anterior cingulate, orbitofrontal cortex, and amygdala. These BHF responses correlated in time and amplitude with frontal midline theta recorded on the Fz scalp EEG contact across trials. These results address the neural mechanisms of control over impulsive decisions.

Disclosures: **E.H. Smith:** None. **B. Kundu:** None. **B. Philip:** None. **T. Davis:** None. **J.D. Rolston:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.06/BB33

Topic: H.02. Human Cognition and Behavior

Title: Effect of heart rate reserve on prefrontal cortical activation in healthy older adults while dual-task walking

Authors: *A. BISHNOI[1], G. CHAPARRO[2], M. HERNANDEZ[1];
[1]Kinesiology and Community Hlth., Univ. of Illinois At Urbana-Champaign, Urbana-Champaign, IL; [2]California State Univ. Dominguez Hills, Carson, CA

Abstract: Heart rate reserve (HRR) has been shown to be associated with cardiovascular function in older adults, but much is still unknown how the heart rate reserve level affects cognitive control while dual-task walking. In this study, we aim to determine the relationship between heart rate reserve level and change in prefrontal cortical (PFC) activation patterns evaluated by functional near-infrared spectroscopy (fNIRS) between low HRR group (LHRR) and high HRR group (HHRR) while dual-task walking. This is a cross-sectional study design. Twenty-three older adults were divided into two groups: LHRR (N=9, age=74.8±7.2, 0 females) and HHRR (N=14, age: 63.3±9, 5 females), based on heart rate reserve cut off of 55, which was calculated by the difference of maximum heart rate and resting heart rate. Participants completed baseline cognitive and motor testing before starting the dual task paradigm on the treadmill. In the paradigm, participants completed a single task walking and dual-task walking with Modified Stroop Color and Word test (MSCWT). This condition was completed in order of easiest to most difficult (Neutral, Congruent, Incongruent, Switching). The results of the linear mixed model analysis revealed that the older adults with LHRR are limited in the recruitment of PFC resources in increasingly challenging cognitive tasks while walking, compared to older adults with HHRR (Figure 1, $p < .001$), while controlling for age and gender differences. These findings provide evidence that HRR in older adults is related to cognitive function. This study is the first to investigate the effect of HRR on brain activation changes in healthy older adults and provides a potential link between low HRR and cognitive impairments in older adults. Keyword(s): fNIRS, Prefrontal cortical activation, HRR

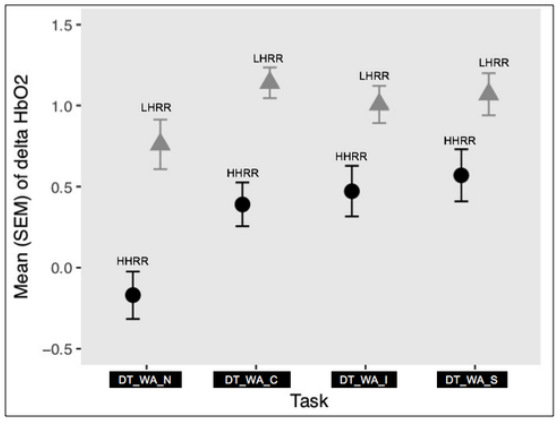

Figure 1: Mean (SEM) increase of the delta PFC (Dual task HbO2- Single task HbO2 level) in two groups (LHRR vs HHRR) with increasing difficulty of dual task walking condition (Neutral (DT_WA_N), Congruent (DT_WA_C), Incongruent (DT_WA_I), Switching (DT_WA_S)).

Disclosures: **A. Bishnoi:** None. **M. Hernandez:** None. **G. Chaparro:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.07/BB34

Topic: H.02. Human Cognition and Behavior

Support: National Institute of Advanced Industrial Science and Technology

Title: The lateralization of mirror neurons in theinferior frontal gyrus during motion observation and execution

Authors: *F. ZHANG[1], S. IWAKI[2];
[1]Natl. Inst. of Advanced Industrial Sci., Tsukuba, Japan; [2]Natl. Inst. Adv Indust Sci. & Tech., Tsukuba, Japan

Abstract: Mirror neurons, which first reported in the macaque monkey, fire both during observation and the execution of similar action. However, the existence of mirror neuron system (MNS) in humans is not clear. The significant clusters responding to both observation and action have been reported in Brodmann area 44 that located in the inferior frontal gyrus (IFG), but the activation of neural system represents the common neural code or overlapping but distinct populations is still in debate. The purpose of our study is to investigate the presence and the role

of mirror neurons for motion observation and execution. We conducted a functional magnetic resonance imaging adaptation paradigm with separate left or right hand movement to define the presence and lateralization of MNS activation. In the further study, we applied the representational similarity analysis in MNS to analyze the response patterns with representational dissimilarity matrix (RDM). Our result revealed the right lateralization, not left lateralization of MNS in IFG. Furthermore, the result of RDM showed the similar brain activity patterns when participants were required to execute the hand movement with the ipsilateral or contralateral hand after they observed the video. This result suggested that when the MNS was activated by motion observation, the activity was right lateralized no matter whether the subsequent motion is completely agree with the observed motion. The result from RDM provided the supplemental evidence for the right lateralization of motor components of MNS in IFG.

Disclosures: F. Zhang: None. **S. Iwaki:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.08/BB35

Topic: H.02. Human Cognition and Behavior

Support: NIDA F32 DA045451
Brown University Department of Neuroscience Connors Fellowship
NIGMS CoBRE P20GM103645
The Robert J. and Nancy D. Carney Institute for Brain Science Innovation Award

Title: Frontostriatal connectivity during abstract sequential control

Authors: *T. H. MCKIM[1], T. M. DESROCHERS[2];
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Abstract: The everyday goal of making coffee intimately intertwines the reward (drinking the perfect cup of coffee) and guiding sequential tasks (e.g., add water and coffee). A change in reward (different coffee) may change your performance of these task sequences. Several fMRI studies using abstract sequential tasks have demonstrated that activity in the rostrolateral prefrontal cortex (RLPFC) is increasingly engaged (ramps up) and is necessary during abstract sequential control (Desrochers et al., 2015; 2019). Ramping dynamics have also been found in the frontal cortex of animals (Donnelly et al., 2015), and dopamine concentration ramping in the striatum scales based on reward magnitude (Howe et al., 2013). Whether reward modulates the ramping signal within the RLPFC and throughout the brain during sequential tasks, and whether frontostriatal circuit connectivity changes as a result during sequential control in humans are

outstanding questions. We tested the hypothesis that progress towards a goal underlies RLPFC ramping, and changes in the associated network by manipulating the value of learned task sequences. Participants kept track of repeated four-item sequences of images that were associated with either high or low reward during fMRI scanning. On each trial, participants indicated whether the image was in or out of a pre-determined order. The sequential task included conditions whereall stimuli were visible (Vis), or all but the last stimulus in the block was occluded by a placeholder image (Occ), requiring sequence monitoring from memory. Elevated reaction times (RTs) at the first position within the sequence demonstrated that participants monitored the presented images as sequences (n = 25; Pos1>Pos2,3,4; $F_{1,24}$=16.097,p<0.001). We observed a trend that sequence value modified performance by decreasing RTs for high value sequences ($F_{1,24}$= 3.38, p=0.078). We found that RLPFC activity ramps up during sequence monitoring, replicating previous results. Preliminary task-based functional connectivity results showed increased connectivity between bilateral lateral prefrontal cortex in the frontoparietal control network, which overlaps with the RLPFC, and the caudate nucleus of the striatum when contrasting high versus low value sequences. We also found decreased connectivity between the specific ramping RLPFC cluster and the nucleus accumbens for the high versus low sequences. Our findings suggest a link between abstract sequential control and reward processing in frontostriatal circuits with similar neural signal dynamics across species that may represent goal-directed behavior.

Disclosures: T.H. McKim: None. **T.M. Desrochers:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.09/BB36

Topic: H.02. Human Cognition and Behavior

Support: NIH/NIDCD K99/R00 DC013828

Title: Continuous tracking of error-related negativity via electrocorticography in humans

Authors: *E. AHN[1], J. PLASS[1], D. BRANG[2];
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Abstract: The error-related negativity (ERN) is thought to be a neural-based performance monitoring mechanism used to identify and correct potential errors. The ERN takes the form of a negative deflection in the ERP response, reaching its peak within 100 ms after an error occurs. While a strong emphasis on the accuracy of one's performance can enhance this ERP response, some studies have reported detection of the ERN even in the absence of any conscious awareness of an error, suggesting that this mechanism is a highly integrated and graded response that our

brain relies on for everyday performance monitoring. The ERN has been detected using a variety cognitive and perceptual tasks, ranging from sentence verification to more complicated motor paradigms. However, research has largely been restricted to non-invasive recordings, limiting our understanding of the neural mechanisms of the ERN. Moreover, previous studies using invasive electrocorticography (ECoG) recordings examined the ERN resulting from single trial-based and discretely time-locked responses using highly artificial tasks like the go/no-go paradigm. Here we sought to study the ERN using ECoG while subjects completed a continuous performance monitoring task using the popular game Flappy Bird, in which subjects adjust the vertical position of a bird to navigate it through a series of pipes to avoid crashing. Using this paradigm in 4 patients, we demonstrate that the ERN can be observed during continuous performance monitoring of errors in a more natural context, localized to the anterior insula and the anterior cingulate cortex, replicating and extending the results of earlier intracranial studies. Additionally, the design of our current study allows continuous monitoring of participants' ERN responses by examining high-gamma activity (indicative of population spiking rates) as the bird moves closer or further from the pipes, reflecting graded changes in the potential for error through changes in crash probabilities. By tracking neural activity during this task, we will identify whether the ERN monitors errors in a continuous manner, reflecting the probability of an error at any given time point, or in a more discrete manner, only when a crash occurs.

Disclosures: **E. Ahn:** None. **D. Brang:** None. **J. Plass:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.10/BB37

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 NS102201

Title: Surprise modulates orienting-related but not strategic post-error processing

Authors: *Y. GUAN, J. R. WESSEL;
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Abstract: Error processing may involve both non-specific, orienting-related processes, as well as error-specific, strategic processes (Wessel, 2018). Recent behavioral findings indicate that motor slowing after errors may be associated with the surprise of the error. For example, Parmentier et al. (2018) found an interaction between surprise processing and post-error slowing in a speeded auditory-visual reaction time task in which (in)correct responses were followed by (un)expected sounds, suggesting surprise as a common underlying mechanism. Here, we test whether surprise differentially affects processes *immediately after error commission* (*N* = 36; *M*

= 19.41 years; 15 males), which should be orienting-related and/or *immediately prior to the execution of the subsequent response* (*N* = 36; *M* = 19.77 years; 11 males), which should be strategic. Using an adapted version of Parmentier et al.'s oddball paradigm, we found the same interaction as Parmentier et al.'s study between the type of trial (post-correct vs. post-error) and the type of tone (standard vs. surprise) when a surprising sound was presented 100ms after the response, but *not* when the sound was presented 100ms following the next stimulus after an error. Since past EEG studies have shown that fronto-central EEG activity during both time periods predicts post-error slowing, we then conducted an EEG study to explore whether error-related signatures are differentially modulated by surprise at the two different time points. Preliminary analyses of a sample of *N* = 10 using time-frequency decomposition showed increased power in fronto-central theta band (4-8 Hz) within 200ms *immediately after the error commission* when a surprise tone was present (Fig. 1), but *not* when the surprising tone was present *immediately prior to the execution of the subsequent response*. These results provide empirical support for theories purporting that error processing entails both orienting and strategic processes, which are differentially related to the neural processing of surprise.

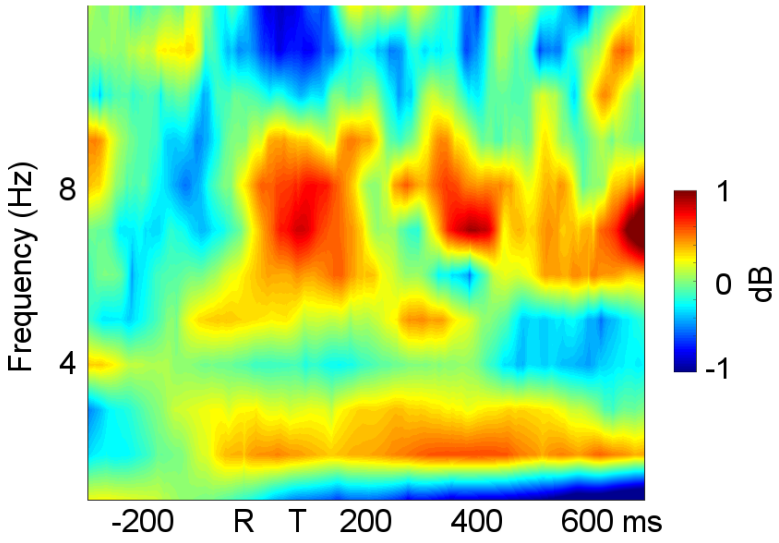

Fig. 1 Grand mean (N = 10) time-frequency plane showing power values at fronto-central sensors relative to a pre-response baseline in the setting where tone presented 100ms post-response onset. Power values were obtained via subtracting power in correct trials from error trials in the suprise tone condtion and the standard tone condition respectively and then subtract the latter from the former. Note the increased power at the theta band (4-8 Hz), which suggests surprise modulates orienting-related post-error processing.

Disclosures: **Y. Guan:** None. **J.R. Wessel:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.11/BB38

Topic: H.02. Human Cognition and Behavior

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Chronic aerobic exercise as a modulator of cognitive control

Authors: *M. L. WUNDER[1], W. R. STAINES[2];
[1]Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada; [2]Kinesiology, Univ. Waterloo, Waterloo, ON, Canada

Abstract: Chronic aerobic exercise may facilitate plasticity in frontal brain regions associated with executive functions. Evidence suggests that more active individuals generally perform better on tasks of inhibitory control; perhaps due to enhanced top-down cognitive control and conflict monitoring occurring pre and post-error. In the present research, we therefore aimed to determine whether chronic aerobic exercise improves the efficacy of top-down cognitive control in response to differing levels of conflict. We hypothesized that enhanced cognitive control and pre-response conflict detection; reflected by a larger N2 ERP component would result in less post-error conflict; reflected by a smaller error-related negativity (ERN) amplitude. Furthermore, more active individuals were expected to respond more accurately and more quickly than non-active controls. 20 participants aged 18-25 were divided into the chronic aerobic exercise (CAE) group or the non-active group based on their responses to the International Physical Activity Questionnaire (2002). To be eligible for the CAE group, participants must be classified as high (>3000 METs); the non-active group was classified as low (<600 METs). Participants then performed a modified arrow-based Flanker task in which conflict was modulated by the distance of the distractors from the target (close or far) and congruency of arrows (incongruent or congruent). EEG was collected while participants performed 800 trials over 4 blocks; 80% of trials were congruent and 20% were incongruent, 50% were close and 50% were far. The ERN and error positivity (Pe) were extracted by subtracting response locked correct from error trials. The N2 was extracted by subtracting stimulus locked congruent from incongruent trials. There was no significant difference between the CAE group and the non-active group in terms of error frequency, reaction time or ERN amplitude. Mixed model ANOVAs with groups (between) and conflict level (within) as factors were used for analysis of ERP components (ERN, Pe, N2). The CAE group showed a significantly larger Pe amplitude ($F_{1,16}$=6.57, p=0.021) compared to the non-active group. Furthermore, close trials (more conflict) elicited a significantly larger N2 amplitude than far trials in the CAE group, but not the non-active group ($F_{1,16}$=9.67, p=0.007). These findings suggest that CAE confers some cognitive benefit regarding the endogenous

processing involved in pre-response conflict detection and the post-error response. These benefits may include enhanced adaptability in the allocation of cortical resources depending on task demands and more efficient evaluation of performance post-error.

Disclosures: M.L. Wunder: None. **W.R. Staines:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.12/BB39

Topic: H.02. Human Cognition and Behavior

Support: NSF STC award CCF-1231216
NIH R01EY026025

Title: Task invariant and task dependent neural processes of conflict resolution during cognitive control

Authors: *Y. XIAO[1], H.-Y. YU[3,4], C.-C. CHOU[3], Y.-C. SHIH[3,4], J. MADSEN[5], D. WEISHOLTZ[6], I. M. REUCROFT[7], N. E. CRONE[8], W. ANDERSON[9], G. KREIMAN[2]; [2]Ctr. for Brain Sci., [1]Harvard Univ., Cambridge, MA; [3]Dept. of Neurol., Neurolog. Institute, Taipei Veterans Gen. Hosp., Taipei, Taiwan; [4]Inst. of Brain Science, Brain Res. Center, Natl. Yang-Ming Univ., Taipei, Taiwan; [5]Dept. of Neurosurg., Boston Children's Hospital, Harvard Med. Sch., Boston, MA; [6]Neurol., Brigham and Women's Hospital, Harvard Med. Sch., Boston, MA; [7]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [8]Neurol., Johns Hopkins Hosp., Baltimore, MD; [9]Dept. of Neurosurg., Johns Hopkins Med. Sch., Baltimore, MD

Abstract: Cognitive control involves simultaneously and flexibly combining multiple sensory inputs with task-dependent goals for decision making. Several tasks have been proposed to examine the mechanisms underlying cognitive control including the Stroop task, the Eriksen flanker task, the multi-source interference task, among many others. Because these tasks have been studied independently, it remains unclear whether any of the neural signatures that correlate with cognitive control reported to date reflect true control mechanisms or whether they are linked to specific sensory or behavioral peculiarities of each task. To address this question, here we directly compared the three tasks against each other in a single session, thereby allowing us to disentangle which neural mechanisms are specific to a given task and which mechanisms reflect task-independent control signals. We examined intracranial field potential recordings from 1196 electrodes in 10 subjects with pharmacologically intractable epilepsy. The behavioral results demonstrated that reaction time for the incongruent condition was significantly longer than that for congruent condition in all three tasks. Consistent with previous work (Tang et al, eLife 2016), we found conflict-selective electrodes that showed distinct gamma activity patterns for congruent

versus incongruent conditions. Interestingly, some electrodes showed task dependent correlates of conflict (i.e. differences between congruent and incongruent trials for some but not all tasks), whereas other electrodes showed task invariant correlates of conflict. These results provide initial steps to dissociate invariant cognitive control mechanisms from task specific sensory and decision processes.

Disclosures: **Y. Xiao:** None. **H. Yu:** None. **C. Chou:** None. **Y. Shih:** None. **J. Madsen:** None. **D. Weisholtz:** None. **I.M. Reucroft:** None. **N.E. Crone:** None. **W. Anderson:** None. **G. Kreiman:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.13/BB40

Topic: H.02. Human Cognition and Behavior

Title: A novel training method of frontal lobe functions - An application of Wisconsin card sorting test and effects on colour stroop task

Authors: *M. SUGANAMI[1], S. ABE[1], K. KOBAYASHI[1], K. IMAMURA[1], M. KOMATSU[2]; [1]Wako Rehabil. Hosp., Saitama, Japan; [2]RIKEN Ctr. For Brain Sci., Saitama, Japan

Abstract: The functions of prefrontal area are often measured by Wisconsin Card Sorting Test (WCST), and the patient with prefrontal area damage usually fails WCST. WCST requires two functions: 1) set shifting among different rules and 2) guessing a hidden rule with partial information. To improve these functions, we have developed the Training of Set Shift (TSS) as an application of WCST. This training is applied as a part of cognitive rehabilitation by a speech therapist (ST), in conjunction with other training methods and with physio therapy and occupational therapy. We have experienced three cases with the prefrontal area lesion with variety of causes, with whom it was possible to conduct TSS. All of them exhibited improvements in the prefrontal area functions. TSS is divided into four parts: 1) pairing, 2) categorising, 3) conjecting and correction (CC), and 4) conjecting and identification (CI). These have different level of cognitive demand and the paring task is the least challenging whereas the CI task is the most demanding. With TSS, just like WCST, subjects are asked to sort a sequence of cards into four piles either in accordance with colour, form, or number, whereas sorting rules are pre-determined by ST. In the pairing task and the categorising task, sorting rules are explicitly shown. Subjects were asked to shift among the different sorting rules passively in the paring task, and actively in the categorising task. With the CC task and the CI task, pre-determined sorting rules are not shown. In these tasks, subjects are asked to guess which sorting rules are used from the feed backs they are given by ST. Cognitive rehabilitation sessions are started from the pairing task and gradually progressed to more demanding tasks. When we

conducted TSS, we administered a colour Stroop task, which is used to assess the prefrontal area function, before and after the TSS. With all three cases, in the first three days, the total reaction time of the colour Stroop task was significantly shortened after TSS ($P<0.05$). Then after several months of training, all three of them exhibited improvement in the Behavioural Assessment of the Dysexecutive Syndrome. One of them was even able to pass the WCST S-F version. These results suggest that, in a convalescent period of prefrontal area damage, TSS might be one of the effective trainings as a part of cognitive rehabilitation.

Disclosures: M. Suganami: None. **S. Abe:** None. **K. Kobayashi:** None. **K. Imamura:** None. **M. Komatsu:** None.

Poster

424. Cognition and Connectivity

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.14/BB41

Topic: H.02. Human Cognition and Behavior

Support: UWEC Student-Faculty Research Collaboration grant

Title: Using independent components analysis to better understand the electrophysiology of error processing

Authors: S. M. WESTERLAND, M. M. LORTIE, S. J. BECKER, S. D. DORTCH, ***D. S. LELAND**;
Psychology, Univ. of Wisconsin-Eau Claire, Eau Claire, WI

Abstract: In two studies of error processing, participants completed a flanker task during EEG recording to invoke response errors and corresponding event-related potential (ERP) components: the error-related negativity (ERN) and error positivity (Pe). The scalp distribution for activity from 125-325 ms suggests that the neural activity that appears as a canonical Pe at posterior electrode sites may also show up as a negative deflection at anterior sites. Other studies have found similar inversions, although usually not as large (e.g. Buzzell et al., 2017; Lo et al., 2015; Schoenberg et al., 2014; Matthewson et al., 2005; Themanson et al., 2006). The inversion could reflect a single dipole (or set of dipoles) whose projections to scalp electrodes yield an anterior negativity together with a posterior positivity (shared dipole hypothesis). Alternatively, the canonical and inverted deflections could have distinct neural generators (distinct dipoles hypothesis). To address this question we have been using independent components analysis (ICA), a mathematical means of separating EEG signals into spatiotemporal components. Finding a common set of ICA components corresponding to both deflections would support the shared dipole hypothesis, while separate sets of ICA components for each deflection would support the distinct dipoles hypothesis. Preliminary findings with a single subject reveal two ICA

components with spatial distributions and peak timings consistent with the Pe. Each of these ICA components includes both anterior and posterior activations, with opposite polarity, in line with the shared dipoles hypothesis. Evidence thus far suggests that the anterior negativity in the Pe time window may be a reflection of the same neural event as the canonical Pe and that it may be a viable added measure for the electrophysiology of error processing.

Disclosures: D.S. Leland: None. **S.M. Westerland:** None. **M.M. Lortie:** None. **S.J. Becker:** None. **S.D. Dortch:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.15/BB42

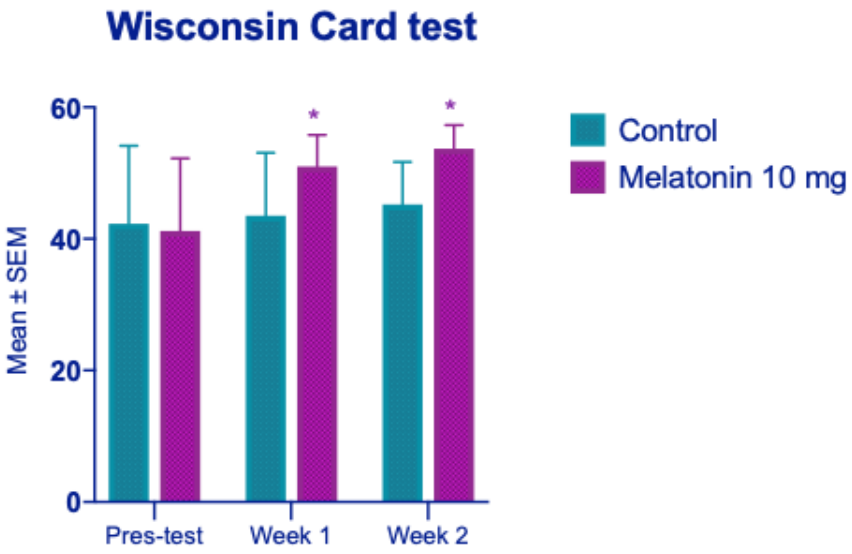
Topic: H.02. Human Cognition and Behavior

Support: UABC-PTC-961

Title: Effect of exogenous melatonin on cognitive functions

Authors: *J. SANCHEZ-BETANCOURT[1], M. AVILA-COSTA[2], C. HERRERA-VERDUGO[3], M. HUERTA-JAUREGUI[3], R. MUÑOZ-JIMENEZ[3], F. CARRILLO-GARCIA[3], M. OLMEDO-LICEA[3], A. MEZA-AMAYA[3];
[1]Fes-Iztacala, UNAM-UABC, ciudad de Mexico, Baja California, Mexico; [2]FES-Iztacala, UNAM, Estado de Mexico, Mexico; [3]UABC, Ensenada, Mexico

Abstract: The use of exogenous melatonin has generally been found to successfully treat sleep disturbances. In recent years it has regained interest due to its favorable effects on learning and memory. In this sense, the benefits of melatonin on other types of processes such as abstract thinking have not been explored. The objective of the present investigation was to determine the effect of melatonin consumption during 2 months on these processes in young adults with an average age of 23.5 years. The sample consisted of 30 participants who were divided into a control group and an experimental group. The experimental group consumed 10 mg of melatonin daily for 2 months. Every three weeks, both groups were evaluated with computerized Wisconsin card tests. The results showed that melatonin had a significant improvement over abstract thinking but not about response times.



Disclosures: **J. Sanchez-Betancourt:** None. **M. Avila-Costa:** None. **C. Herrera-Verdugo:** None. **M. Huerta-Jauregui:** None. **R. Muñoz-Jimenez:** None. **F. Carrillo-Garcia:** None. **M. Olmedo-Licea:** None. **A. Meza-Amaya:** None.

Poster

424. Cognition and Connectivity

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.16/BB43

Topic: H.02. Human Cognition and Behavior

Support: The present work is supported by the "Programme Investissements d'avenir (PIA)" through the French national research Agency (ANR) and is funded by a 4M Euros grant to the November 13 Research Program (for the first two phases 2016-2019)

Title: Resilience after terror: The role of memory suppression

Authors: ***A. MARY**[1], J. DAYAN[1], G. LEONE[1], C. POSTEL[1], F. FRAISSE[1], C. MALLE[1], T. VALLÉE[1], C. KLEIN-PESCHANSKI[2], F. VIADER[1], V. DE LA SAYETTE[1], D. PESCHANSKI[3], F. EUSTACHE[1], P. GAGNEPAIN[1];

[1]Normandie Univ, UNICAEN, PSL Res. University, EPHE, INSERM, U1077 Neuropsychology and Imaging of Human Memory, CHU de Caen, Caen, France; [2]CNRS, Paris, France; [3]CNRS, UMR8209, Univ. Paris I Panthéon Sorbonne, EHESS, Univ. de PSL, Paris, France

Abstract: In the aftermath of a terrorist attack, some people move forward while others remain deeply traumatized, continuously reliving a haunting and intrusive past that they cannot get rid of. Such intrusive memories are widely acknowledged as central to the expression and maintenance of PTSD. Here, we examine using fMRI whether the preservation of the neural functions supporting the ability to voluntarily control and suppress memory retrieval contribute to positive adaptation in the aftermath of Paris November 13 terrorist attack.

fMRI activity was recorded while 55 trauma-exposed participants with PTSD (PTSD; 26 males; 36.5y ± 8.4), 47 trauma-exposed participants without PTSD (non-PTSD; 30 males; 36.5y ± 7.1) and 73 non-exposed healthy participants (35 males; 33.5y ± 11.5) attempt to suppress unwanted memories of previously learnt word-object pairs. Participants self-reported the presence or absence of memory intrusion at the end of each trial, allowing to isolate the conditions that triggered inhibitory control. Functional connectivity between the right anterior medial frontal gyrus, a core node of the inhibitory control system, and memory target ROIs, was investigated using context-dependent psychophysiological interactions. The statistical results were corrected for multiple comparisons using the False Discovery Rate ($P_{\text{-FDR}} < .05$).

Attempt to prevent the unwanted emergence of intrusive memory into consciousness (compared to non-intrusive reminders) was associated with a significant increase in down-regulation for non-PTSD compared to PTSD group in the right rostral hippocampus ($t(100) = -1.9$), and the bilateral parahippocampal cortex ($t(100) < -2.2$), fusiform gyrus ($t(100) < -2.3$) and precuneus ($t(100) < -2.69$). A mixed pattern of findings was observed for the non-exposed group, showing intermediate effects between non-PTSD and PTSD groups. Finally, regression models showed that participants without PTSD who were more effective at downregulating the right rostral hippocampus and right precuneus in response to intrusions had lower intensity of reexperiencing symptoms at the clinical level (Beta = 0.30, [.13 .56] and 0.41, [.11 .82], respectively; bootstrapped 98.6% CI corrected for multiple comparisons).

Our findings highlight the presence of a central dysfunction in the ability to control and regulate unwanted intrusive memory in PTSD. The preservation of inhibitory control capacity to countermand the recollection in the hippocampus of unbidden intrusive memory contributes to a positive adaptation in the aftermath of traumatic experiences and to a good mental health outcome.

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Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.17/BB44

Topic: H.02. Human Cognition and Behavior

Support: MH11173
MH63901

Title: Frontostriatal interactions during cognitive control

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Abstract: The prefrontal cortex is necessary for cognitive control. Specifically, it has been proposed that higher level abstract information is processed in rostrolateral frontal regions whereas more concrete lower level information is processed in caudolateral frontal regions. Recent research has suggested that a rostral-caudal gradient for abstraction may also exist in the striatum, in which the striatum gates relevant contexts into the frontal cortex. Here, we tested whether we could obtain evidence for rostral-caudal fronto-striatal interactions in a task that varied in dimensions of cognitive control. Twenty participants were scanned during functional magnetic resonance imaging (fMRI) while performing a cognitive control task that varied in two dimensions: the level of abstraction and the number of rules that had to be maintained. We found increased activation in a rostral frontal region (left middle frontal gyrus; BA 45) with increased abstraction of the task, replicating earlier findings. Additionally, when the number of rules was increased, we observed increased activation in the inferior frontal sulcus (BA 44) and the dorsal premotor cortex (BA 6). To assess fronto-striatal connectivity during cognitive control demands, we performed an analysis of generalized psychophysiological interaction (gPPI) for the cognitive control dimensions of our task. Our analysis revealed that the inferior frontal sulcus was coupled with the middle portion (body) of the striatum when the number of rules were increased; and middle frontal gyrus was coupled with the head of the caudate, but not the tail, as the abstraction of the task increased. These results demonstrate that frontal-striatal interactions are organized along a rostral-caudal axis of cognitive control.

Disclosures: D.A. Vogelsang: None. **J. Riddle:** None. **M. Desposito:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.18/BB45

Topic: H.02. Human Cognition and Behavior

Support: National Institutes of Health R01MH110311

Title: Neural dynamics across human prefrontal cortex supporting different levels of abstraction

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Abstract: Task abstraction refers to the hierarchical nesting of processes, from those relating to abstract goals through to specific actions. Abstraction can take a number of forms, such as temporal, relational, or rule abstraction. Neural underpinnings of task abstraction critically involve the lateral prefrontal cortex (PFC), where abstract information is thought to be represented in anterior areas and concrete information in posterior areas, forming a rostral-caudal hierarchy. However, different views have emerged over the apex of this hierarchy, with original accounts placing the frontal pole at the apex and more recent accounts placing the mid-dorsolateral PFC at the apex. Because recent work in macaques suggests that beta rhythms (13-30Hz) and gamma rhythms (>30Hz) respectively support abstract and concrete rule processing, such neural signatures for rule abstraction may better characterize the rostro-caudal hierarchy. We analyzed electrocorticography recordings from epilepsy patients performing a rule abstraction task with a hierarchical structure. Patients needed to integrate abstract rules (specifying the relevant dimension of forthcoming cues and its associated concrete rule set) and concrete rules (mapping features to actions), to respond correctly. Depth electrodes were located across the rostro-caudal axis of the PFC, with regional designation based on the Automated Anatomical Labeling Atlas. After artifact and epileptiform activity removal, we re-referenced data using a bipolar derivation from sequential channels, and applied the Hilbert transform to the bandpass filtered data to calculate power spectra. Preliminary results suggest complex oscillatory patterns emerge across the PFC during rule processing. Orbitofrontal frontal cortex, and rostral PFC more generally, showed beta and lower frequency activity after the onset of the abstract rule cue, which persisted until the behavioral response was executed. Similarly, superior frontal cortex showed strong beta activity after the abstract cue onset. In comparison, the middle frontal gyrus showed greater gamma activity, especially in response to the concrete rule cue. Inferior frontal cortex similarly showed increased gamma activity. Selectivity for particular abstract or

concrete rules was evident in high gamma activity across much of lateral PFC. Our data suggest a link between rule processing and beta activity in rostral and superior PFC and gamma activity in caudal and inferior PFC. This is consistent with a rough topography of rule representations across the entire lateral PFC, and distinct roles for beta and gamma activity in rule abstraction.

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Poster

424. Cognition and Connectivity

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Program #/Poster #: 424.19/BB46

Topic: H.02. Human Cognition and Behavior

Support: Office of the Director of National Intelligence (ODNI), Intelligence Advanced Research Projects Activity (IARPA); Contract 2014-13121700004; PI: Aron Barbey

Title: Accelerometer-measured physical activity is positively related to functional connectivity of the dorsal attention network in young adults

Authors: *D. M. PINDUS[1], C. E. ZWILLING[2], J. S. JARRETT[2], T. TALUKDAR[3], H. S. SCHWARB[4], C. H. HILLMAN[6], N. J. COHEN[5], A. F. KRAMER[6], A. K. BARBEY[4]; [1]Dept. of Kinesiology and Community Hlth., [2]Beckman Inst. for Advanced Sci. and Technology,, Univ. of Illinois at Urbana-Champaign, Urbana, IL; [3]Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois at Urbana-Champaign, Champaign, IL; [4]Beckman Inst. for Advanced Sci. and Technol., [5]Dept. of Psychology, Univ. of Illinois at Urbana-Champaign, Urbana, IL; [6]Dept. of Psychology, Northeastern Univ., Boston, MA

Abstract: Physically active young adults efficiently modulate attentional control, showing task-related increase in the activation in frontal and parietal brain regions. In older adults, physical activity (PA) interventions can enhance resting state functional connectivity globally and in cognitively salient brain networks. Whether daily PA may benefit functional connectivity in resting state brain networks in young adults has not been explored. We evaluated the associations between accelerometer-measured PA and graph theory measures of global and local efficiency of 7 intrinsic functional connectivity networks (ICNs; default mode (DMN), frontal-parietal (FPN), dorsal attention (DAN), ventral attention, limbic (LN), somatomotor and visual). We hypothesized that higher levels of moderate-to-vigorous PA (MVPA; min/day) will be related to greater efficiency of information transfer locally in cognitively salient ICNs (DMN, FPN and DAN) and globally, between these and all other networks. We used baseline data from 201 subjects (M_{age}=24.5 ±5.6 yrs, 106 females) from the INSIGHT trial (NCT02780739). Following

preprocessing, the mean fMRI BOLD time series were extracted from subjects' grey matter voxels using the Craddock parcellated brain atlas as a mask. A subject-wise functional connectivity matrix reflecting pairwise Pearson correlations between the mean BOLD time series signals obtained from nodes defined by the Craddock's atlas was computed, Fisher's Z-transformed and standardized to Z-scores. Z-scores associated with significant positive correlations within each subject's whole brain functional connectivity matrix (Bonferroni corrected thresholds) were re-scaled to represent connection weights ranging from 0 to 1. Based on these weights, weighted connectivity matrices representing functional connectivity between nodes of 7 ICNs were computed and measures of global (an inverse of an average path length of neighboring nodes within the network) and local efficiency (average efficiency within the network) were derived. After accounting for age, sex, education and aerobic fitness, greater PA volume (accelerometer counts/day) and more daily MVPA were related to *greater* local efficiency in the DAN, while greater PA volume was related to *lower* local efficiency in the LN. Our results suggest that more physically active young adults show more efficient transfer of information within DAN at rest, which supports effortful control. Decreased local efficiency in the LN further suggests that PA may preferentially bias resting state functional connectivity in support of goal directed, compared to automatic and reward driven, cognitive processing.

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Poster

424. Cognition and Connectivity

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Topic: H.02. Human Cognition and Behavior

Support: Vetenskapsradet VR 721-2014-2468
VR 521-2014-3224
Barncancerfonden MT2017-0013
Swedish government under the ALF agreement ALFGBG-726541

Title: Studying executive functions during mental fatigue using functional near infrared spectroscopy (fNIRS)

Authors: *G. H. KUHN[1], S. SKAU[1], I. H. JONSDOTTIR[2], L. BUNKETORP-KÄLL[3], B. JOHANSSON[1];
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Abstract: Mental fatigue after mild traumatic brain injury (TBI-MF) and in patients with exhaustion disorder (ED) is characterized by pronounced decline in cognitive performance after moderate cognitive activity. In this study, we investigated brain activity in the frontal cortex during prolonged mental activity in TBI-MF patients (n=20), ED patients (n=20) and controls (n=20). We chose a test-retest design of six neuropsychological tests in conjunction with fNIRS to study brain activation changes in the frontal cortex during prolonged cognitive activity. The TBI-MF group performed comparatively worse than the controls during the Stroop-Simon test and did not improve as much as the controls on Digit Symbol Coding (DSC). ED patients felt subjectively more fatigued during the procedure and performed worse in the mental control task PASMO compared to controls. For fNIRS imaging, a Stroop-Simon test showed no change over time, but the controls had more activity in larger parts of the frontal cortex compared to TBI-MF and ED patients. For the Symbol Search and DSC, the TBI-MF group had more frontal brain activation than controls. ED patients activated the PFC more in the sustained attention task OPATUS-CPTA. In the Symbol Search the ED patients had higher activity in left PFC and in the Digit Symbol Coding more activation in the right frontal cortex. As the TBI-MF subjects were studied more than 5 months after injury and ED subjects were diagnosed more than 3 years earlier, our data indicate that the problems persist longer after initial diagnosis than previously known.

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Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.21/BB48

Topic: H.02. Human Cognition and Behavior

Title: Prospective and retrospective metacognitive judgments: Neural evidence that when a judgment is made determines the type of information used

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Abstract: How do you think you will perform on the test tomorrow? How did you perform on the test yesterday? The information that we use to make metacognitive judgments can vary depending on subtle differences in perspective. If asked to judge future performance, we may attempt to retrieve information we know will be on the test or attempt to practice some of the material. If asked to judge past performance, we may use our sense of fluency for the test questions or our subjective sense of how easy the test was. The current study used neuroimaging to test how the brain supports prospective and retrospective metacognitive judgments.

Participants completed a probabilistic category learning task in which they learned to classify Gabor patches into two categories based on their spatial frequency. In between learning runs, they made prospective and retrospective judgments of whether they would classify specific Gabor patches correctly. In the prospective trials, they would see a Gabor patch, rate their likelihood of correctly categorizing it, and then categorize the stimulus. In the retrospective trials they would categorize the stimulus and then rate it. During prospective judgments, activation in the hippocampus and right rostrotrolateral PFC (rlPFC) tracked the objective uncertainty in the task. This result indicates that prospective judgments more attuned to the actual uncertainty associated with each Gabor patch. During retrospective judgments, the ventral striatum and right rlPFC were active and tracked participants' subjective confidence ratings. TThese results suggest that both types of judgment rely (at least partly) on similar metacognitive processes, but may use different sources of information to inform that process. Prospective judgments may rely on information from more intrinsic factors such as the objective uncertainty of the Gabor patches while retrospective judgments may rely on more internal, subjective cues.

Disclosures: T. Kelley: None. **M.J. Serra:** None. **B.D. England:** None. **T. Davis:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

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Program #/Poster #: 424.22/BB49

Topic: H.02. Human Cognition and Behavior

Title: A two component model of human intelligence within the lateral frontal cortex

Authors: *W. R. TRENDER[1], R. BRAGA[2], A. HAMPSHIRE[1];
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Abstract: Human intelligence has been the topic of intense research since the late 1800s. One of the ways in which this research has manifested itself is in the investigation into variability between individuals' abilities to perform cognitive tasks. This is important because this variability may provide insight into the mechanisms by which our brains perform higher cognitive functions. Furthermore, although we are likely far from the truth, in the future this insight could provide a framework with which we can better understand intellectual disability and improve overall cognitive function.
In the present study we have investigated concepts of intelligence in the context of functional connectivity (FC) within the lateral frontal cortex (LFC). The LFC is known to be important in many aspects of cognition such as working memory, attention and response inhibition. However, we do not know how changes in activation and FC within the LFC relate to differences in cognitive ability.
To attempt to answer some of these questions we have used functional magnetic resonance

imaging (fMRI) and cognitive task data from 402 subjects in the Human Connectome Project (HCP). Correlations between different LFC regions time courses (TCs) were used as response variables to predict variability within the behavioural task data. A separate, seed based, whole brain analysis was performed using correlations between and within functional connectivity maps for each LFC region as response variables in a partial least squares (PLS) model to predict variation in the behavioural task data.
We found that, using the local LFC connections, the individual differences in cognitive ability could be represented by two components which explained 2.45% and 2.7% of the variability in the data respectively. No significant components were extracted from the whole brain within network coherence analysis. One significant component was extracted from the whole brain between network coupling analysis, explaining 1.21% of the variance in the behavioral data. These results provide evidence for a model of intelligence that contains distinct functional networks that work together to produce behavioural outcomes.

Disclosures: W.R. Trender: None. **R. Braga:** None. **A. Hampshire:** None.

Poster

424. Cognition and Connectivity

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Program #/Poster #: 424.23/BB50

Topic: H.02. Human Cognition and Behavior

Support: R01MH110831
P50MH094258

Title: Single neurons in human medial frontal cortex signal conflict post-action

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Abstract: Goal-directed behaviors require monitoring of on-going actions and evaluating action outcomes. The medial frontal cortex (MFC) is crucially involved such evaluative processes and plays a pivotal role in learning the target and amount of cognitive control to be exerted based on monitored outcomes. A prominent example is conflict monitoring, in which case the MFC is thought to monitor conflict between co-activated neural representations of competing actions or goals. Such monitoring signal is assumed to have an early onset before an eventual action is committed, since competition of representations should ensue as soon as processing of goal-relevant stimulus information starts. However, given the prominence of evaluative signals in the MFC, it is likely that conflict levels can also be represented after an action has been completed.

The temporal smoothing of BOLD signal obfuscates the exact timing of conflict monitoring and dissociation of pre- and post-action thus requires techniques with high spatial and temporal resolution. We report single neurons in human dorsal anterior cingulate cortex (dACC) and pre-supplementary motor area (pre-SMA) that signal conflicts after action execution. We recorded single neuron activity from epilepsy surgical candidates. We investigate such post-action conflict signals with two tasks: the color-word Stroop task and the Multi-source Interference Task (MSIT). Since a subset of patients performed both tasks in the same session, a direct comparison of single-neuron activity in both tasks is thus possible. We confirmed the existence of conflict signals triggered by stimulus onset in both tasks as previously reported. In addition, we found a significant proportion of MFC neurons that signaled conflict immediately before action onset. However, the post-action conflict neurons constitute a different class of neurons than these two types of conflict neurons with pre-action activation. The post-action conflict signal could not be explained simply by continuation of stimulus-onset-triggered or pre-action conflict. Importantly, despite their similar timings and overall dynamics, post-action conflict signals in Stroop and MSIT are carried by two separate populations of neurons, which highlights the disparate nature of these signals. In addition, we also found correlation between the spike rates of these neurons and behavioral measures in the tasks. The post-action conflict signals could represent a metacognitive mechanism that evaluates difficulty encountered during action performance and enables learning and optimization of such actions.

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Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.24/BB51

Topic: H.02. Human Cognition and Behavior

Title: Stroop interference and 5HTTLPR polymorphism between heavy smokers

Authors: *H. AGUILAR-ZAVALA, J. NEGRETE-DIAZ, D. VARGAS-QUINTANA, F. SOTO;
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Abstract: Introduction: Seven millions of people in the world dead to cause of smoker's habits. Smokers dead earlier, although world effort to limit the tobacco's use, the nicotine dependence is stronger. The genetic factors can explicate about dependence mechanism. The 5HTTLPR polymorphisms give risk to drugs use and abuse, in addition was related with cognitive disturbance, but there are few studies about link between 5HTTLPR polymorphism and smoker's habits. Objective: The main of this study was to compare the polymorphisms 5HTTLPR,

psychologic factors and attention skills between heavy smokers and no smokers. Methods: 55 subject adults were included, 28 heavy smokers and 27 no smokers. General data, family income and perceived stress were asked and the attentional skills from Stroop Colors Word test was evaluated. Blood sample was collected (10 ml) for DNA extraction and genotype classification of 5HTT gen polymorphisms. The subjects were grouping by allelic variants "S" (44pb deletion) (59 subjects) and "L" (528pb) (51 subjects). Results: Subjects with S allele show higher scores in attention ($\tilde{X}$=6.11) than subjects with L allele ($\tilde{X}$=6.11) (Z=0.012,p<0.02); In addition, 48% of smokers with S allele show lower age of initiation to tobacco habit (Z=-2.26,p<0.03). Furthermore, this results only keep similar in women with S allele. Conclusion: Subjects with S allele are better in resistance of interference Stroop, but begin to smoke early than others with allele L. The S allele only has effect in women.

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Poster

424. Cognition and Connectivity

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Topic: I.07. Data Analysis and Statistics

Support: This work was supported in part by a Grant to CABMC (Control of Animal Brain using MEMS Chip) funded by Defense Acquisition Program Administration (UD140069ID)
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Title: Inter personal functional connectivity in low beta band is associated with emotional convergence during watching movie

Authors: *H. KIM[1], P. SEO[1], S. HER[1], S. CHOI[1], J. CHOI[2], K.-H. KIM[1];
[1]Biomed. Engin., Yonsei Univ., Wonju, Korea, Republic of; [2]Neurosurg., UCLA, Los Angeles, CA

Abstract: Introduction: Recent studies demonstrated that physiological responses of interacting individuals become synchronized. However, it is unknown how the neural activities of individuals are synchronized and whether this inter-personal neural synchrony occurs from task similarity or emotional convergence. Here we tried to investigate the relationship between inter-personal neural synchrony and emotional convergence level during watching affective movies individually or simultaneously.

Methods: Fifteen subjects (22.8±1.66 years old, 10 males) watched affective movie clips individually in the first experiment, and sixteen pairs of healthy subjects with same sex (22.5±1.65 years old, 8 male pairs) watched the movie clips simultaneously in the second experiment. 62-channel EEGs were recorded. Immediately after watching each video clip, the subjects reported their emotional responses as valence levels in 9-point Likert scale. Emotional convergence scores were calculated as the inverse of absolute value of the difference between the self-ratings of the two subjects in a pair. Source current densities were estimated on cortical surface using weighted minimum norm estimation from surface EEGs. Inter-personal functional connectivity was quantified by the phase-locking values (PLVs). The association between PLV and emotional convergence score was investigated by Spearman correlation for the 16 pairs. The distribution of correlation from randomly-shuffled pairs was generated from 1,000 surrogate data in order to verify the significance of the association between PLV and emotional convergence.
Results: The Spearman correlation was significantly higher than zero for alpha- and beta-bands. This implies that the interpersonal functional connectivity is significantly correlated with emotional convergence, even when the two subjects did not watch the movie simultaneously. Comparing the first and second experiments, however, the second experiment, i.e. simultaneous watching, resulted in significantly higher correlation. The association between PLV and emotional convergence was concentrated in frontal, left temporal, paracentral, and occipital areas, in low beta-band.
Discussion: The results showed that shared emotional experience during simultaneous watching condition enhances the functional connectivity of cortical activities of two persons in low-beta band, probably due to the activity of mirror neuron system.

Disclosures: H. Kim: None. **P. Seo:** None. **S. Her:** None. **S. Choi:** None. **K. Kim:** None. **J. Choi:** None.

Poster

424. Cognition and Connectivity

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Topic: I.07. Data Analysis and Statistics

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Title: Changes in event-related potential due to vigilance reduction by long-term driving

Authors: *P. SEO[1], H. KIM[1], S. HER[1], S. CHOI[1], J. CHOI[2], K.-H. KIM[1];
[1]Yonsei Univ., Wonju, Korea, Republic of; [2]Neurosurg., UCLA, Los Angeles, CA

Abstract: Our purpose was to investigate the changes in event-related potentials (ERPs) due to the vigilance reduction during long-term driving. Twenty undergraduate and graduate students participated in the experiment (18 males, 2 females, age: 23.9±1.4 years, average driving experience: 5.1±1.4 years). The participants took enough sleep (> 8 hours) in the night before the experiment, and did not take caffeine, nicotine, or other drugs on the day of the experiment. Experiments were conducted at 7:00 pm, after normal daytime activities of typical undergraduate students.
The participants were instructed to keep lane during driving on the straight road with two lanes. Two kinds of unexpected situation occurred randomly every 30±0.0764 seconds: sudden stopping of preceding vehicle, and sudden secession from the lane. 64 channel EEG were recorded along with steering wheel angle, acceleration/brake pedal operation, and driver's vehicle position in the lane.
The EEG signals were segmented according to the onsets of the unexpected events and classified into high or low vigilance conditions according to the response time (RT) (high vigilance: short RT, low vigilance: long RT, compared to the average RT). The ERP waveforms were obtained by averaging single-trial EEG waveforms, and major ERP components were defined according to the polarity and latency of peaks in the waveforms. The amplitudes and latencies of each component were statistically compared between high and low vigilance conditions.
Two ERP components were identified characterizing significant differences between high and low vigilance. The first one was at 300 ms after the unexpected event onset, representing the perceptual performance reduction due to the fatigue of long-term driving. Despite the huge differences between the two types of unexpected events, the characteristics of neural activity changes according to the vigilance level were not essentially different. Another ERP component was identified, which showed amplitude reduction for lower vigilance condition during a broad interval from 400 to 700 ms in parietal-occipital area. This corresponds to P300, which is recognized to show amplitude decrease for inattentive state. It is expected that cumulative fatigue of long-term driving causes attentional dysfunction as well as impaired sensory perception.

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Poster

424. Cognition and Connectivity

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Title: Abnormal cortical activities of idiopathic REM sleep behavior disorder patients during visuospatial attention revealed by deep neural network

Authors: *S. HER[1], D. YEO[1], H. KIM[1], P. SEO[1], K. CHA[1,2], S. CHOI[1], J. CHOI[3], J.-A. LIM[4,2], J.-I. BYUN[5,2], T.-J. KIM[6,2], K.-Y. JUNG[2], K.-H. KIM[1];
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Abstract: Idiopathic REM sleep behavior disorder (IRBD) is a sleep disorder which is characterized by dream enactment behavior and the loss of muscle atonia during REM sleep, and known to be associated with high risk of developing neurodegenerative diseases. Many IRBD patients are suffering from cognitive impairments, particularly visual perception, visuospatial abilities, attention, and executive function. However, neurophysiological mechanism of these cognitive impairments has not yet been identified. We investigated the oscillatory cortical activities of IRBD patients during attentional task, and tried to identify the spatiotemporal characteristics of abnormality compared to normal controls using machine learning. 15 IRBD patients and 17 healthy control subjects performed attentional network test while 60 channel EEGs were recorded. The subjects were requested to respond as quickly as possible to press a direction key corresponding to the middle arrow direction of the target stimulus. Source current densities on the cortical surface were estimated using weighted minimum norm estimate method. We tried to investigate the differences in single-trial cortical activities between IRBD patients and normal controls using a pattern classifier based on deep neural network (DNN). In addition, the critical information for the pattern classification was obtained by applying layer-wise relevance propagation (LRP) method to the trained DNN. The DNN consisted of four fully-connected layers of the units with rectified linear activation function. The time-series of single-trial event-related current densities at 100 cortical vertices formed the input to the DNN. The relevance score, i.e. the contribution of each input unit to the classifier's decision, was obtained by LRP method, which yielded the information on the time and location showing major difference between IRBD and control. The classification accuracy was as high as 73.8±1.3% (sensitivity: 70.4±2.9%, specificity: 76.8±2.7%). The activities of visual areas within 150-250 ms epoch were found to be the most critical for the classification of the two groups. This is in accordance with our previous ERP studies using Posner cueing task. Another important feature was the right frontal activity within 250-300 ms, which is judged to represent visuoperceptual dysfunction IRBD patients, due to top-down processing of frontal area. This is in line with previous studies which showed cortical thickness reduction in frontal area and lingual/fusiform gyri in IRBD.

Disclosures: **S. Her:** None. **D. Yeo:** None. **H. Kim:** None. **P. Seo:** None. **K. Cha:** None. **S. Choi:** None. **J. Choi:** None. **J. Lim:** None. **J. Byun:** None. **T. Kim:** None. **K. Jung:** None. **K. Kim:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.28/BB55

Topic: I.07. Data Analysis and Statistics

Support: Hasso Plattner Design Thinking Research Program (HPDTRP)

Title: Higher flexibility in functional connectivity dynamics is associated with higher creativity and lower inhibition

Authors: ***H. XIE**[1], S. JAHANIKIA[1], C. GENIESSE[1], R. BEATY[2], N. SONALKAR[1], M. SAGGAR[1];
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Abstract: Creative thinking is crucial to scientific and technological innovation. While many studies approach creativity as either a divergent or convergent construct, we adopted a novel paradigm - creative foraging task (CFT; Hart et al., 2017) - to study the neurobehavioral relation between creativity and exploration/exploitation. Functional MRI data (N=29) were acquired while the participants were engaged in the CFT, where participants reorganized ten connected squares into unique and interesting solutions (Fig. 1). After preprocessing (fMRIprep v1.2.8), dynamic functional connectivity (FC) was estimated and the multi-slice community detection was used to quantify flexibility in FC. High FC flexibility implies higher rate of brain regions switching community memberships and has been linked with memory performance and learning. Here, we tested whether FC flexibility during CFT is related with creativity (using Alternate Uses Task and CFT performance) and executive functioning. Although no significant relation was observed between CFT performance (e.g., number of shapes created) and FC flexibility, a significant positive relation between FC flexibility during CFT and AUT fluency was observed (rho = 0.47, p = 0.025). Further, a negative relation between FC flexibility during CFT and cognitive inhibition (rho = -0.44, p = 0.035) was observed. In sum, our preliminary results suggest higher variation in brain dynamics is associated with higher creativity and lower inhibition.

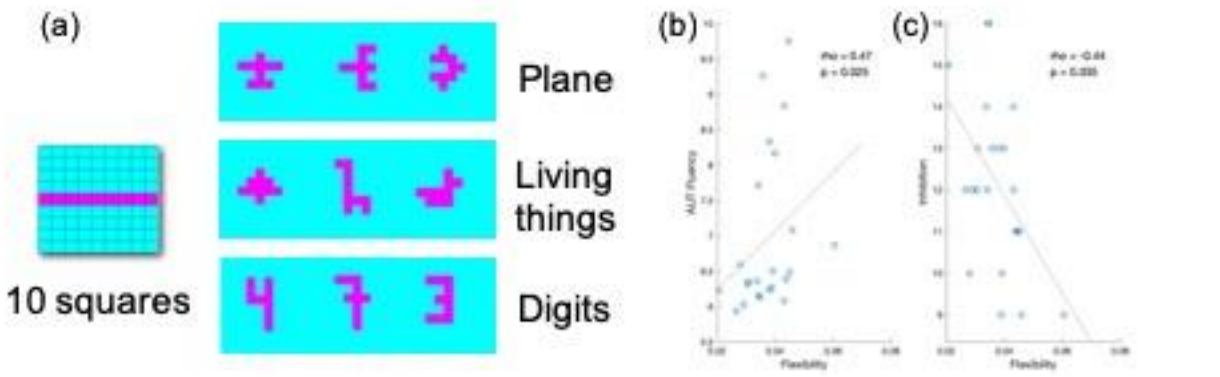

Figure1: (a) Creative foraging task. Subjects used a mouse to reorganize ten squares within a ten by ten space, with the only rule being all squares must be connected (diagonal connection not allowed). A total number of 36,446 unique solutions exists and our participants created 2,149 unique shapes during the 10 minutes' task. (b) AUT fluency vs FC flexibility. (c) Cognitive inhibition vs FC flexibility.

Disclosures: H. Xie: None. **S. Jahanikia:** None. **C. Geniesse:** None. **R. Beaty:** None. **M. Saggar:** None. **N. Sonalkar:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.29/BB56

Topic: H.02. Human Cognition and Behavior

Support: SFB1280

Title: In search of the extinction network: A multi-center study

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Abstract: Extinction learning is defined as the gradual decrease in the conditioned response with continuous presentation of the conditioned stimulus in the absence of the aversive stimulus. Converging behavioural and physiological evidence indicate that extinction is a new type of learning. However, the neural mechanism subserving this new learning process is far from understood. Indeed, although a few studies suggest that the extinction of learned fear responses involve changes in brain connectivity within an extended neural network, the exact brain structures that define this network remain largely elusive. In this ongoing project, we have

collected over two-hundred resting-state fMRI and diffusion weighted data shortly before participants engaged in various extinction learning paradigms. Our initial aim is to find structural and functional connectivity patterns that relate to the extinction learning network. Second, we intend to test whether structural and functional connectivity patterns predict inter-individual variations in the extinction efficacy. Finally, we also compare different preprocessing pipelines, both in-house as well as existing automatic implementations. Preliminary findings reveal correlations among several regions, including the hippocampus, amygdala, ventro-medial prefrontal cortex, anterior cingulate gyrus and cerebellum. These regions have been previously associated with the extinction learning network. Current work is in place to measure other types of connectivity, in particular Granger causality and spectral dynamic causal modelling, which will provide information about causal links within this network. Furthermore, we also aim to model behavioural and electrodermal activity responses during extinction learning, which will be used as indicators of learning efficacy.

Disclosures: C. Gomes: None. **F. Labrenz:** None. **H. Quick:** None. **D. Timmann-Braun:** None. **N. Axmacher:** None.

Poster

424. Cognition and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 424.30/BB57

Topic: H.02. Human Cognition and Behavior

Title: Episodic past, future, and counterfactual thinking in relapsing-remitting multiple sclerosis patients

Authors: *F. DE BRIGARD[1], O. AYALA[2], D. BANTA[1], L. DUARTE[2], A. LOZANO[2], J. GARCIA[3], P. MONTAÑÉS[2], S. W. DAVIS[4];
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Abstract: Multiple sclerosis (MS) is a progressive disease characterized by widespread white matter lesions in the brain and spinal cord. In addition to well-characterized motor deficits, MS results in cognitive impairments in several domains, including attention, working memory, executive functions and, critically, episodic memory. Recent studies have also revealed that patients with MS exhibit deficits in episodic future thinking, i.e., our capacity to imagine possible events that may occur in our personal future. Both episodic memory and episodic future thinking have been shown to share cognitive and neural mechanisms with a related kind of hypothetical simulation known as *episodic counterfactual thinking*: our capacity to imagine alternative ways in which past personal events could have occurred but did not. However, the extent to which episodic counterfactual thinking is affected in MS is still unknown. The current

study sought to explore this issue by comparing performance in mental simulation tasks involving either past, future or counterfactual thoughts in 22 non-depressed relapsing-remitting MS patients and 22 matched controls. All participants underwent MRI structural imaging, and a thorough neuropsychological assessment prior to engaging in a mental simulation task. This task consisted in randomly generating episodic past, future and counterfactual thoughts in response to prompts. The contents of the mental simulations for all participants were assessed using an adapted version of the autobiographical interview. We found that, relative to controls, patients showed mark reductions in the number of internal details across all mental simulations. However, they show no differences in the number of external and semantic-based details, suggesting that the deficit is more specific to episodic than to semantic memory. Additionally, diffusion-weighted imaging tractography was used to estimate the integrity of several canonical fiber pathways. While we found that declines in fractional anisotropy in all white matter tracts—including primary motor (i.e., corticospinal tract) as well as retrieval-related pathways (i.e., uncinate fasciculus, corpus callosum)—correlated with deficits in tests of executive function, working, and episodic memory, tractography measures of streamline values for the superior longitudinal fasciculus correlated with more detailed counterfactual simulations in MS patients relative to controls. These findings further illuminate the brain basis of hypothetical simulation deficits in MS.

Disclosures: F. De Brigard: None. **O. Ayala:** None. **D. Banta:** None. **L. Duarte:** None. **A. Lozano:** None. **J. Garcia:** None. **P. Montañés:** None. **S.W. Davis:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.01/BB58

Topic: B.09. Network interactions

Support: MAGNET program of the Israel Innovation Authority grant (61450)

Title: Frontal asymmetry in ADHD and its modulation by TMS treatment

Authors: A. AVNIT, U. ALYAGON, *A. ZANGEN;
Life Sci., Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel

Abstract: Abnormal functional brain asymmetry and interhemispheric coherence have been previously implicated in attention deficit hyperactivity disorder (ADHD). However, as current evidence for adult ADHD is scarce and inconclusive, it is not clear whether these neuromarkers proceed into adulthood. We investigated these abnormalities in adults ADHD by comparing a cohort of 99 ADHD and 78 non-clinical individuals. By combining TMS with EEG, we additionally examined right-frontal cortical excitability, indicated by TMS-evoked potential

(TEP), and interhemispheric connectivity, reversely indicated by right-to-left interhemispheric signal propagation (ISP). Moreover, we examined the relation between aberrant frontal asymmetry and a response inhibition deficiency in ADHD, measured as stop-signal reaction time and the N200 ERP components. The modulation of the above measures by a clinically effective multi-session TMS treatment was also examined. We found that, compared to controls, the ADHD group demonstrated elongated SSRT, reduced N200 right-frontal-asymmetry, weaker TEP, and stronger ISP. Moreover, in the ADHD group, N200 right-frontal-asymmetry positively correlated with SSRT and with TEP. Additionally, the effective TMS treatment decreased interhemispheric Alpha-power coherence, while its clinical effect was predicted by spontaneous low-frequency right-asymmetry measured at baseline. However, no differences between the ADHD and control groups in spontaneous resting-state asymmetry or in interhemispheric functional connectivity were observed. We conclude that: (1) In adult ADHD, frontal asymmetry abnormalities are apparent during response inhibition performance, but not for spontaneous brain oscillations; (2) Frontal interhemispheric connectivity abnormalities, previously observed in ADHD children, may normalize with age; (3) Abnormal frontal asymmetry is related to a key cognitive deficit in ADHD and to the clinical effect of TMS treatment, and may originate from reduced right-frontal excitability; (4) An effective TMS treatment might improve executive cognitive functioning, as indicated by decreasing interhemispheric Alpha coherence.

Disclosures: A. Avnit: None. **U. Alyagon:** None. **A. Zangen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Brainsway. F. Consulting Fees (e.g., advisory boards); Brainsway.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.02/BB59

Topic: H.02. Human Cognition and Behavior

Support: BBSRC

Title: Functional reorganization for executive functions in deaf individuals

Authors: *B. MANINI[1,3], V. VINOGRADOVA[1], B. WOLL[3], M. EIMER[4], D. CAMERON[2], J. SAADA[5], V. CARDIN[3,1];
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Abstract: Recent studies have described the functional reorganization of working memory networks in deaf individuals (Cardin et al., 2017; Ding et al., 2015). Specifically, the posterior superior temporal cortex, involved in auditory processing in hearing individuals, is recruited for Visual Working Memory (VWM) tasks in deaf participants. Furthermore, hearing and deaf participants show different functional connectivity profiles between temporal and frontoparietal regions. These results suggest that early auditory experience may impact the neural profile underlying cognitive processing. It is still unclear if this reorganization is ascribable to some specific aspects of VWM (namely, visual information storage) or if a broader functional reorganization occurs in the brain circuits involved in executive functions as a consequence of deafness. To address this, we studied brain regions involved in executive function in congenitally or early deaf and hearing individuals. We hypothesized reorganization of executive function networks as a consequence of early sensory loss.
Profoundly or severely deaf individuals and hearing control participants underwent MRI while performing tasks tapping into four executive functions: working memory, inhibition, task-switching, and planning. Images were realigned, co-registered, normalized, and smoothed (8 mm FWHM Gaussian kernel) following standard pre-processing procedures in SPM12.
Our results showed different functional profiles of activation between the groups. Specifically, there were differences in the extent of activation of temporal and frontoparietal regions between the two groups. These results suggest that neural plasticity and early auditory deprivation may reshape the cortical networks underlying higher-order cognitive functions.

Disclosures: B. Manini: None. **V. Vinogradova:** None. **B. Woll:** None. **M. Eimer:** None. **D. Cameron:** None. **J. Saada:** None. **V. Cardin:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.03/BB60

Topic: H.02. Human Cognition and Behavior

Support: R.11012/08/2017-HR

Title: Hyperactivation of inferior frontal gyrus inhibits hyperactivity in ADHD: A quantitative EEG study

Authors: *T. BATABYAL[1], S. P. MUTHUKRISHNAN[1], C. LEON[1], R. SAGAR[2], P. TAYADE[1], R. SHARMA[1], S. KAUR[1];
[1]Physiol., [2]Psychiatry, All India Inst. of Med. Sciences, New Delhi, New Delhi, India

Abstract: Background:-Attention deficit hyperactivity disorder (ADHD) is the most common neurobehavioral disorder of childhood associated with impaired attention, excessive motor

activity and impulsivity.The neural signature of attentive mechanisms is largely dominated by alpha band oscillations and its inhibitory function has been recently suggested. Thus it is closely linked to two fundamental functions of attention (suppression and selection). Hence, we wanted to explore the pathophysiological relevance of cortical sources in the alpha band which are different between ADHD as compared to age-matched controls during resting state.
Methodology:- Resting state EEG data was acquired from 10 ADHD (mean age ± SD =11.1yrs ± 2.42, 9 males, 1 female) and age-matched controls (mean age ± SD =12 yrs ± 3.65, 8 males, 2 females) using 128 channel high density electrodes. Data was preprocessed in the frequency range of alpha (8 to 14 Hz) and source analysis was done using sLORETA algorithm. Statistical analysis of cortical source activity was performed using Statistical Non Parametric Mapping (SnPM) in sLORETA and cortical sources showing voxel activity above a statistically significant ($p<0.05$) with the critical threshold value of $t=3.772$ has been discussed.
Results:- Precentral gyri, superior frontal gyri, inferior frontal gyri and medial frontal gyri in frontal lobe and post central gyri in parietal lobe showed significantly higher activity in ADHD during resting. Maximum change was found to be localized at inferior frontal gyri in the frontal lobe (MNI coordinates 40,25,10; max $t = 4.656$, $p = 0.012$).
Discussion:- The motor cortical areas of pre-central and post-central gyri have shown higher activity in ADHD as compared to controls. This could be the neural basis for hyperactivity in ADHD. In order to overcome this behavioural symptom, inferior frontal gyrus has shown highest activity in ADHD which is known as a response inhibition centre. This could be one of the neural compensatory mechanism in ADHD wherein hyperactivity of inferior frontal gyrus in alpha frequency range causes inhibition of the motor cortical areas of pre-central and post-central gyri thereby suppressing hyperactive behaviour during resting state.

Disclosures: T. Batabyal: None. **S.P. Muthukrishnan:** None. **C. Leon:** None. **R. Sagar:** None. **P. Tayade:** None. **R. Sharma:** None. **S. Kaur:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.04/BB61

Topic: H.02. Human Cognition and Behavior

Title: Effects of repetitive head impacts on cognitive functions in collegiate contact sport athletes

Authors: *C. R. VUELVAS-OLMOS[1,2], N. Y. CORTÉS-ALVAREZ[1,3], P. J. FLORES-MORENO[1,2], A. VUELVAS-OLMOS[4], J. GUZMÁN-MUÑIZ[5,3], N. A. MOY-LÓPEZ[4,3], F. ROJAS-LARIOS[1,2];

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Abstract: Introduction: Contact sport practice has been rise worldwide. Contact sports are sports that emphasize or require physical contact between players. Currently soccer, football, rugby and box are the most practiced sport in the world, however, in these sports direct or indirect contact of the head are implicated. According previous studies, head impacts constant while playing contact sports may lead to a variety of worrisome outcomes, even, an increased the susceptibility to concussion, chronic traumatic encephalopathy risk, changes in the brain with just a season in a sport contact, even when the player is not outward signs of a concussion, including neurocognitive deficits and brain matter changes at magnetic resonance imaging.
Aim: To determine the effects of contact sport practice on cognitive performance in collegiate sport athletes.
Material and method: Participants were 20 collegiate soccer players and 20 noncontacts collegiate sport athletes, who practiced along 6 months uninterrupted and without record of TBI. All athletes were assessed using the CogState Battery, which consists of six cognitive tasks that measure control motor visual, associated peer learning, psychomotor function, attention, working memory and executive function.
Results: There no found significant differences between contact sport group compared to noncontact sport group, according to the sex, age and years of education. The contact sport athletes performed more poorly at two cognitive domains than noncontact sport athletes: Associated peer learning (contact group: accuracy (ACC) 1.39±0.22 vs noncontact group: ACC 1.78±0.18; p=0.001) and working memory (contact group: Movements per second (MPS) 1.47±0.3, correct moves (CMV): 45±6 vs noncontact group: MPS 1.60±0.24, CMV: 51±4; p=0.003). There not found significant differences in the others cognitive domains.
Results: Therefore, repetitive head impacts may negatively impact the associated peer learning and working memory functions in soccer players; even when there are no outward signs of an injury. Further studies are necessary, mainly because in Mexico it has not been issued or published in the declaration of the national health policy for the evaluation and management of the heads.

Disclosures: C.R. Vuelvas-Olmos: None. **N.Y. Cortés-Alvarez:** None. **P.J. Flores-Moreno:** None. **J. Guzmán-Muñiz:** None. **N.A. Moy-López:** None. **F. Rojas-Larios:** None. **A. Vuelvas-Olmos:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.05/BB62

Topic: H.02. Human Cognition and Behavior

Support: Institute of Education Sciences, U.S. Department of Education, through grant R305B150008 to Carnegie Mellon University

Title: Effects of cognitive-motor training on executive function and resting prefrontal cortex connectivity in preschool children: An fNIRS study

Authors: *C. ENG[1], M. POCSAI[1], F. FISHBURN[4], N. C. WILLIAMS[2], D. CALKOSZ[3], E. THIESSEN[1], A. FISHER[1];
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Abstract: Studies utilizing functional near infrared spectroscopy (fNIRS) show that prefrontal cortex (PFC) connectivity at rest plays an important role in the development of executive functions (EF). Studies with older populations show that Exergames (concurrent cognitive and motor stimulation) improve EF. Yet, little is known about the effects of Exergame training on EF and the associated neural substrates in children below school age. This study investigated the effects of Exergame training on resting PFC connectivity and EF of 4 to 5 year olds. Participants were first blocked on sex, classroom, and age, and then were randomly assigned to either the Exergame or Control condition: 21 children formed the Exergame group (M = 5.06 ± .65 years; 8 females), while 20 children (M = 5.06 ± .64 years; 8 females) in the Control group engaged in typical classroom activities. The 1-week Exergame intervention included two 20-minute consecutive sessions. The Exergame was projected onto a wall with a connected nonslip game mat and children responded by stepping left or right on the game mat's arrows in accordance with the goals of the game. PFC resting connectivity, performance on 2 EF tasks (Flanker and Day-Night), and teacher ratings of EF (BRIEF) were collected before the intervention (pretest), after the intervention (posttest), and 1 month later (delay test). PFC connectivity was assessed using *Inscapes*: a movie paradigm of abstract shapes without a narrative that elicits patterns of resting connectivity, while mitigating fidgetiness. Teachers and researchers who administered the EF tasks were blind to condition assignment. Oxy-hemoglobin concentration was recorded using a 10-channel continuous wave fNIRS system (TechEN CW6, 8 detectors, 4 sources) on the PFC, and connectivity was computed between all channel pairs at pre, post, and delay tests. A repeated measures ANOVA on each of the Z-transformed connectivity values was conducted, correcting for multiple comparisons. Results indicate that Exergame training significantly increased PFC inter-hemispheric connectivity, increased intra-hemispheric connectivity in the left PFC, Flanker Task accuracy, Day-Night Task accuracy, and BRIEF scores from pre to posttest (all ps < .001), while no significant differences were found in the sex/classroom/age-matched control group in PFC connectivity or EF assessments from pre to posttest. These Exergame training induced changes in PFC connectivity correlated with improved EF skills. The improvements in EF persisted over a 1 month period. This study provides novel insights into the associations between Exergame training, PFC connectivity, and EF development in preschool children.

Disclosures: C. Eng: None. **M. Pocsai:** None. **F. Fishburn:** None. **N.C. Williams:** None. **D. Calkosz:** None. **E. Thiessen:** None. **A. Fisher:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.06/BB63

Topic: H.02. Human Cognition and Behavior

Support: KHIDI Grant HI18C1166

Title: A resting-state fNIRS study in early childhood and early adolescence

Authors: *S. EOM[1], **J. CHOI**[2], J.-M. KIM[2], M. LEE[3], D.-J. YI[4];
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Abstract: Resting-state functional near-infrared spectroscopy (fNIRS) is a potential technique for the study of brain functional connectivity (FC) and networks in children. The present study aimed to investigate functional brain activation patterns in network organization during development from early childhood to early adolescence using resting state functional Near Infrared Spectroscopy (rs-fNIRS).
This study enrolled 71 healthy normal participants, including 44 young children (7.7 ± 1.1 years, age range of 6.0-10.1 y; 17 boys) and 27 early adolescents (11.1 ± 0.8 years, age range of 11.1-13.0 y; 8 boys). Measurements were performed by NIRSIT (OBELAB Inc, Korea) which utilizes 24 lasers source and 32 photo detectors with multiple source-detector spacing. For representing individual distance matrix, density parameter is calculated after applying threshold value at the distance matrix. Moreover, relating neuropsychological tests for working memory and processing speed such as Digit Span, Coding, and Symbol Search from Wechsler Intelligence Scale for Children-fourth edition, and for executive function such as Children's Color Trails Test (CCTT) were performed. fNIRS data and behavioral measure were analyzed with normality test and independent t-test as an inter-group factor and correlation analysis was conducted among fNIRS, behavioral and neuropsychological data. Subjects were classified into two groups by their cognitive functional level as low- and high- group based on their scores in the neuropsychological tests. And then, we compared functional connectivity matrices of low- and high score groups at the level of cognitive function.
In terms of development, early adolescents showed significantly high level of density parameter in rs-fNIRS compared to early children in cognitively low function group, particularly in Coding ($p<0.05$), and CCTT interference ($p<0.01$). However, no significant differences on rs-fNIRS parameters were shown in high function level group between early children and early adolescents. Furthermore, significant discrimination in CCTT interference based on rs-fNIRS density between low and high function group was found ($P<0.01$) in early adolescents.

Our results suggest that re-fNIRS parameters are associated with different functional activation patterns in the frontal subregions based on not only cognitive level but also developmental aspects. fNIRS could be a potential measure as an effective tool to investigate the contributions of developmental prefontal function of executive functioning in early children and early adolescents.

Disclosures: **S. Eom:** None. **J. Choi:** None. **J. Kim:** None. **M. Lee:** None. **D. Yi:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.07/BB64

Topic: H.02. Human Cognition and Behavior

Support: Northern Norway Regional Health Authority, 5154\PEP1012-11
Helgeland Hospital Trust

Title: Working memory, inhibitory control, and set-shifting in an unmedicated ADHD cohort diagnosed in adulthood

Authors: *V. A. GRANE[1,2], A.-K. SOLBAKK[2,1,3], T. ENDESTAD[2,1];
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Abstract: Objective: To examine whether core executive control functions are reduced in unmedicated adults with Attention Deficit Hyperactivity Disorder (ADHD) compared to match healthy controls. **Method:** Performance on tasks demanding memory span and working memory (Digit Span), interference control and response inhibition (Color Word Interference Test; CWIT-Inhibition), and set-shifting (Trail Making Test; TMT and CWIT-Switching) was assessed in newly diagnosed patients with ADHD (n=36) and in healthy controls (n=34). The associations of test scores to self-reported symptoms (Achenbach System of Empirically Based Assessment, ASEBA) and executive function in everyday life (Behavior Rating Inventory of Executive Function; BRIEF-A) were also examined. **Results:** The groups did not differ in immediate memory span, but the ADHD cohort performed significantly worse than controls when there was an additional demand on working memory. Statistically controlling for individual differences in information processing speed, using an independent simple reaction time measure, did not influence the result. The ADHD group performed inferiorly to the control group on the baseline psychomotor speed conditions of the TMT, but the most pronounced difference appeared on the set-shifting condition. Results on the interference control/response inhibition task (CWIT-Inhibition) did not distinguish the groups, but there was a tendency for the ADHD group to perform more poorly when a concurrent demand on attentional switching was introduced

(CWIT-Switching). For the ADHD group, prolonged CWIT-Switching task completion time was significantly associated with increased self-reported problems with behavioral regulation (BRIEF-A). **Conclusion:** ADHD-related reductions of attentional set-shifting and working memory capacity, but not inhibitory control, support the growing literature suggesting that adult ADHD may not be associated with an overall impairment in executive functioning, but rather with difficulty in specific aspects of executive function. Accordingly, clinical assessment should ideally span a wide range of executive control tests, including the fundamental control functions studied here.

Disclosures: V.A. Grane: None. **A. Solbakk:** None. **T. Endestad:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.08/BB65

Topic: H.02. Human Cognition and Behavior

Support: Simons Foundation Autism Research Initiative Award #220843
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Title: Time course of induced oscillatory neural activity during Stroop conflict in healthy adults and children

Authors: *C. L. DALE[1], L. B. HINKLEY[1], K. G. RANASINGHE[2], T. L. LUKS[1], A. M. FINDLAY[1], N. J. POJMAN[2], P. BUKSHPUN[2], T. THIEU[2], T. P. ROBERTS[3], P. MUKHERJEE[1], E. H. SHERR[2], S. S. NAGARAJAN[1];
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Abstract: A long history of research utilizing Stroop interference tasks has documented poorer performance when task-directed responses conflict with overlearned or automatic responses in healthy adults and youth. Electrophysiological and BOLD-derived cortical sources associated with conflict-related processing implicate frontal, parietal, and anterior cingulate areas, the latter occurring 400-450 ms post-stimulus. These evoked methods emphasize low frequency activity that is tightly coupled to stimulus onset, minimizing neural processes with higher temporal variability that may help describe the neural underpinnings of cognitive conflict. Given that efficiency of executive functions and fidelity of frontal regions are developing in youth, induced

spatiotemporal patterns of activity are of interest. Healthy adults (N = 54, 18-55 years) and typically-developing youth (N = 42, 7-17 years) underwent a cognitive assessment battery and whole-head magnetoencephalography recording (MEG, CTF Systems, Vancouver BC) during a Color Naming Stroop Task. Using adaptive spatial beamforming methods implemented in NUTMEG (bil.ucsf.edu/nutmeg), the difference between cortical oscillatory power induced by Incongruent versus Congruent stimuli was analyzed at each voxel over time within 5 frequency bands. Cortical power differences were submitted to within- and between-group SnPM analyses, as well as correlated with performance on both Stroop and cognitive assessment tasks. Typical Stroop performance decrements were obtained in each age group, with youth both slower and less accurate than adults. MEG results indicate a spatial sequence of frontal conflict-related activation in adults, predominately in theta and high gamma bands, initiated as early as 125 ms and continuing through the response. Patterns of high gamma activity indicate a more complex, re-entrant pattern of conflict activity in lateral and medial frontal areas, as well as inferior parietal lobe. Youth showed limited activation in lower frequency bands, but strong high gamma activity localized to medial frontal and cingulate areas from 200 ms through 475 ms. Overall, data reveal a spatiotemporal evolution of conflict-related neural activity in multiple frequency bands as executive functions mature.

Disclosures: C.L. Dale: None. **L.B. Hinkley:** None. **K.G. Ranasinghe:** None. **T.L. Luks:** None. **A.M. Findlay:** None. **N.J. Pojman:** None. **P. Bukshpun:** None. **T. Thieu:** None. **T.P. Roberts:** None. **P. Mukherjee:** None. **E.H. Sherr:** None. **S.S. Nagarajan:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.09/BB66

Topic: H.02. Human Cognition and Behavior

Title: Neuropsychological assessment of executive functions in juvenile offenders

Authors: *P. ZARATE GONZALEZ, J. VAZQUEZ-RAMIREZ, N. SANDOVAL-FLORES, F. BENITES-SERRATOS, D. PAZ-TREJO, H. SANCHEZ-CASTILLO;
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Abstract: Different perspectives and theories in cognitive neuroscience have been tried to explain juvenile delinquency and risk behavior in adolescents, especially in those who committed homicide, kidnap, rape and extremely violent behavior. Nowadays two perspectives spread light through the issue. On the one hand, the arguments about cerebral immaturity bring us a closer look into the PFC, in which the role played by the density of gray and white matter is crucial in the process of decision making in a risky situation; on the other hand, the brain imbalance model provides a new paradigm that questions the adolescent's own immaturity,

placing the asynchrony between cortical and subcortical areas as an explanatory framework. In this study, we performed a neuropsychological assessment of the executive functions in juvenile offenders from the General office for juvenile offenders in Mexico City (DGTPA). The sample consisted of 30 male (mean 16 years old) and 6 female (mean 17 years old) adolescents (N=36) convicted mostly for homicide, robbery and kidnap. We employed a neuropsychological battery called BANFE (neuropsychological battery of executive functions and frontal lobes) that links prefrontal, orbitomedial and dorsolateral areas with specific executive functions. The results indicate alterations in a small group of participants in both, anatomical and functional areas and overall executive functioning, these results only showed significant differences between female and male in those executive functions related with orbitomedial and dorsolateral areas. The hypothesis of emotional valence is highly associated with risky behavior in adolescents as a mediator of decision making in almost all executive functions and displace the hypothesis of neurological immaturity in the commission of juvenile delinquency.

Disclosures: P. Zarate Gonzalez: None. **J. Vazquez-Ramirez:** None. **N. Sandoval-Flores:** None. **F. Benites-Serratos:** None. **D. Paz-Trejo:** None. **H. Sanchez-Castillo:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.10/BB67

Topic: H.02. Human Cognition and Behavior

Support: NSF Graduate Research Fellowship
UCLA start up funds

Title: Do different self-control skills in youth share a common neural substrate

Authors: *J. F. GUASSI MOREIRA[1], A. S. MENDEZ LEAL[1], Y. WAIZMAN[1], N. EMILIA[1], J. A. SILVERS[2];
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Abstract: The acquisition of self-control is an inherently developmental process that occurs over the course of decades. To date, much research has examined the neurodevelopmental bases of self-control across a range of contexts. However, virtually no work has addressed whether the neural bases of self-control across different contexts stem from a common neural substrate, or whether they are instantiated differently (i.e., marked by similar or disparate population coding). We address this issue in an ongoing study in which a sample of youth—exposed to varying degrees of stress—completed three tasks while undergoing functional magnetic resonance imaging (fMRI). Forty-three youth (age range: 10-22) completed two self-control tasks under affectively 'hot' conditions: a cognitive reappraisal task, which involved using a cognitive

strategy to regulate negative affect, and a driving simulation task, which involved weighing potential risks and rewards during decision making. After preprocessing and cleaning the data, we conducted a whole-brain multi-voxel pattern searchlight analysis (neighborhood size = 75 voxels, naïve Bayes classifier, leave-one-out cross validation) to reveal brain regions where the population codes could discriminate between tasks. The analysis identified clusters in the bilateral dorsolateral prefrontal cortex, dorsal anterior cingulate cortex, and bilateral superior parietal lobule. Importantly, clusters were not simply obtained due to chance, as they survived the 67% classification accuracy threshold (obtained using a binomial reference distribution). That activity from these regions—consistently implicated in self-control tasks such as those used here—were found to discriminate between tasks suggests that the neural code underlying the development of self-control varies across affective contexts. In other words, the neural basis of brain regions involved in self-control is not comprised of a common substrate and appears to vary as a function contextual demands. Future analyses will seek to identify instances where the opposite is true: where the neural code is indeed similar across different contexts of self-control.

Disclosures: **J.F. Guassi Moreira:** None. **A.S. Mendez Leal:** None. **Y. Waizman:** None. **N. Emilia:** None. **J.A. Silvers:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.11/BB68

Topic: H.02. Human Cognition and Behavior

Support: NIH R01 MH069456
NSF GRFP

Title: Neural approach for understanding event segmentation in early development

Authors: *T. S. YATES, L. J. SKALABAN, C. T. ELLIS, N. B. TURK-BROWNE; Psychology, Yale Univ., New Haven, CT

Abstract: In order to structure and scaffold experience, humans segment continuous input into discrete events. Much of the prior research on event segmentation has been in adults. Across individuals, there is consistency in where they identify boundaries during naturalistic experience, and these boundaries are reflected in state changes in neural activity patterns. Different parts of the cortical hierarchy segment experience at different timescales: for example, events tend to be relatively short in duration (i.e., more boundaries) in early visual areas and long in duration (i.e., fewer boundaries) in higher-order association cortex. Here we explore whether an analogous neural state approach can be used to study event segmentation in infants and toddlers who are pre-verbal or otherwise unable to perform behavioral event segmentation tasks. How young

children perceive and segment events is largely unknown, so if successful, this has potential to reveal fundamental insights about their experience of the world. For example, it is possible that infants have similar event structures to adults in early visual areas, reflecting similar sensory processing, but lack longer event structures in association cortex, which are thought to track memory, understanding, and narrative. Infants could also exhibit event boundaries at different time points or be less reliable than adults in what constitutes an event boundary. Our approach is to compare the fMRI activity of infants and adults watching the same short cartoon movie. Adult passive movie viewing data were obtained from a larger fMRI dataset collected from our lab. Consensus event boundaries were determined behaviorally by an independent sample of adults. Using a hidden Markov model, we replicated previous findings of a decrease in the optimal number of events higher in the cortical processing hierarchy, even in a considerably shorter narrative. We also found overlap in the timing of event boundaries between this neural approach and the boundaries identified behaviorally by others. Indeed, fMRI activity patterns were more correlated within vs. across behavioral events in the precuneus and posterior cingulate cortex. Having quantified neural event structure in adults, we are now investigating the fMRI activity of infants watching the same movie to determine at which timescales they segment events, how these boundaries compare to adults, and where in the brain they are represented.

Disclosures: **T.S. Yates:** None. **L.J. Skalaban:** None. **C.T. Ellis:** None. **N.B. Turk-Browne:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.12/BB69

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R15 MD010201-01

Title: Association between lung function and neurocognitive assessments in urban minority children

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Abstract: Epidemiological studies have found associations between exposure to ambient particulate matter (PM) and adverse respiratory and neurocognitive outcomes. Children are particularly vulnerable to the effects of ambient PM since their CNS is still in development, especially in regions related to executive functions, which develop significantly between the ages of 6 to 10 years. It has been reported that children exposed to high levels of traffic related air pollutants (TRAP) had lower scores in attention and working memory. However, not much is

known about the relationship between cognitive changes and lung function in children. Therefore, the main goal of this research is to evaluate the relationship between lung function and neurocognition in Puerto Rican children living in urban areas with heavy traffic. We hypothesized that children with reduced lung capacity will score lower in cognitive tasks related to executive functions. To test this hypothesis, we conducted a secondary data analysis of previously collected respiratory and cognitive data of children ages 6 to 9 living near two main sources of traffic TRAP. For the analysis, we selected the days and participants for which lung function was measured prior the administration of the cognitive test (i.e. Cognitive Assessment System 2). Results showed significant associations between forced vital capacity and the planning construct ($r=0.881$, $p=0.03$), specifically in the connections subtest (a version of the trail-making test). Similar results were observed in a replicate study. Additional research is needed to understand the effects of pulmonary function on cognitive skills, especially in vulnerable populations such as children.

Disclosures: L.B. Mendez: None. **H.J. Rosa-Lopez:** None. **N.G. Medina:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.13/BB70

Topic: H.02. Human Cognition and Behavior

Title: Polygenic score of intelligence is more predictive of crystallized than fluid performance among children

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Abstract: Intelligence is a strong correlate of educational, occupational and health outcomes. Twin and Genome Wide Association Studies in adults have revealed genetic associations with intelligence to be moderately heritable and related to the neurobiology of the brain. We aimed to better understand these genetic associations with intelligence in the context of the developing brain. Specifically, we questioned if genetic associations of intelligence a) loaded on specific factors of cognition (i.e. fluid vs crystallized) and b) related to differences in cortical brain morphology measured using MRI scans. To do this we generated a genome-wide polygenic score of intelligence (GPSI) for the Adolescent Brain Cognitive Development (ABCD) baseline data, which consists of 11,875 nine and ten year olds across the US. We found that the GPSI was a highly significant predictor of estimates of both fluid ($t=7.1$, $p=1.2\times10^{-12}$) and crystallized

(t=15.0, $p=3.5x10^{-50}$) cognition, with greater predictive power for crystallized than fluid (z=4.9, $p=8.7x10^{-7}$). This indicates a stronger loading of GPSI on crystallized cognition. GPSI was significantly correlated with total cortical surface area (t=5.3, $p=1.4x10^{-7}$), but not mean thickness (t=0.25, p=0.8). Vertex-wise analyses showed that the surface area association is largely global across the cortex. The stronger association of GPSI with crystallized than fluid measures is consistent with recent findings that more culturally dependent measures of cognition are more heritable. These findings in children provide new evidence relevant to the developmental origins of previously observed cognitive loadings and brain morphology patterns associated with GPS of intelligence.

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Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.14/BB71

Topic: H.02. Human Cognition and Behavior

Title: Neurobehavioral trait and state signatures in child-victims of peer bullying

Authors: *I. SOLIS[1], Y. KIM[1], C. M. MCGINNIS[1], P. G. LESNIK[1], L. SERNA[2], K. T. REWIN CIESIELSKI[1,3];
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Abstract: Introduction: Child-Victims (CVs) of peer bullying persistently exposed to physical and psychological victimization, and social exclusion present with low self-esteem, suicidal ideations, somatic and psychological disorders, reduced life achievements and violent acts of desperation, including school shootings. Early prevention is an urgent call and poses two major questions: (i) How early in childhood is bullying experienced, and therefore, when to begin the first preventive efforts, and (ii) What are the primary risks factors for victimization in CVs, and therefore what neurobehavioral phenomena must be targeted by preventive efforts. Searching for the answers we conducted two studies. **Methods:** *Study I:* Structural interviews comprising 50 questions in 18 local preschools with 20 instructors of children ages 4-6, that focused on the content of earliest episodes of child-peer bullying, and *Study II*: 34 CVs ages 6-17, with matched, non-involved, control-children (CC). Examination of a two-factorial model of neurobehavioral CVs characteristics was conducted: (i) *Trait Signatures* (TS), the crystalized, long-term neurobehavioral markers from early life; and (ii) *State Signatures* (SS), the psychological, somatic, and cognitive acute consequences of ongoing victimization. A neuropsychological

battery of tests, clinical parental interviews, and resting state brain EEG (reported elsewhere) were applied. **Results:** Among TS, CVs as compared to CC, displayed early life history of high anxiety, compulsive behaviors, visual-spatial perception/attention deficits, limited vocabulary and various diverse characteristics, such as exceptional high school performance, and extremes of physical attractiveness. Among SS, CVs displayed higher levels of ongoing depressive moods, low visual-spatial memory/conceptualization, and higher rates of ongoing traumatic experiences compared to CC. **Conclusion:** The cognitive-social-emotional profile of developmental Trait Signatures calls for translational programs of prevention with children at risk for bullying victimization at an early preschool age aiming to increase self-esteem and skills of social communication, ability for top-down inhibitory control of emotions and behaviors, and acceptance of diversity.

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Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.15/BB72

Topic: H.02. Human Cognition and Behavior

Support: ERC-ADG-2015

Title: Functional dynamics of the infant brain

Authors: *S. NAIK[1], D. BATTAGLIA[2], G. DEHAENE-LAMBERTZ[1];
[1]Developmental Neuroimaging Group, UNICOG, Neurospin, Paris-Saclay, France; [2]INS, Univ. Aix-Marseille, Marseille, France

Abstract: Compared to adults, infant visual Event Related Potentials (ERPs) are highly variable across trials in terms of latency of the known ERP components, making it difficult to interpret the subject-specific ERPs. While the traditional ERP studies focus on the mean activity--discarding the across-trial variability as noise-- this variability is important for improving our understanding of the maturation of functional dynamics in infants. Using multivariate topographic analysis on high-density EEG recordings, we explored the variability of responses to face stimuli in 2-6 month old infants (N=39, Mean age: 14.15 +/- 4.79 weeks) to understand how this variability is adjusted throughout the course of development. Specifically, we show that the latencies of the single trial events most resembling to known target ERP components (e.g. P1- or P400-like topographies) fluctuate from one trial to the next, but the **distributions** of these latencies gradually **concentrate in time and reduce their spread around the mean** throughout the age of maturation. Furthermore, using multivariate topographic analysis, we defined a

measure of dissimilarity across trials and observed the time-evolution of this measure to understand the stimulus driven differences (variability) across trials while the infants process lateralized face stimuli; passively looking at the fixation. We prove the existence of **durations of reduced inter-trial variance**– or Variability Quenching Events (VQE)– following stimulus presentation at this early age. We observe that the latencies of infant VQEs are later than those of the adults. Moreover, these VQE events align with the developing latencies of P1 and P400 response components, being possibly involved in their maturation dynamics. Furthermore, we observed **transient 'slowing down'** or intra-trial variance quenching **in single-trial,** onset of which briefly aligned in time with the onset of VQEs-- possibly suggesting the existence of stable temporal window for information integration. Interestingly, infants exhibited stable intra-trial patterns for longer durations than adults and overall sluggish dynamics, unlike in adults where the slowing down was concentrated around the peri-stimulus durations. In future, understanding the relationship between transiently stable dynamics and task-related responses may help unravel the role played by response variance in the emergence and development of ERP responses.

Disclosures: S. Naik: None. **D. Battaglia:** None. **G. Dehaene-lambertz:** None.

Poster

425. Development, Cognition, and Connectivity

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Program #/Poster #: 425.16/BB73

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant HD075865

Title: Brain and cognitive sequelae of preterm birth: Baseline findings from the ABCD study

Authors: *F. HAIST, N. AKSHOOMOFF;
Psychiatry, UC San Diego, La Jolla, CA

Abstract: Children born preterm are at increased risk of disrupted perinatal brain development and subsequent neurodevelopmental impairments. Even "healthy preterm" children with a benign neonatal course and without focal brain injury show diffuse changes in white matter but little is known about how these affect cortical development and associated cognitive functions as children enter adolescence. Large scale studies may provide valuable information to address this. Here, we used data from 9 and 10 year-olds from in the Adolescent Brain Cognitive Development (ABCD) study (NDA Release 2.0) to evaluate major white matter tract integrity, regional subcortical gray and white matter volume, and cognitive test performance (NIH Toolbox Battery) across three birth groups: Full Term (FT; ≥ 37 wks gestation; Max N=10,099), Late Preterm (LPT; 33-36 wks; Max N=1262), and Very Preterm (VPT; 28-32 wks; Max

N=341). We used general linear model univariate and multivariate tests that controlled for age and sex. In terms of mean diffusivity (MD) within select white matter tracts, we found significant birth term group effects within the corpus callosum, bilateral fornix, bilateral anterior thalamic radiations, and right hemisphere cingulate. Within each of these regions, the preterm groups (LPT & VPT) had lower MD values than FT children, suggesting that reduced white matter integrity is present in most children born preterm. The exception was in the left hemisphere cingulate, with no significant group differences. There were significant volumetric differences, where both preterm groups had significantly enlarged lateral ventricles and reduced brainstem volume than the FT group. The VPT group had significantly reduced whole brain volume, cerebellar white matter, cerebral white matter, and thalamic volume than both the LPT and FT groups. On the NIH Toolbox, the VPT group scored significantly lower than the FT group for both the Crystalized (verbal) and Fluid (performance) composite measures, while the LPT scored significantly lower only on the Crystalized measure. The large sample afforded by the ABCD cohort provides a unique opportunity to explore the long-term sequelae of preterm birth.

Disclosures: F. Haist: None. **N. Akshoomoff:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.17/BB74

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant1640909

Title: ASD and ADHD motor neurodevelopmental disorders

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Abstract: From childhood to adulthood our brains keep changing as we learn. Neurodevelopmental disorders in children refer to deficits or delays in the developmental process. An important example is Autism Spectrum Disorders (ASD). Despite its high prevalence, most clinical diagnoses of ASD remain subjective in nature. Similarly, Attention Deficit Hyperactive Disorders (ADHD) involve developmental deficiencies. There are no general and effective treatments for this psychiatric Neurodevelopmental Disorders (NND).

There is a significant need to find objective NDD assessments biomarkers. In our previous work, we introduced a quantitative biomarker (s-Peaks) based on a statistical analysis of natural motions at millisecond time scales (Wu et al 2018). We used these biomarker in ASD subgroups that have idiophatic origins. The biomarker provides a precise screening tool for ASD severity with unprecedented accuracy. It also provided a quantitative connection between the way individuals move and to their psychiatric scores (Wu et al. 2018). This connection suggest that the motor feature we found in ASD may be a core element characterizing generally NDDs. Here we have extended our ASD biomarker studies to ADHD individuals. Recently DSM-5 noted that there are a number of co-morbidities between ASD and ADHD. They did not include, however, motor deficiencies. Here we compare ASD against ADHD to find how they are different or similar in terms of our s-Spikes movement biomarker (Wu et al. 2018). We also included Typically Developing (TD) individuals as controls o compare to ASD and ADHD. We developed a battery of movement protocols by using bleu tooth sensors that can capture motions with high frequency (www.xsens.com & http:apdm.com). These sensors are mounted on the subject's bodies (i.e. gloves, torso suits including up to six sensors). The subjects considered have developing or already developed motor skills. The ASD-ADHD and TD individuals followed motor protocols, like normal reaching or walking. These motions provide the necessary kinematic information to extract the statistical properties to extract the statistical properties characterizing in ASD and ADHD individuals. We will present detailed statistical analyses of the results found.

Disclosures: J.V. Jose: None. **J.I. Nurnberger:** None. **M.H. Plawecki:** None. **A. Phadnis:** None. **K. Doctor:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.18/BB75

Topic: H.02. Human Cognition and Behavior

Title: Mental abacus skilled kindergarteners have enhanced attentional and spatial abilities

Authors: *D. CHENG[1], X. ZHOU[2];
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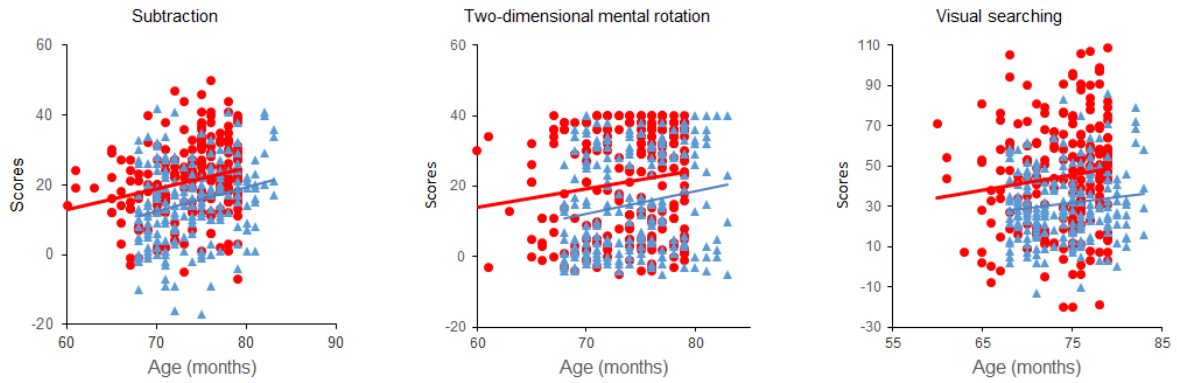
Abstract: In China and other Asian countries, it is popular for children to learn mental abacus skills. Mental abacus skilled children or adults can perform fast and accurate calculation based on mental image of an abacus. Studies consistently show that mental abacus learning can promote arithmetic abilities and numerical processing abilities. However, ti is controversial that whether there is transfer effects of mental abacus learning on general cognitive abilities. The

current investigation examined the group differences between mental abacus skilled kindergarteners and controls in general cognitive abilities. A total of 442 kindergarteners participated in the investigation, half being mental abacus skilled (passing the Test for Level 7 on the Standard Mental Abacus) and the other half being without any experience of abacus and mental abacus. The results showed that mental abacus skilled kindergarteners performed better in continuous geometric form searching task and two-dimensional mental rotation task, and the group differences still existed after controlling for other cognitive abilities (i.e., intelligence, reaction and decision speed, and precision of approximate number system). The findings suggest that mental abacus skilled kindergarteners have enhanced attentional and spatial abilities, and mental abacus learning before formal schooling could effectively promote the development of children's attentional and spatial abilities.



Disclosures: **D. Cheng:** None. **X. Zhou:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.19/BB76

Topic: A.09. Adolescent Development

Support: The Research Council of Norway, project number 229142.
The Northern Norway Health Authority, grant number PFP1140-13.

Title: Combined analysis of cortical surface area and cortical thickness in adolescent anorexia nervosa

Authors: *P. M. ASLAKSEN[1], A. D. MYRVANG[2], J. H. ROSENVINGE[2], T. ENDESTAD[3], K. STEDAL[4], Ø. RØ[4], T. R. VANGBERG[2];
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Norway; [4]Regional Dept. for Eating Disorders, Div. of Mental Hlth. and Addiction, Oslo Univ. Hosp., Oslo, Norway

Abstract: The aim of the present study was to test whether cortical surface area (CSa) and cortical thickness (CTh) were significantly different in girls with anorexia nervosa (AN) compared to healthy controls (HC). Previous studies have separately suggested that AN is accompanied by reductions in CTh and cortical surface architecture. To our knowledge, no previous study has concomitantly tested how CTh and CSa is affected in patients with AN. 30 inpatients with restrictive AN and 28 age matched healthy controls participated in the study. The mean age of the AN patients was 15.8 years and 16.2 for the HC group. MR scanning was performed in two 3T-scanners (Siemens Skyra and Phillips Achieva), with voxel sizes of 1x1x1mm and slice thickness of 1mm in both scanners. The AN patients were scanned approximately 4 weeks after admission. The Freesurfer 6.0 software was used to preprocess MR data for CTh and CSa. The Permutation Analysis of Linear Models (PALM) was used to perform statistical analysis of the data. The statistical analyses were performed with threshold free cluster enhancement, FWER-corrections across modalities (CTh and CSa) and contrasts, and 1000 permutations with tail-approximations. The results showed that both CTh and CSa separately were significantly reduced in AN patients compared to the HC group when controlling for age and scanner-type. The reductions in CTh were widespread across the cortical surface, but most pronounced in the superior frontal and the cingulate cortices. A non-parametric combination analysis (Fisher) performed with PALM showed that the reduction of CTh and CSa in AN patients compared to the HC was strongest in the superior temporal, the precuneus and the posterior cingulate cortices. In sum, the present study suggests that cortical thickness and cortical surface area are differentially affected in patients with AN.

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Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.20/BB77

Topic: A.09. Adolescent Development

Title: Investigation of intergroup bias in two neuromaturationally distinct age cohorts: An ERP study

Authors: *R. M. HANNA[1,2], T. J. BOSCH[3,1], K. A. FERCHO[4,1], L. A. BAUGH[3,1]; [1]Ctr. for Brain and Behavior Res., Vermillion, SD; [2]South Dakota State Univ., Brookings, SD; [3]Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD; [4]Aviation Safety, AAM-510, FAA Civil Aerospace Med. Inst., Oklahoma City, OK

Abstract: It is well established that humans are a social species. Not only are we social, when compared to other primate species we are superlative in measures of reciprocal and non-reciprocal altruism, complexity and breadth of our social networks, and empathy towards conspecifics and heterospecifics alike. However, our prosocial nature is not without boundaries. Like many of our primate cousins (e.g., Rhesus Macaques, Chimpanzees, &c.) we subdivide our social world into ingroups and outgroups. Ingroups are continually updated and play a significant role for us across the lifespan. At no other time in life is the importance of the ingroup more acutely felt or observed than during adolescence/young adulthood. Between the ages of 12 and 25 peer salience, along with risk-taking, reward seeking, and novelty seeking increases. The developmental mismatch hypothesis of the dual systems model of brain development posits that these adolescent typical behaviors result from earlier maturing subcortical structures (NAcc and AMG) "overriding" phylogenetically newer structures of the PFC, specifically the dlPFC, dACC, and OFC. Many of the primary structures implicated in "the social brain." While studies (primarily fMRI) have been conducted to understand social cognition across the lifespan, few ERP studies explicitly investigate intergroup bias from a developmental mismatch perspective. The present study does exactly this. Using notional groups based upon each subjects' political affiliation with non-noxious initiation we investigated the temporal differences in ingroup vs. outgroup processing. Following a separate behavioral training session, subjects were asked to view images of ingroup members or novel outgroup (that held an opposing political ideology) members that were paired with congruent or incongruent conservative vs. liberal stereotype statements. Subjects needed to evaluate group affiliation, assess the statement and indicate the likelihood that the person pictured made the statement within 5000 ms. Results (maximal over frontocentral electrodes) replicate previous findings that show increased P2 amplitude for outgroup images and increased N2 amplitude for ingroup images, but only for the 30-35-year-old age cohort. For both congruency and group conditions, these results were reversed in the 18-19 year-old age cohort. These findings support increased early categorization of ingroup peers in adolescents. However, because these trends are from a pilot study with low sample sizes (18-19 n=16; 30-35 n=4) further investigation is necessary.

Disclosures: **R.M. Hanna:** None. **T.J. Bosch:** None. **K.A. Fercho:** None. **L.A. Baugh:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.21/BB78

Topic: A.09. Adolescent Development

Title: Brain maturity: The structural connectome and its relationship with psychiatric symptoms in youth

Authors: *A. LUNA[1], C. JIOOK[2,1], J. POSNER[2,1];
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Abstract: Evidence for the role of abnormal neuromaturation in psychiatric disorders is emerging and warrants further investigation. Accurate measurement of neuromaturation can be used to characterize psychiatric disorders. We present a multimodal age prediction model using stacked ensemble machine learning (SEML). We evaluate the utility of brain predicted age differences (BrainPAD) in characterizing the relationship between abnormal neuromaturation and psychiatric symptoms in youth (ages 5-18). Using the Child Behavioral Checklist (CBCL) to assess symptoms, we hypothesized that subjects with greater symptoms would have greater BrainPAD.

T1-weighted structural and diffusion MRI from the Healthy Brain Network (N=498) were used to estimate morphometry (Freesurfer v6.0) and white matter (WM) structural connectomes (MRtrix3). A machine learning framework (H2O), was used to predict brain age based on connectomic and morphometric features. BrainPAD was defined as predicted age minus chronological age. Participants with a CBCL Total score in the normal range (T-score < 60; N=263) were used for model development (train/test = 80%/20%). 5-fold cross validation was performed to train, validate, and optimize the model, followed by testing on the hold-out set (20%). The model was then used to predict brain age in participants with a CBCL Total score in the borderline/clinical range (CBCL Total T-score > 59, N=235).

Mean absolute errors (MAE) for each SEML model were 1.653 (morphometry), 1.395 (connectome), and 1.162 years (morphometry + connectome). Generalized linear modeling (GLM) (y = all brainPAD scores), adjusted for age, sex, and race, revealed a negative association with CBCL Total raw score [t(234) = -2.10, p=0.03]. GLM with only negative brainPAD values (< -1.162) revealed a negative relationship with thought problems [t(72)= -3.09, p=0.003], externalizing [t(69)= -2.48, p=0.016], and total scores [t(69)= -2.24, p=0.028). ANOVA revealed group differences in internalizing [F(2, 280)=4.052, p = 0.01], and withdrawn/depressed raw scores [F(2, 280)=5.628, p = 0.006]. Post hoc Tukey HSD revealed higher mean internalizing (p-adj=0.013) and withdrawn/depressed scores (p-adj = 0.015) for Negative vs Normal brainPAD.

The most accurate age prediction in youths was achieved using both the WM structural connectomes and morphometry. The significant association between negative brainPAD and neuropsychiatric symptoms suggests that aberrant behavior in youth may be rooted in delayed neuromaturation. Future research could examine whether brainPAD predicts onset of psychiatric disorders, as well as factors that contribute to alterations of brain age.

Disclosures: A. Luna: None. **C. Jiook:** None. **J. Posner:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.22/BB79

Topic: A.09. Adolescent Development

Title: Developmental increase in 1/f slope in EEG oscillatory power

Authors: *D. CELLIER[1], J. RIDDLE[2], K. HWANG[1];
[1]Univ. of Iowa, Iowa City, IA; [2]Psychiatry, Univ. of North Carolina, Chapel Hill, Chapel Hill, NC

Abstract: Differences in neural electrophysiology across development have been hypothesized as a potential correlate of neurocognitive abilities and biomarkers of psychiatric or neurodevelopmental disorders. Results from prior EEG studies have found age- or disease-related differences in the amplitude of theta- or alpha-band oscillations, as well as the ratio of theta- vs beta-band power (theta-beta ratio; Arns et al., 2011). However, prior research has not taken into account the 1/*f* background signal that is ubiquitous in electrophysiological recordings. The 1/*f* background signal in EEG is thought to reflect the excitation-inhibition balance in spontaneous neural activity (Peterson et al., 2018), and changes in the slope of 1/*f* background signal have been associated with a decline in cognitive performance in aging adults (Voytek et al., 2015). The developmental change in 1/*f* signal, however, has not been addressed. In the present study, we analyzed a high-density resting-state EEG dataset from the Child Mind Institute (Langer et al., 2017) which consists of 40 healthy subjects between the ages of 9 and 25 years. We first defined EEG electrode clusters of interest in the frontal cortex and the parietal-occipital cortex. We then calculated the power spectral density of canonical frequency bands (theta: 4-8 Hz, alpha: 8-12 Hz, and beta: 18-25 Hz). Next, we used the FOOOF toolbox (Peterson et al. 2018) to calculate the 1/*f* background signal. We were interested in whether 1/*f* background signal would serve as a better predictor of age than the theta-beta ratio. In a multiple regression model, we found that the slope of the 1/*f* background noise was negatively associated with age (beta = -2.71; p = 0.046), indicating that 1/*f* slope becomes steeper with age. Importantly, the amplitude of alpha-, theta-, and beta-band did not significantly correlate with age after accounting for age-related differences in 1/*f* slope, nor did the theta-beta ratio. Therefore, our results suggest that, compared to conventional band-limited measures, accounting the contribution from 1/*f* slope is a more parsimonious and informative approach to tracking both typical and atypical development of intrinsic neural oscillations.

Disclosures: D. Cellier: None. **J. Riddle:** None. **K. Hwang:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.23/BB80

Topic: A.09. Adolescent Development

Support: NIH Grant 5U01DA041134-04

Title: White matter microstructure and childhood head injury: Results from the adolescent brain cognitive development (ABCD) study

Authors: *C. SHETH[1], R. HUBER[1], E. MCGLADE[1], P. RENSHAW[2], D. A. YURGELUN-TODD[2];
[1]Psychiatry, [2]Univ. of Utah, Salt Lake City, UT

Abstract: Adolescence is an important period in human development characterized by rapid brain maturation. The Adolescent Brain Cognitive Development (ABCD) study is a longitudinal, multi-site study that has recruited nine-ten year old children with the goal of investigating factors that influence the developmental trajectories across childhood and adolescence. Over the last decade, there has been increasing concern about the potential sequelae of concussion and head injury in children. Studies have indicated that head injuries may increase risk for physical and mental health-related problems. Furthermore, injury to the developing brain may alter subsequent maturation and impact neurobehavioral and cognitive development. Advanced magnetic resonance (MR) neuroimaging techniques such as diffusion tensor imaging (DTI) and restriction spectrum imaging (RSI) have been increasingly used to characterize underlying brain changes that correlate with patterns of behavioral change associated with head injury. Previous DTI studies of childhood head injury have concluded that forces of acceleration and deceleration lead to vulnerability of white matter tracts within fronto-temporal, limbic, and projection fibers. We examined data from the ABCD study from 3818 participants (first data release) to investigate whether presence of a head injury was associated with white matter abnormalities. Head injury was assessed by the Modified Ohio Traumatic Brain Injury Screen instrument completed by the parent. Five hundred and four parents reported a head injury that caused their child to be evaluated in the emergency room and 3314 parents endorsed no head injury. Results from DTI and RSI analyses showed lower fractional anisotropy (FA) (p=0.03) and neurite density (ND) (p=0.04) in the sub-adjacent white matter associated with the left rostral anterior cingulate cortex (rACC) in participants with head injury. Both reductions in FA and ND are thought to reflect compromised white matter integrity. ND is driven primarily by myelinated axons and the observed lower ND may be associated with decreased density or smaller average caliber of myelinated axons and thus may be related to changes in myelin integrity. DTI provides a measure of white matter tract orientation however RSI is more resistant to signal contamination by crossing fibers. The FA and ND results converge to suggest white matter abnormalities in the left rACC are associated with head injury in this age group. Alterations of white matter integrity may be related to reported changes in emotional and cognitive domains after head injury given that the ACC is a central hub for emotion regulation and cognition.

Disclosures: C. Sheth: None. **R. Huber:** None. **E. McGlade:** None. **P. Renshaw:** None. **D.A. Yurgelun-Todd:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.24/BB81

Topic: A.09. Adolescent Development

Title: Identifying behavioral and neural predictors of caffeinated soda intake in childhood using a large database from the ABCD study

Authors: *M. KWON[1], ***H. KIM**[1], J. YANG[1], J. HUR[1], T.-H. LEE[3], W.-Y. AHN[2]; [2]Dept. of Psychology, [1]Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Virginia Polytechnic Inst. and State Univ., Blacksburg, VA

Abstract: Caffeine is the most frequently used substance among children, and caffeinated soda is the most preferred routes of caffeine administration. Even though early caffeine overdose is known to have long-term negative effects in youth including higher vulnerability to a Substance Use Disorder (SUD) in adolescence and adulthood, the effects of soda consumption on cognitive and neural functions in childhood remain largely elusive. Here, we examined the behavioral and neural correlates of regular soda consumption on reward processing, impulsivity, and working memory, which are well-known to be associated with SUDs. We used a large database (N = 2001, age = approximately 10-11 years old) from the Adolescent Brain and Cognitive Development (ABCD) Study, which is a 10 years longitudinal cohort study in the US measuring comprehensive predictors and outcomes related to mental and physical health across childhood and adolescence. We classified participants into the soda drinking group and the control group (no soda consumption at all) and compared their behavior and fMRI patterns. Hierarchical linear regression approaches were used to identify measures with significant group differences, and machine learning approaches (LASSO and elastic net) were used to identify the multivariate patterns of behavioral and neural predictors for caffeinated soda intake. Preliminary results suggest that (1) frequent caffeinated soda drinking is associated with higher impulsivity and impaired working memory performance, and (2) the frequency of soda drinking was associated with increased activation in the hippocampus, amygdala, and dorsolateral prefrontal cortex during the emotional N-back task. These patterns were only found in males whose average soda intake was higher than females. In sum, our results revealed specific behavioral and neural predictors of caffeinated soda intake using a large database, and suggest that regular caffeinated soda intake in childhood is associated with impaired cognitive functions.

Disclosures: **M. Kwon:** None. **H. Kim:** None. **J. Yang:** None. **J. Hur:** None. **T. Lee:** None. **W. Ahn:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.25/BB82

Topic: I.07. Data Analysis and Statistics

Support: CONACYT grant 330142

Title: Topological abnormal load in ADHD functional brain network

Authors: *Z. GRACIA TABUENCA, J. DIAZ-PATIÑO, I. ARELIO, S. ALCAUTER;
Univ. Nacional Autónoma de México, Querétaro, Mexico

Abstract: Attention Deficit Hyperactivity Disorder (ADHD) is a developmental disorder characterized with difficulty to control the own behavior. Its symptoms have been associated with lower fronto-parietal functional connectivity (Elton et al., 2013), however many questions remain at the hierarchical brain network level. This study focuses on analyzing the ADHD functional brain network as a topological space.
Sample consisted of 81 control (38 male, age: 7.2 - 18.0 years old) and 99 (78 male, age: 7.2 - 17.6 years old) from the ADHD200 dataset (Milham et al., 2012), who underwent a resting-state fMRI scan (3Tesla, TR = 2s, 180 volumes). After standard preprocessing and spatial normalization to a standard space, average fMRI time series were extracted from 264 anatomical regions of interest (ROI) and a Pearson's cross-correlation (r) matrix was calculated. These ROIs can be divided into 14 functional networks. Based on the connectivity distance (1-r), the gradual process to connect every isolated ROI (1-r = 0) into a one single component in the 264 shorted steps was calculated. This process is called Rips filtration. The whole set of distances was sum up for each dataset, which can be interpreted as a connectivity rate at the brain network level. Group inference was assessed with a logistic regression including sex, age, and average head-motion as confounding variables. Results were corrected for multiple comparison with a False Discovery Rate (FDR $q < 0.05$).
Rips filtration sum showed higher scores in the ADHD group ($z = -3.02$, $p = 0.0025$) at the whole brain level. This pattern was also found between subcortical and auditory ($z = -3.36$, $p = 7.92e-04$) and salience ($z = -3.44$, $p = 5.71e-04$) networks.
Results showed higher functional connectivity in the ADHD group, contrary to previous studies (Tomasi et al., 2012; Elton et al., 2013), however these works only measured bivariate relationships between brain regions, instead of taking into account the brain network as a topological space. These results showed different topological properties in the ADHD brain network that could not been addressed by the standard functional connectivity methods, and can contribute to the better understanding of the physiopathology of this neurodevelopmental disorder.

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Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.26/BB83

Topic: H.02. Human Cognition and Behavior

Title: The evolution of narrative processing during development

Authors: *S. S. COHEN, C. BALDASSANO;
Psychology, Columbia Univ., New York, NY

Abstract: Development is marked by an increase in the proficiency with the variable domain of day to day life. This knowledge growth may create more coherent neural representations of life experiences, and a more abstract and semantic organization of the events within them. Here we look at the changes in neural processing with age in the Healthy Brain Network (HBN) dataset. Functional magnetic resonance imaging (fMRI) data was recorded from 233 children (80 female) whose ages ranged from 5 to 21 while they viewed two video animations. Videos were used because they allow for the assessment of brain dynamics evoked by multidimensional stimuli in which each moment dynamically depends on what preceded it. In order to assess changes in narrative processing in a data-driven way (without an explicit stimulus model), we explore how both inter-subject correlation (ISC) and stable event patterns change with maturation and other phenotypic variables. Preliminary results indicate that ISC increases with age in the auditory cortex (from 0.51 to 0.60, p = 5e-4), intraoccipital sulcus (from 0.45 to 0.52, p = 0.003), fusiform gyrus (from 0.50 to 0.57, p = 0.002), and posterior middle temporal gyrus (from 0.43 to 0.50, p = 0.005). These results indicate that age, and the increase in life experiences that accompany it, induce more coherent neural responses in both sensory and higher-level processing regions.

Disclosures: S.S. Cohen: None. **C. Baldassano:** None.

Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.27/BB84

Topic: H.02. Human Cognition and Behavior

Support: CONACyT Grant Fronteras de la Ciencia No. 783

Title: Parietal hyper-connectivity is associated with low math performance during adolescence

Authors: *R. A. ABREU-MENDOZA[1], M. PINCUS[1], Y. CHAMORRO[2], E. MATUTE[2], M. J. ROSENBERG-LEE[1];
[1]Rutgers Univ., Newark, NJ; [2]Inst. de Neurociencias, Univ. de Guadalajara, Guadalajara, Mexico

Abstract: In childhood, hyper-connectivity of the intraparietal sulcus (IPS) with frontal, parietal and temporal-occipital regions has been associated with poor math achievement in both rest (Jolles et al., 2016) and task (Rosenberg-Lee et al., 2015). However, no research to date has examined whether this same connectivity pattern is observed in later stages of development. Furthermore, most research has focused on comparing the lower end of the math ability spectrum to the middle; thus, little is known about the brain connectivity patterns of individuals with very high math performance.

This study aimed to determine whether whole-brain functional connectivity of the bilateral IPS, during rest, was modulated by math performance in adolescence. We collected task-free fMRI data of 42 adolescents (21 females, mean age = 16.42 years, SD = 0.78) with a wide range of math performance levels (1st - 99th percentile), as measured by the Math Computation subtest from the WRAT-4 (Wilkinson & Robertson, 2006). All participants had an IQ ≥ 80 on a short form of the WISC-IV (Wechsler, 2007), a reading speed score above the 10th percentile in the Reading a Text Aloud subtest from the Neuropsychological Assessment for Children (ENI; Matute et al., 2007) and did not meet the diagnostic criteria for Attention-Deficit/Hyperactivity Disorder. To conduct the connectivity analysis, we used as seed regions the right and left IPS, defined by combining the right and left hIP1, hIP2, and hIP3 from the Jüelich histological atlas. Math performance was strongly correlated with IQ (r = .68), thus we used residualized math scores as our covariate of interest. Our main prediction was that lower math performance scores would be associated with higher connectivity between the IPS and other math-relevant brain regions.

Consistent with previous research, low math performance was correlated with higher connectivity from left IPS to a set of frontal (supplementary motor area, SMA), parietal (superior parietal lobule), and visual areas (inferior occipital gyrus and lingual gyrus). While connectivity from the right IPS to the right SMA was also associated with worse math performance, connectivity of this seed to left inferior parietal lobule was positively correlated with better math performance. Overall, these results suggest that across childhood and adolescence, hyper-connectivity of IPS may be a biomarker of low mathematical abilities.

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Poster

425. Development, Cognition, and Connectivity

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 425.28/BB85

Topic: H.02. Human Cognition and Behavior

Support: Templeton Foundation
University of Chicago Center for Practical Wisdom

Title: Using EEG to evaluate an early childhood intervention

Authors: *K. J. YE[1], A. SAMEK[3], K. J. YODER[2], J. DECETY[2], A. HORTACSU[1], J. A. LIST[1]; [1]Econ., [2]Psychology, Univ. of Chicago, Chicago, IL; [3]Econ., USC, Los Angeles, CA

Abstract: Early childhood interventions — such as preschool — are now observed to have a large impact on academic skills and Kindergarten readiness of disadvantaged children. While researchers and policymakers have argued for expanded preschool access, exactly *why* and *how* preschool works has remained a black box. This paper provides the first evidence that a large-scale early childhood intervention can change an important biological input to the education production function, children's executive function brain activity. We take advantage of the Chicago Heights Early Childhood Center (CHECC) RCT that randomized children to a free preschool or to a control group for a year. Following the intervention, we collected electroencephalogram (EEG) measures to assess the children's brain activity as a complement to ongoing behavioral assessments. We recorded event-related potentials (ERPs) from 72 4- to 6-year-old children (36 treated, 36 control; 33 female, 39 male) who participated in CHECC while they completed an academic and an executive function task, a modified Peabody Picture Vocabulary Test (PPVT) and Animal Go-Nogo (AGNG), respectively. Our main outcome measures are academic and executive function ERP components: N4, which measures language processing, and N2 and P3, which measure attention and effort. We find evidence that the CHECC preschool had an impact on executive function brain activity during AGNG: compared to children in the control group, the treated children had lower N2 mean amplitude ($p<0.05$) measured at the Cz electrode. Furthermore, the N2 mean amplitude is predictive of longer-term executive function skills. Three years after the end of the CHECC intervention (and after the EEG data collection), we recruited children from our EEG sample to participate in a behavioral follow-up assessment of their academic and executive function skills. We find that our EEG measures collected are predictive of children's scores in the three-year follow-up, above and beyond the predictive power of baseline abilities. All results use linear regressions with gender, age, race, and baseline ability controls.

Disclosures: K.J. Ye: None. **A. Samek:** None. **K.J. Yoder:** None. **J. Decety:** None. **A. Hortacsu:** None. **J.A. List:** None.

Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 426.01/CC1

Topic: H.02. Human Cognition and Behavior

Support: NIH/NINDS UH3-NS100543

Title: Understanding the role of cortico-cerebellar circuits in supporting cognitive control

Authors: *M. AVERY[1], B. CAMPBELL[2], N. MATTHEWS[2], A. G. MACHADO[3], K. B. BAKER[4];
[1]Case Western Reserve Univ., Cleveland, OH; [2]Cleveland Clin. Lerner Col. of Med., Cleveland, OH; [3]Ctr. Neurolog. Restoration, [4]Dept. of Neurosci., Cleveland Clin., Cleveland, OH

Abstract: The cerebellum is thought to play an important role in movement and cognition through its widespread interactions with cerebral cortical and subcortical structures (Romer et al. 2018). We have shown previously that chronic stimulation of the dentate nucleus (DN), a major ascending output pathway of the cerebellum, can modulate cortical excitability and lead to improvements in stroke recovery through enhanced functional reorganization of motor circuitry (Baker et al. 2010). That work is currently being translated to humans, with chronic, post-stroke patients undergo unilateral implantation of an eight-channel deep brain stimulation (DBS) lead in the DN contralateral to the affected cerebral hemisphere. As part of that procedure, the proximal end of the lead is externalized for a period of time post-surgery, which allows for the recording of DN local field potentials in conjunction with scalp EEG. Here, we present data addressing how communication between the cerebellum and cortex is coordinated in order to facilitate cognitive control. Patients performed an anti-saccade task in which they had to saccade towards or away from a visually-presented cue. Saccading away from the cue is thought to actuate cognitive control mechanisms partially mediated by frontal cortex and cortico-cerebellar circuits. DN LFP and scalp EEG data were time-locked to task events. Our results suggest that patients exhibit deficits in the anti-saccade task, marked by slowed reaction time and errors in voluntary but not involuntary saccadic movements. Electrophysiological recordings from the DN and EEG surface electrodes showed task-mediated modulation of activity in both the DN and frontal cortex during anti-saccade trials. Specifically, we saw frequency-specific alterations in the beta band during anti-saccade trials. This suggests that stimulation within the beta frequency may be able to promote improvement in certain aspects of cognition. Future experiments will incorporate stimulation of the DN at various frequencies to assess frequency-specific improvements or impairments in performance.

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Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 426.02/CC2

Topic: H.02. Human Cognition and Behavior

Support: NIMH Grant R01MH118500

Title: Does layer 5 of the cortex project to the thalamic reticular nucleus? Implications for core and matrix thalamocortical circuits and sleep spindles

Authors: A. YAZDANBAKHSH, ***B. ZIKOPOULOS**;
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Abstract: Two distinct thalamocortical (TC) circuits with reciprocal components can be identified in mammals: The core TC circuit, prevalent in sensory thalamus, drives activity focally in the middle cortical layers. In turn, these core thalamic neurons are innervated by small 'modulatory' cortical axon terminals from pyramidal neurons in layer 6 (L6). The matrix TC circuit, prevalent in high-order thalamus, has a complementary organization: large axon terminals from cortical layer 5 (L5) pyramidal neurons drive activity of matrix thalamic neurons that, in turn, innervate broadly and modulate the superficial cortical layers. Situated strategically between the thalamus and cortex, the inhibitory thalamic reticular nucleus (TRN) intercepts all TC communication. Projections from sensory or motor cortices to TRN terminate exclusively as small boutons and originate from L6, akin to core TC circuits. No studies have shown direct projections to TRN from cortical neurons in L5 that participate in matrix circuits. However, in comparison with other cortices, prefrontal cortices issue substantial projections to the thalamus from L5 and send similar driver-like projections to TRN, which terminate as large boutons and could potentially originate from L5. These large prefrontal axon terminals are similar to cortical boutons in the caudate nucleus and the amygdala, which originate mainly from L5. Based on this indirect evidence we tested the hypothesis that cortical L5 neurons project to TRN in matrix networks, by constructing a computational TC circuit that included core and matrix components with an optional cortical L5 to TRN projection (L5-TRN ON/OFF). Based on the features of TC circuits, our model was able to simulate relay and filtering of signals, and could initiate and propagate spindle oscillations. Activation of TRN neurons with L5-TRN ON in our model initiated spindle generation with different powers, depending on the level of cortical feedback and involvement of model core vs. matrix. Our preliminary findings are in agreement with hypotheses that spindles can be classified in core-generated, matrix-generated or mixed types, depending on the pathways involved in their generation, but only if L5-TRN is ON. Simulation

results indicate a more diffuse nature of spindles in matrix compared to core, with the mix type showing intermediate properties, suggesting that shifts in the engagement of distinct TRN, core, and matrix circuits may underlie typical sleep spindle generation and states of vigilance. Disruption of TC-TRN circuit balance may underlie seizures, atypical sensory reactivity, and deficits in sleep and attentional gating seen in autism and schizophrenia.

Disclosures: A. Yazdanbakhsh: None. **B. Zikopoulos:** None.

Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 426.03/CC3

Topic: H.02. Human Cognition and Behavior

Support: NIH R01 NS102201
NIH T32GM108540
Iowa Neuroscience Insitute Program of Excellence funds

Title: Motor inhibition and motor-based response conflict share a common neural mechanism involving the subthalamic nucleus

Authors: *D. A. WALLER[1], A. SINGH[2], A. ESPINOZA[2], N. S. NARAYANAN[2], J. R. WESSEL[1];
[1]Psychology, Univ. of Iowa, Iowa City, IA; [2]Neurol., Univ. of Iowa Col. of Med., Iowa City, IA

Abstract: Recent research suggests that the same neural circuit underlying outright motor inhibition may also be involved during motoric response-conflict, induced by paradigms like the Simon task. This circuit, implemented in a fronto-basal ganglia network, relies on the subthalamic nucleus (STN) and internal segment of the globus pallidus (GPi) in the basal ganglia to implement inhibitory control. So far, the differential contributions of these two regions to motor inhibition and the resolution of response-conflict are unclear. The current study aimed to illuminate the causal role of both regions in motor inhibition and response conflict using deep brain stimulation (DBS). Eighteen patients with DBS implanted bilaterally in the STN (N = 9) or GPi (N = 9) completed a stop-signal and Simon task in both the DBS ON and DBS OFF states. Control samples of young, healthy adults (N = 21) and Parkinson's (PD) patients with no DBS (N = 20) were collected. Scalp EEG data was also collected from all subjects. Stop-signal reaction time (SSRT), a measure of outright stopping in the stop-signal task, was longer on average for PD patient controls (264ms) than healthy controls (223ms). Though DBS patients had SSRTs longer than both control groups, SSRTs for the ON state (307ms) were closer to both control groups than OFF state SSRTs (338ms), and therefore closer to the healthy baseline. SSRT was faster in the ON compared to the OFF state in both GPi ($t(8) = 2.17$, $p = 0.03$) and

STN patients ($t(8) = 1.80$, $p = 0.055$), which is in line with prior literature. In contrast, DBS only reduced the Simon reaction-time effect, a measure of response conflict, in STN patients ($t(8) = 2.62$, $p = 0.015$). The degree of response conflict in the Simon task was calculated by subtracting congruent trial RT from incongruent trial RT and dividing by congruent RT to yield a normalized difference score. These Simon effect scores with DBS ON (.079) more closely resembled Simon effect scores of healthy participants (.086), while DBS OFF scores (.095) were closer to scores of PD controls (.098). The Simon effect scores and SSRTs for STN patients with DBS on were positively correlated ($R^2 = 0.66$, $p = .008$): subjects with shorter SSRTs also yielded less conflict interference. These results indicate a common mechanism shared by outright motor inhibition and resolution of motor-based response conflict, which appears to rely on the STN and not the GPi. This aligns with theories that DBS as treatment for movement disorders may work by interrupting a pathologically overactive inhibitory basal-ganglia pathway, restoring it to a closer-to-baseline state.

Disclosures: D.A. Waller: None. **A. Singh:** None. **A. Espinoza:** None. **N.S. Narayanan:** None. **J.R. Wessel:** None.

Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 426.04/CC4

Topic: H.02. Human Cognition and Behavior

Title: Hippocampal connectivity with sensorimotor cortex during volitional finger movements: Evidence for volitional control

Authors: *D. D. BURMAN, B. PETROVIC, J. MEYER;
Northshore Univ. Healthsystem, Evanston, IL

Abstract: Cognitive control refers to brain processes involved in regulating behavior according to internal goals or plans. This study examines whether hippocampal connectivity with sensorimotor cortex during paced movements shows a pattern of spatial and temporal selectivity required for cognitive control. Functional magnetic resonance imaging activity was recorded from thirteen right-handed subjects during a paced, non-mnemonic (repetitive tapping) motor task. Connectivity was examined from psychophysiological interactions in hippocampal activity during two analyses: the first identified motor interactions relative to rest, whereas the second identified differential motor activity between adjacent fingers. Connectivity was observed in both pre- and postcentral gyrus, but only postcentral connectivity was topographical, coincident with finger representations identified in a previous study. Differences in the magnitude of connectivity were observed between finger representations, representing spatial selectivity for the target of movements; the postcentral representation of the moving finger invariably showed

greater connectivity than adjacent fingers. Furthermore, the magnitude of connectivity within a pre- or postcentral finger representation was largest when its finger moved, representing temporal selectivity for movement. While the hippocampus is known to be sensitive to spatial and temporal features of the environment, consistent with its role in learning and memory, the pattern of spatial and temporal selectivity of hippocampal connectivity observed in this study occurred during volitional movements in the absence of motor learning or recall. Spatial and temporal selectivity of connectivity during volitional movements meets the criteria for cognitive control adapted from oculomotor studies, suggesting a role for the hippocampus in motor control.

Disclosures: D.D. Burman: None. **B. Petrovic:** None. **J. Meyer:** None.

Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 426.05/CC5

Topic: H.02. Human Cognition and Behavior

Title: Characterizing higher-order thalamo-cortical projection patterns in humans

Authors: *A. M. HOWELL[1], S. WARRINGTON[2], J. JI[1], B. ADKINSON[1], C. FONTENEAU[1], S. N. SOTIROPOULOS[2], J. D. MURRAY[1], A. ANTICEVIC[1];
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Abstract: Thalamic systems form the nexus of virtually all brain-wide information flow. Specifically, higher-order thalamic systems, such as the mediodorsal nucleus of the thalamus (MDThal), exhibits extensive projections to prefrontal cortex (PFC), which support higher-order executive computations and behavior. However, higher order thalamo-cortical projections in humans remain understudied. Recent non-human primate work in rhesus macaques has demonstrated an organized pattern of MDThal-PFC projections whereby most PFC regions display topographically-ordered projections to MDThal sectors. However, one PFC area - namely cingulate area 24 (CA24) - exhibits a unique projection pattern, which spans all of MDThal. This type of anatomical mapping has not been characterized in humans, which is critical to inform functional mechanisms of how MDThal-to-PFC projection may subserve higher cognition. The current study tests if MDThal-PFC projection patterns established in rhesus macaques generalize to humans. Using diffusion weighted imaging (DWI) we computed a whole-brain probabilistic tractography (wbPT) (Behrens et al., 2007) solution, from which we quantified MDThal-PFC projections using Human Connectome Project data (n=337 unrelated; HCP-1200 release). We leveraged recently-developed neuroanatomically informed constraints on the DWI wbPT solution to more specifically examine MDThal-PFC diffusivity. In turn, we

tested if CA24-to-MDThal tractography exhibits a focal or diffuse projection patterns in humans. This thalamic tractography result provides key constraints for interpreting how higher order PFC-to-thalamus functional interactions may be shaped in humans, which are shaped across neurodevelopment and profoundly altered by mental illness. Ultimately, establishing a unified multi-modal cortico-thalamic topography in humans is vital to inform how brain-wide information flow shapes complex behavior.

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Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 426.06/CC6

Topic: H.02. Human Cognition and Behavior

Support: NIMH R01 Research Project Grant MH112734 (MWM)
NIMH K23 Research Career Development Award MH092696 (MWM)
Kansas Center for Autism Research and Training (KCART) Research Investment Council Strategic Initiative Grant (MWM)

Title: Neurocognitive processes associated with reduced top-down control of prepotent eye movements in autism spectrum disorder

Authors: *S. E. KELLY[1,2,3], L. M. SCHMITT[4], J. A. SWEENEY[5], M. W. MOSCONI[1,2,3]; [1]Clin. Child Psychology, The Univ. of Kansas, Lawrence, KS; [2]Schiefelbusch Inst. for Life Span Studies, [3]Kansas Ctr. for Autism Res. and Training (KCART), Univ. of Kansas, Lawrence, KS; [4]Div. of Developmental and Behavioral Pediatrics, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [5]Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Impairments in inhibitory control (IC), or the ability to suppress a dominant behavioral response, are common in individuals with autism spectrum disorder (ASD). Multiple psychological and neurophysiological processes contribute to successful IC, though the extent to which these distinct processes are affected in ASD is not known. We previously have documented that individuals with ASD show a reduced ability to proactively delay response onset during a manual stop-signal task which contributes to failures inhibiting contextually inappropriate responses. Relative to manual movements, eye movements are highly automated, more difficult to inhibit, and more closely linked to discrete neurophysiological processes. Characterizing IC of eye movements in ASD may provide key insights into spared and affected psychological and neurophysiological processes. Sixty individuals with ASD aged 5-29 years and 63 age- and gender-matched typically developing controls completed an oculomotor stop-

signal task (i.e., countermanding). During this task, the majority of trials were GO trials, on which participants made rapid eye movements (i.e., saccades) toward peripheral targets (12 degrees to the left or right of center). The remaining trials were STOP trials, on which a stop signal appeared at variable intervals following the peripheral target (i.e., stop signal delays) to cue the participant to inhibit the saccade. Stopping accuracy (i.e., the percent of STOP trials successfully inhibited), estimated reaction time of the stopping process (SSRT), and reaction time slowing on GO trials (RT slowing) compared to a baseline RT task were examined. Individuals with ASD exhibited reduced stopping accuracy and RT slowing and faster SSRTs compared to controls. For both groups, stopping accuracy was positively related to RT slowing and not related to SSRT. Increased age was associated with higher stopping accuracy and RT slowing, and these relationships did not differ across groups. The results indicate that individuals with ASD show a reduced ability to inhibit and proactively delay prepotent eye movements, while reactive stopping abilities are unaffected. Impaired IC was strongly and selectively associated with deficits in their ability to strategically delay response onset rather than reactively inhibit responses. These findings implicate reduced top-down control via fronto-striatal inhibition of brainstem circuitry in ASD, provide new targets for addressing clinical issues of IC, and suggest that tests of proactive control of eye movements may be useful for testing treatment efficacy and clarifying neurophysiological mechanisms of key clinical outcomes in ASD.

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Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 426.07/CC7

Topic: H.02. Human Cognition and Behavior

Support: NIH/NIMH Grant RO1 MH102266-01A1

Title: Mapping neurodevelopmental trajectories of thalamo-cortical systems across the mental health spectra

Authors: C. FONTENEAU[1,2], A. HOWELL[1,2,3], A. KOLOBARIC[1,2], L. J. JI[1,2,3], G. REPOVS[5], N. D. WOODWARD[6], *A. ANTICEVIC[1,2,3,4];
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Abstract: Brain networks linking cortex to thalamus are critical for cognitive, sensory, and motor function. Dysruption of thalamocortical networks has been implicated in the pathophysiology of neurodevelopmental disorders, including psychosis, and mechanisms of clinical phenotypes, especially cognitive impairment. More specifically, patients with schizophrenia and bipolar disorder consistently show a combination of thalamic hypo-connectivity with the prefrontal cortex and thalamic hyper-connectivity with the sensorimotor cortex. However, critical knowledge gaps remain with respect to the normal developmental trajectory of thalamocortical networks and onset of thalamocortical disturbances in psychosis. In addition, very little is known about how individual differences in thalamocortical connectivity relate to cognitive impairment in individuals expressing psychosis spectrum symptoms. With this in mind, the aim of our study was to characterize the thalamocortical network development across the mental health spectra. Using multi-modal neuroimaging data, we assessed the functional and structural connectivity in healthy and at-risk participants from several available large-scale cross-sectional datasets (Philadelphia Neurodevelopmental Cohort (n=1601, ages 8-21); Cambridge Center for Ageing and Neuroscience (n=656, ages 18-88); Nathan Kline Institute-Rockland Sample (n=932, ages 6-85); Pediatric Imaging, Neurocognition, and Genetics dataset (PING: N=1239, ages 3-20)). Furthermore, our study also characterized the association between individual differences in thalamocortical connectivity and executive functions, in order to put forth a mechanistic model of normal variation in cognitive function and cognitive impairment in youth with psychosis spectrum symptoms. In particular, we focused on the medial-dorsal nucleus of the thalamus known to play a critical role in executive functions. Identifying the neural correlates of psychosis spectrum symptomatology may provide intervention targets for improving outcomes in youth with psychosis spectrum symptoms, biomarkers for stratifying at-risk individuals, and inform etiological and dimensional models of psychosis.

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Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 426.08/CC8

Topic: H.02. Human Cognition and Behavior

Support: NSERC

Title: A 20-minute single-bout of aerobic exercise optimizes psychological arousal and improves executive function

Authors: *N. AYALA, M. D. HEATH;
Univ. of Western Ontario, London, ON, Canada

Abstract: A single-bout of aerobic and/or resistance training provides a short-term 'boost' to executive function. This improvement has been linked to increased regional cerebral blood flow and a concomitant increase in task-dependent activity within frontoparietal networks. It is, however, possible that the observed post-exercise benefit is additionally related to a general enhancement in psychological arousal; that is, the improvement is attributed to both low- and high-level executive benefits. To address this issue, we employed pupillometry to examine whether psychological arousal contributes to an exercise-induced improvement in executive function. Notably, changes in pupil size during a goal-directed saccade task have been reliably shown to reflect overall arousal (i.e. tonic locus ceruleus neural activity) as well as task-dependent neural activation (i.e. phasic neural activity) within the superior colliculus (SC)- a region providing retinotopic coordinates for saccades and mediated via direct projections from frontal executive networks. In the present study, participants (N=20) completed a 20-minute aerobic exercise session (via cycle ergometer at 80% HR_{max}). Pre- and post-exercise oculomotor performance and pupil dynamics were assessed via pro- and antisaccades. Prosaccades are a stimulus-driven response requiring a saccade to veridical target location, whereas antisaccades entail a response mirror-symmetrical to target location and are mediated via an extensive frontoparietal executive network. Results showed: (1) a pre- to post- exercise decrease in pro- and antisaccade reaction times ($p=0.041$), (2) decreased post-exercise tonic pupil size ($p<0.001$), and (3) increased task-dependent phasic pupil dilations ($p=0.045$). Accordingly, the results demonstrate that saccadic performance is associated with a decrease in arousal- closer to an optimal level of arousal for task engagement- and augmented phasic recruitment of neural resources following aerobic exercise. Accordingly, we propose that improved post-exercise executive function is- in part- related to enhanced psychological arousal.

Disclosures: **N. Ayala:** None. **M.D. Heath:** None.

Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

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Topic: H.02. Human Cognition and Behavior

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Title: Neuromodulatory brainstem activity predicts intrinsic co-fluctuations in cortical activity

Authors: *R. L. VAN DEN BRINK[1], O. COLIZOLI[1], J. W. DE GEE[2], T. H. DONNER[1]; [2]Neurophysiol. und Pathophysiology, [1]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: The ascending arousal systems of the brainstem send widespread projections to the cortex, from which they release modulatory neurotransmitters that alter the dynamics of their cortical target circuits. As such, these brainstem systems are ideally situated to induce coordinated state change across widespread cortical regions. Here, we used fMRI in humans to examine co-fluctuations between cortex-wide activity and multiple neuromodulatory brainstem nuclei: the basal forebrain (BF), ventral tegmental area (VTA), substantia nigra (SN), dorsal raphe (DR), and locus coeruleus (LC). We characterized co-fluctuations between these nuclei themselves, their relationship with pupillary indices of arousal, and examined their contribution to cortical activity fluctuations in two datasets and behavioral contexts: (i) trial-to-trial fluctuations of rapid responses evoked by a challenging perceptual choice task (2AFC random dot motion discrimination, N=15); and (ii) ongoing fluctuations during fixation of an otherwise blank screen ('rest', N=24). We used anatomical atlases to delineate brainstem nuclei, physiological noise regression to suppress respiratory and cardiac artifacts, and seed-based partial correlation analysis to evaluate co-fluctuations (with pupil or cortical dynamics) unique to each brainstem nucleus.We found positive partial correlations between pupil diameter and VTA and DR (task), SN (rest), LC and BF (task and rest). In both behavioral contexts, activity in most brainstem nuclei co-varied with activity in other brainstem nuclei and in widespread cortical regions. Task-evoked responses in all nuclei showed exclusively positive correlations with the cortex, with the strongest correlations for BF and LC. Evoked responses in the VTA and SN showed small correlations when locked to stimulus onset but more widespread correlations when locked to performance feedback. Remarkably, correlations between DR and cortex dissociated between behavioral contexts: positive correlations during task, but negative correlations during rest. Our findings suggest that neuromodulatory brainstem arousal systems control widespread cortical state changes on different timescales. We delineate, for the first time, the unique contribution of individual neuromodulatory systems to such changes. Our results also indicate that brainstem activity is an important factor shaping intrinsic co-fluctuations in cortical activity, which are often interpreted as expressions of intra-cortical network interactions.

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Poster

426. Subcortical-Cortical Interactions

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 426.10/CC10

Topic: H.02. Human Cognition and Behavior

Support: R01MH108654

Title: Parasympathetic arousal related cortical activity is associated with attention during cognitive task performance

Authors: *A. D. BARBER[1], M. JOHN[2], P. DEROSSE[1], M. L. BIRNBAUM[1], T. LENCZ[1], A. K. MALHOTRA[1];
[1]Feinstein Inst. for Med. Res., Manhasset, NY; [2]Mathematics, Hofstra Univ., Hempstead, NY

Abstract: Parasympathetic arousal is associated with states of heightened attention and well-being. Arousal may affect widespread cortical and subcortical systems across the brain, however, little is known about its influence on cognitive task processing and performance. Healthy adult participants (n=20) underwent multi-band echo-planar imaging (TR = 0.72 seconds) with simultaneous pulse oximetry recordings during the performance of the Multi Source Interference Task (MSIT), the Oddball Task (OBT), and during rest. Processing speed on both tasks was robustly related to heart rate. Participants with slower heart rate responded faster on both the MSIT (33% variance explained) and the OBT (25% variance explained). Within all participants, trial-to-trial fluctuations in processing speed were robustly related to the heartbeat-stimulus interval, a metric that is dependent both on the concurrent heart rate and the stimulus timing with respect to the heartbeat. Models examining the cardiac-BOLD response revealed that a distributed set of regions showed arousal-related activity that was distinct for different task conditions. Across these cortical regions, activity increased with slower heart rate. Arousal-related activity was distinct from task-evoked activity and it was robust to the inclusion of additional physiological nuisance regressors into the models. For the MSIT, such arousal-related activity occurred across visual and dorsal attention network regions. For the OBT, this activity occurred within fronto-parietal regions. For rest, arousal-related activity also occurred, but was confined to occipital regions. The pulvinar nucleus of the thalamus showed arousal-related activity during all three task conditions. Widespread cortical activity, associated with increased parasympathetic arousal, may be propagated by thalamic circuits and contributes to improved attention. This activity is distinct from task-evoked activity, but affects cognitive performance and therefore should be incorporated into neurobiological models of cognition and clinical disorders.

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Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.01/CC11

Topic: H.02. Human Cognition and Behavior

Support: University of Basel, Switzerland

Title: The neural basis of inter individual differences in episodic memory performance

Authors: *L. GEISSMANN, D. COYNEL, D. J. DE QUERVAIN;
Univ. of Basel, Basel, Switzerland

Abstract: Background. Group-based fMRI activation studies have revealed several brain regions related to episodic memory (EM) by showing voxel clusters that are more active during encoding of subsequently remembered vs. not remembered items. However, much less is known about the neural basis of the substantial variability in EM performance across subjects. Here, we investigated inter-individual differences in EM performance using a conventional voxel-based approach and a network-based approach. The latter approach might be particularly well-suited as cognition stems from networks of interacting brain regions, rather than from isolated brain regions.
Methods. In an fMRI task, healthy young subjects (n = 1434) encoded 72 pictures and were asked to freely recalled them after 10 min. First, the fMRI data were decomposed using a group-based ICA (d = 60 components [ICs]) to extract task-based functional connectivity networks. This decomposition was conducted in two separate subsamples (n = 590 and n = 580) to test for the ICA solution's stability. Time-courses for all 60 ICs were extracted for each subject with dual regression and used to estimate the individual responsivity of the networks to the presented stimuli, by means of linear models. We then calculated responsivity scores for each subject, each IC, and each voxel, to correlate them with EM (brain behavior correlation). Responsivity scores were based on encoding-specific first-level contrast estimates and available for all voxels throughout the brain (voxel-based approach) and all 60 ICs (network-based approach). Individual responsivities were then correlated with EM performance (*FWE*-corrected).
Results. The voxel-based approach revealed brain behavior correlations in the left precuneus/PCC, OFC, SFC, left cerebellum, and bilateral hippocampal formation. The network-based approach revealed brain behavior correlations in 6 FCNs. These FCNs partly match well-known resting-state networks, such as the default mode network and executive control network, and were reproducible in both subsamples (r > |.7|).

Conclusions. This study reports a neural basis of inter-individual differences in EM performance using two different approaches. Compared to the voxel-based approach, the network-based approach revealed additional brain behavior correlates of EM. EM relies on sensory processing and cognitive integration during encoding. This fits with brain regions covered by the 6 FCN.

Disclosures: L. Geissmann: None. **D. Coynel:** None. **D.J. De Quervain:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.02/CC12

Topic: H.02. Human Cognition and Behavior

Title: Hierarchical bayesian analyses reveal individual differences in the neural dynamics of response inhibition

Authors: *M. F. MOLLOY, G. BAHG, Z.-L. LU, B. M. TURNER;
Psychology, The Ohio State Univ., Columbus, OH

Abstract: Response inhibition is widely used to examine differences in executive functioning between different groups, often comparing stopping abilities between clinical populations and healthy controls. However, individual variability has been found to be intrinsic to cognitive control tasks. Hence, an examination of response inhibition would ideally involve an accurate estimation of both group- and individual-level effects. Hierarchical Bayesian analyses account for individual differences by simultaneously estimating group and individual factors and compensate for sparse data by pooling information across subjects. Hierarchical Bayesian models are thus an ideal tool for studying response inhibition, especially when analyzing neural data. We constructed hierarchical Bayesian models of the functional magnetic resonance imaging (fMRI) neural time series, constructing hierarchies across conditions, subjects, and regions of interest (ROIs). In previous work, we demonstrated that in models of the BOLD time series data, constructing a hierarchy across conditions and subjects provided the best balance between model fit, generalizability, and constraint. The hierarchical structure constructed across ROIs provides additional information about functional connectivity. Here, we applied this modeling technique to response inhibition to explore the sources and prevalence of individual differences within the neural dynamics of response inhibition. First, we ran a simulation study to compare our hierarchical Bayesian models to a conventional GLM. We found that the models we constructed out-performed a standard analysis in separating signal from noise in fMRI data, especially when accounting for individual and trial-to-trial variability. Second, we applied our model to go/no-go and stop-signal fMRI data from eleven participants (mean age = 24.6 years; 5 females and 6 males). The simultaneous group and individual estimates revealed the different dynamics in going, not going, and stopping on a group level while preserving individuality. Finally, analyses

of coactivation between ROIs estimated by the models demonstrated the prevalence of individual differences within functional connectivity across the two tasks. The ubiquity of individual differences in neuroscience and cognitive processes is becoming clear. We demonstrate the utility of hierarchical Bayesian modeling in not only accounting for these differences, but using these differences to help us further understand cognition.

Disclosures: **M.F. Molloy:** None. **G. Bahg:** None. **Z. Lu:** None. **B.M. Turner:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

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Program #/Poster #: 427.03/CC13

Topic: H.02. Human Cognition and Behavior

Support: the National Natural Science Foundation of China (31730038)
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the Guangdong Pearl River Talents Plan Innovative and Entrepreneurial Team grant #2016ZT06S220

Title: Partitioning heritability analalysis unveils the genetic architecture of human brain functional connectivity patterns

Authors: *J. FENG, G. XUE;
State Key Lab. of Cognitive Neurosci. and, Beijing, China

Abstract: Resting state functional connectivity profile has been increasingly shown as an important endophenotype that is tightly linked to human cognitive functions and psychiatric diseases, yet the genetic architecture of this multidimensional trait is barely understood. Using a unique sample of 1534 unrelated, young and healthy Chinese Han individuals, we revealed significant heritability of functional connectivity patterns in the whole brain and different subnetworks. We further proposed a partitioned heritability analysis for multidimensional functional connectivity patterns, which revealed the common and unique enrichment patterns of genetic contribution to brain connectivity patterns for several gene sets linked to brain functions. In particular, we found that genes linked to functional connectivity (FC) or expressed preferentially in the central nervous system (CNS) showed enriched contribution to heritability in almost all significantly heritable networks, whereas the SNPs associated with intelligence (IQ) or educational attainment (EA) showed enrichment primarily in cognitive control networks including medial frontal network, frontoparietal network and their combinations. Interestingly, gene sets associated with different psychiatric conditions, including autism (ASD), attention deficit hyperactivity disorder (ADHD), and schizophrenia (SCZ), showed both common and

unique patterns of enrichment. SCZ-associated SNPs only showed enrichment in the medial frontal network, ASD and ADHD-associated SNPs both were enriched in the frontoparietal network, additionally, ASD-associated SNPs were enriched in the executive control network (the combination of medial frontal and frontoparietal networks). This novel knowledge of the genomic architecture of neuroimaging endophenotypes provides new insight to link genes, brain and behaviors, which will allow us to better understand the genetic and cognitive mechanisms underlying various psychiatric conditions, and eventually guide the developmental of effective diagnoses and treatments.

Disclosures: J. Feng: None. **G. Xue:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.04/CC14

Topic: H.02. Human Cognition and Behavior

Title: Mixed handedness predicts academic achievement of university students

Authors: *G. PLUCK[1], P. BRAVO MANCERO[2];
[1]Univ. San Francisco De Quito, Quito, Ecuador; [2]Univ. Nacional de Chimborazo, Riobamba, Ecuador

Abstract: Hand preference is linked to cerebral hemisphere dominance. This is seen, for example, in language lateralization. People with a preference for their right hand are highly likely to have language lateralized to the left hemisphere. For people with a preference for their left hand, language lateralization on the right hemisphere is much more common. However, the implications of variation in hemisphere dominance and lateralization are poorly understood. In children, having no hand preference, i.e. being at the 'hemispheric indecision point', appears to have a negative impact on cognitive processes, as reflected in lower school grades. In contrast, laboratory studies with adults have shown that mixed handedness, i.e. not showing a strong preference for either left or right hands, is associated with better declarative memory. We studied the association of mixed handedness with academic grades in a sample of 120 undergraduate students (60 engineering and 60 psychology). All were assessed with the Edinburgh Handedness Inventory, a self-report measure of hand preference which provides a continuous measure of mixed handedness, and measures of intelligence (Matrix Matching Test) and psychological distress (General Health Questionnaire-28). The latter were included as both tend to correlate with academic grades and may mask associations between handedness and academic achievement. Sex was covaried due to the differences between the groups. The results confirmed that intelligence was positively correlated ($r = .154$, $p < .05$) and psychological distress was negatively correlated with academic grades ($r = -.159$, $p < .05$). There was a positive but non-

significant correlation between mixed handedness scores and academic grades. However, with intelligence and psychological distress scores covaried, the partial correlation between mixed handedness and grades was significant ($r = .204$, $p < .05$). The correlation of greater mixed handedness associated with higher academic grades appears to be independent of the material studied as it is similar in magnitude in both the Engineer ($r = .193$) and Psychology ($r = .203$) groups. Although a relatively weak association, and only revealed with potential confounders controlled for, the results suggest a benefit to mixed handedness. This association is likely therefore to be a consequence of low hemispheric dominance, which seems to provide some small advantage in educational achievement. This could possibly act through more efficient cognitive processing. Nevertheless, further research is needed to replicate the current finding and identify mechanisms through which mixed handedness may provide an advantage in higher education.

Disclosures: G. Pluck: None. **P. Bravo Mancero:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.05/CC15

Topic: H.02. Human Cognition and Behavior

Support: NIH Intramural Research Program 1ZIAMH002783

Title: Inter-subject representational similarity analysis reveals phenotype-specific patterns of brain activity during movie watching

Authors: *E. S. FINN[1], A. KHOJANDI[1], D. A. HANDWERKER[1], P. J. MOLFESE[1], *P. BANDETTINI[2];
[2]Section on Functional Imaging Methods, [1]NIMH-NIH, Bethesda, MD

Abstract: Naturalistic tasks, such movie watching, are an ecologically valid way to study brain function in human neuroimaging experiments. Such stimuli evoke patterns of brain activity that are, by and large, highly consistent across subjects, yet there exist meaningful individual differences atop this shared variance. Here, we use inter-subject representational similarity analysis to determine if and how similarity of brain activity during movie watching is related to similarity of behavioral phenotypes. Subjects (n = 89 unrelated; Human Connectome Project) underwent fMRI scanning (~60min, 7T, voxel size = 1.6 mm^3, TR = 1s) while viewing a series of video clips from documentary or Hollywood-style films. Subjects also completed several behavioral measures outside the scanner. We calculated inter-subject correlation (ISC) for each of 268 nodes in a whole-brain atlas (brain similarity), and related this to behavioral similarity for various traits modeled using two different distance functions (Fig 1, left column). Results

showed widespread representational similarity between brain responses and phenotypes, the nature of which differed by trait (Fig. 1). For some traits, this relationship was best modeled by a Euclidean distance function, in which subjects look most similar to their nearest neighbors, regardless of absolute score—e.g., subjects with more similar responses on the NEO Five-Factor Personality Inventory had more similar brain responses in several regions, irrespective of their absolute trait scores (extraversion, openness, etc.). For other traits, similarity scaled monotonically (increasing or decreasing) with absolute score, such that, for example, pairs of subjects with faster reaction times on an emotion recognition task showed higher ISC than pairs of subjects with slower reaction times across much of the brain. We conclude that inter-subject similarity of brain responses during movie watching shows nuanced relationships with phenotypes, suggesting the potential for a movie-based "stress test" to draw out individual variation of interest.

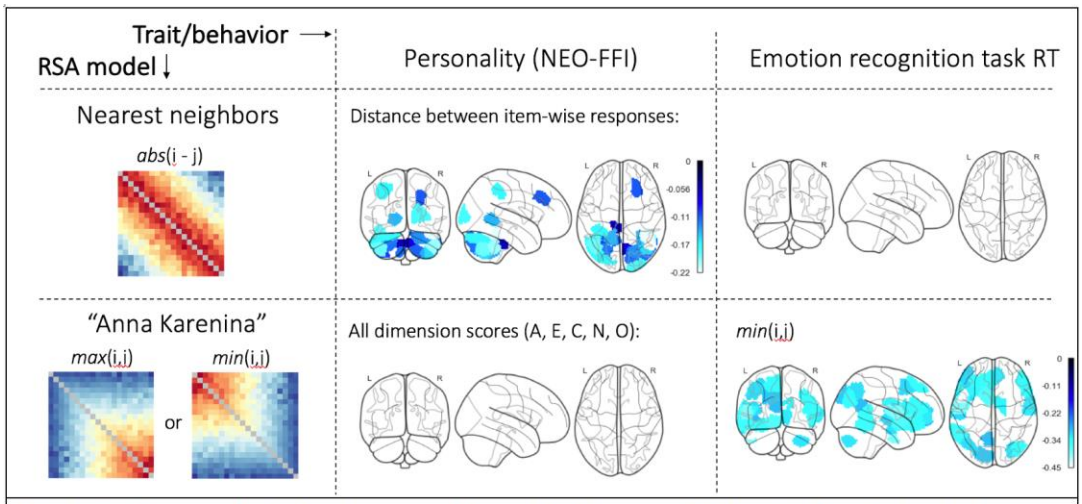

Fig. 1. Capturing relationships between inter-subject brain and behavioral similarity. Left column: simulated inter-subject correlation matrices, where each row (*i*) and column (*j*) corresponds to a subject, and subjects are ordered by score on a behavioral phenotype. Warm (cool) colors denote higher (lower) predicted similarity of brain response for two different potential RSA models ("nearest neighbors" and "Anna Karenina"). Some traits (e.g., personality) are better modeled by a "nearest neighbors" distance function (middle panel, top row; cool colors reflect the expected inverse relationship between Euclidean distance and brain similarity), while other traits or behaviors (e.g., reaction time on an emotion recognition task) are better modeled by an "Anna Karenina" distance function, in which all fast responders are alike, while all low responders are less similar (again, cool colors reflect the expected inverse relationship, such that subject pairs with a lower minimum reaction time show more similar brain responses). All results calculated based on a 268-node whole-brain atlas, with nodewise representational similarity p-values determined via permutation test (n = 10,000) and displayed at $p < 0.05$, FDR corrected.

Disclosures: **E.S. Finn:** None. **A. Khojandi:** None. **D.A. Handwerker:** None. **P.J. Molfese:** None. **P. Bandettini:** None.

Poster

427. Personalized Brain Signatures

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Program #/Poster #: 427.06/CC16

Topic: H.02. Human Cognition and Behavior

Support: NIH/NIA R01 AG038465

Title: Predictive utility of functional connectivity vs. voxel activation

Authors: *C. G. HABECK, Q. R. RAZLIGHI, Y. STERN;
Cognitive Neurosci. Div., Columbia Univ., New York, NY

Abstract: Functional connectivity has become a major focus of cognitive neuroimaging research in the last 1.5 decades, for both resting-state as well as task-related imaging. Given the abundance of task-related data, empirical comparisons of the predictive utility of voxel activation vs. functional connectivity are easy to undertake and enable rigorous apples-to-apples comparison in identical held-out data. We investigated the differential predictive utility of both modalities, for different ages and cognitive outcomes. Two hundred and forty six participants aged 20 to 77 underwent fMRI for 12 cognitive tasks, pertaining to 4 cognitive domains: episodic memory (MEM), fluid reasoning (FLUID), perceptual speed (SPEED), and vocabulary (VOCAB) (Habeck, Gazes et al. 2016, Habeck, Eich et al. 2018). Functional connectomes were computed after scrubbing and motion-confound regression to arrive at 264 x 264 task-and-subject connectivity matrices (Power, Cohen et al. 2011, Power, Barnes et al. 2012, Carp 2013). Standard first-level time-series modeling was performed with FSL to derive task-and-subject maps. Data for each cognitive domain were stacked, resulting in 246*3=738 task-and-subject observations. We then performed extensive split-sample simulations to probe quasi-replication with 1000 iterations, for which 240 observations were randomly sampled as a training data set, and 60 different observations were randomly picked as the test set, repeatedly. Training and test sets are independent within, but not across, iterations. We ran a simple PCA-regression to estimate a regression model which could then be applied to the held-out data to venture a prediction of the cognitive outcome of interest. The predictive utility in the held-out data was quantified with the Predicted Residual Sum Of Squares (PRESS) statistic between predicted and actual outcomes. Apart from the single-modality predictions, a combined average ("vote") was considered too. We found that connectivity only showed superior predictive utility for select cognitive outcomes and age groups considered in this study, and thus did not prove superior across the board. The results confirm the continued importance of voxel activation data, at least for empirical success of brain-behavior prediction. Further, regardless of data modality, out-of-sample prediction showed better success for younger than older participants, suggesting that both

task-related activation and connectivity determine task performance in older participants to a lesser degree than in younger participants.

Disclosures: C.G. Habeck: None. **Q.R. Razlighi:** None. **Y. Stern:** None.

Poster

427. Personalized Brain Signatures

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.07/CC17

Topic: H.02. Human Cognition and Behavior

Support: CNRS MI DEFISENS GRANT

Title: Physiological and cognitive factors underlying perceptual variability in a sensory system

Authors: M. MANTEL, C. MANESSE, A. FOURNEL, C. ROUBY, C. FERDENZI, ***M. BENSAFI**;
Ctr. De Recherche En Neurosciences De Lyon, Bron, France

Abstract: Perceptual variability is a characteristic of sensory experience: the same environmental stimulus can lead to distinct behavioral responses in different individuals. An issue raised and not yet clearly elucidated in behavioral and cognitive neuroscience concerns the role of physiological and cognitive factors in this variability. What is the weight of age, gender, culture, motivation, etc. in this perceptual diversity? The present study attempted to answer this question in a sensory system where variability is the rule, namely olfaction. The study of perceptual variability raises two methodological challenges: the first deals with sample size, and the second with the representativeness of the used stimulations. Here, we considered these 2 challenges by setting up a large-scale study in more than 5000 individuals who were asked to rate 8 representative odors (i.e food, non-food, pleasant, unpleasant odors, etc.) in an online psychophysical experiment. Participants were asked to identify and rate the odorants along several perceptual dimensions (intensity, familiarity, pleasantness, irritation and edibility). A large number of factors that could potentially influence olfaction were also collected, including sex, age, BMI, cultural background, culinary habits, smoking habits, sensory diseases, etc. Preliminary analysis showed than 77% of the variance in perceptual ratings could be explained by two main factors. The first one was highly correlated to odor hedonics and edibility. The second factor was highly correlated to irritation evoked by the odors. This reinforces the idea that both olfactory and trigeminal dimensions intervene perception of smell. We also found some sources of variability in the individual space: identification performances were higher among women and correlated to neophilia scores. Moreover, age, a variable known to highly influence olfactory abilities, was not related to identification abilities but was rather correlated to the perceptual experience of odors, as they were more pleasant, but also less intense and irritating as

the participants got older. These preliminary results contribute to draw a portrait of olfactory perception and its diversity among a large population.

Disclosures: **M. Bensafi:** None. **M. Mantel:** None. **A. Fournel:** None. **C. Manesse:** None. **C. Rouby:** None. **C. Ferdenzi:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.08/CC18

Topic: H.02. Human Cognition and Behavior

Title: The brain network of personality traits and cognition

Authors: L. PIRETTI[1], T. CERNI[1], D. CHECCHI[2], R. JOB[1], ***R. RUMIATI**[3];
[1]Dept. of Psychology and Cognitive Sci., Univ. of Trento, Trento, Italy; [2]Dept. di Economia, Mgmt. e Metodi Quantitativi, Univ. degli Studi di Milano, Milano, Italy; [3]Area of Neurosci., SISSA, Trieste, Italy

Abstract: Personality plays an important role in determining individual differences in academic and life outcomes[1]. However, the mapping between personality-related brain areas and cognition is still poorly understood. Here we investigated whether the grey matter (GM) of personality-related brain areas might correlate with cognitive skills. 83 participants underwent t1-weighted structural magnetic resonance imaging and were administered the Big Five Inventory[2]. Literacy and numeracy were also assessed in a participants' subset. First, voxel-based morphometry (VBM) was performed to investigate the brain areas associated with each personality trait. Results showed that local GM concentration in the occipital cortex bilaterally, right supramarginal gyrus and left dorsolateral prefrontal cortex (dlPFC) inversely correlated with neuroticism. Openness directly correlated with the local GM in right medial orbitofrontal cortex and left cerebellum, and inversely with right amygdala and hippocampus. Moreover, GM inversely correlated with agreeableness in the occipital cortex bilaterally, and conscientiousness within left postcentral gyrus. By extracting from each peak of activation the average GM concentration within an 8mm sphere. Second, to test eventual links between personality-brain areas and cognitive skills, the region of interest (ROI) derived from VBM analysis was correlated with 43 participants' literacy and numeracy scores. Among the clusters associated with neuroticism, only the local GM concentration in the left dlPFC (r = -0.31, p < .05) inversely correlated with the numeracy score. In addition, grey matter concentration in right amygdala and hippocampus, that inversely correlated with openness, also inversely correlated with both numeracy (r = -0.32, p < .05) and literacy scores (r = -0.37, p < .05). Our results suggest that GM of specific brain areas is associated with specific personality traits which, in turn, are linked to participants' cognition.

References:
1. Borghans, L., Golsteyn, B. H., Heckman, J. J., & Humphries, J. E. (2016). What grades and achievement tests measure. *PNAS*, *113*, 13354-13359.
2. John, O. P., Donahue, E. M., & Kentle, R. L. (1991). The big five inventory—versions 4a and 54.

Disclosures: L. Piretti: None. **T. Cerni:** None. **D. Checchi:** None. **R. Job:** None. **R. Rumiati:** None.

Poster

427. Personalized Brain Signatures

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Program #/Poster #: 427.09/CC19

Topic: H.02. Human Cognition and Behavior

Title: Tracking gray matter footprints of neurally distributed cognitive functions

Authors: E. AKPAN, G. E. KOCH, *M. N. COUTANCHE;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The gray matter volume of particular brain regions can reflect a person's cognitive and affective functioning, as well as their experience. Anatomical metrics from neuroimaging scans have been successfully related to individual differences in navigational experience, anxiety, developmental IQ, prior physical activity, and more. When a cognitive function of interest is closely linked to a specific brain region, an anatomical analysis can target a particular region-of-interest. In cases where cognitive functions are represented across widely distributed brain regions, however, a whole-brain exploratory approach is the most frequently used method. This method has a number of limitations, particularly the strong multiple-comparison correction required, which limits its potential for detecting brain-to-behavior relationships. We report the development of a novel method that leverages a massive synthesis of functional results to measure the distributed "gray matter footprint" (GMF) underlying a cognitive function. This approach is both data and hypothesis driven, as it draws on a synthesis of thousands of studies to identify distributed voxels that are associated with a particular cognitive function. Specifically, Neurosynth, an automated meta-analytical online resource that synthesizes functional imaging data from thousands of studies, is combined with a participant's segmented gray matter map to measure the volume of gray matter associated with a cognitive function. We provide a proof-of-concept by predicting individuals' reported semantic memory usage from their semantic-memory GMFs. A leave-one-subject-out support vector regression trained on subjects' GMF can predict the semantic memory scores of untrained participants, supporting the method's predictive potential.

Disclosures: **E. Akpan:** None. **G.E. Koch:** None. **M.N. Coutanche:** None.

Poster

427. Personalized Brain Signatures

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.10/CC20

Topic: H.02. Human Cognition and Behavior

Title: Assessment of individual differences in navigation by diffusion MRI connectometry

Authors: *J. M. SMITH, E. R. CHRASTIL;
UCSB, Santa Barbara, CA

Abstract: Navigation is arguably one of the most important behaviors associated with survival. Although a common, optimized strategy among individuals within a species could be advantageous, in reality, navigation has an incredible degree of variability, both between individuals and between groups. This variability is poorly understood, largely due to the complexity of the systems involved and lack of methods for studying individual differences in cognition.The current study is part of a multi-modal imaging approach to assess individual differences in navigation. Subjects explored an unfamiliar virtual maze environment and were tasked with remembering the location of objects throughout the maze. To assess performance, subjects were asked to start at one object and navigate to a target object on each trial. During this phase, all of the objects were replaced with identical red spheres, thus, a memory of the environment is required to successfully navigate to the correct object. Diffusion spectrum imaging was conducted just prior to the navigational task (139 directions, TR=4300, TE=100.2ms, b-max=4990). After scanning, the diffusion images were preprocessed with QSIPrep and analyzed with the connectometry toolbox software DSI Studio. Connectometry is a permutation based approach that provides higher reliability and replicability with less false positives than standard diffusion imaging analysis. Preliminary analysis on a subset (n=48) of our subject pool found extremely apparent tracks associated with high task performance that project from the middle of the brain into the cerebellum ($P = 0.002$, $t > 3.00$, $FDR < 0.05$). An alternative fiber bundle, which appears to terminate in the same cerebellar region, is negatively correlated, suggesting an alternative pathways in those who performed poorly. Previous findings of cerebellar activity suggests its role in navigation is related to self-motion information processing. The results of our current study provide evidence that the cerebellum is one of the key brain regions responsible for differences in navigational capability. These findings suggest promise for diffusion connectometry as a useful toolbox in analyzing individual differences in cognition.

Disclosures: **J.M. Smith:** None. **E.R. Chrastil:** None.

Poster

427. Personalized Brain Signatures

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Topic: H.02. Human Cognition and Behavior

Support: Funds of the University of Basel (to A.P. and D.J.-F.d.Q.).

Title: Genes to memory: Multi-level omics data integration of quantitative trait loci linked to memory in two independent samples

Authors: *V. VUKOJEVIC[1], V. FREYTAG[1], A. MILNIK[1], A. PAPASSOTIROPOULOS[1], D. J. DE QUERVAIN[2];
[1]Div. of Mol. Neurosci., [2]Div. of Cognitive Neurosci., Univ. of Basel, Basel, Switzerland

Abstract: A large amount of phenotypic variability of complex traits, e.g. cognitive performance, is driven by the underlying genetic architecture. Therefore, understanding the genetic basis of the quantitative trait variation, e.g. quantitative trait loci (QTL), is one of the major challenges in current biology of complex cognitive traits. In the present study we performed a genome wide analysis of DNA methylation and expression cis-QTLs (meQTLs and eQTLs, respectively) in a European sample of healthy young adults (N = 533). We validated our findings in an independent sample of similar size and origin (N = 319), and further investigated the association of the significant loci with episodic and working memory performance across two samples. Finally, we extended our analysis with the metabolomics data in both samples, and identified those QTLs that share conserved effects on cognitive traits from DNA to metabolite level. The cross-investigation of cis-meQTLs in the discovery and replication sample revealed 154'135 unique meQTLs (corrected for 1.4×10^{11} tests; 448'243 CpGs, 313'505 SNPs). These meQTLs were significantly associated to 19'929 unique CpGs, targeting a total of 8'599 genes (FDR of 5%). The same set of SNPs was additionally enriched in eQTLs: 37'721 SNPs associated to 6'578 transcripts (4'330 genes, 67% concordance with meQTLs overlapping genes; FDR of 5%). Data integration with the metabolome data assessed in the same samples (771 metabolites, 54'619 metabQTLs; FDR of 5%) revealed a significant proportion of QTLs shared from "genes to metabolites" (3'692 shared QTLs, 945 unique genes; targeting 89% of the measured metabolites). A second level analysis revealed that these shared QTL CpGs were also related to cognitive phenotypes across two investigated European samples (255 SNPs/42 metabolites and 172 SNPs/25 metabolites, for episodic and working memory respectively). Our findings suggest that there a significant proportion of multi-level omics QTLs that also show an enrichment of significant hits in association analyses with complex traits, like cognitive performance. This is of special importance for studies that rely on proxy tissues, like in the case of cognition and psychiatric disorders, and might provide novel biomarkers or drug targets.

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Poster

427. Personalized Brain Signatures

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Topic: H.02. Human Cognition and Behavior

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NIH Grant R01NS32979
NIH Grant R01NS06424
McDonnell Center for Systems Neuroscience Grant 22-3920-36239P
NIH Grant F32NS092290

Title: Heritability of individual variant sub-types in functional brain networks

Authors: ***B. A. SEITZMAN**[1], C. N. LESSOV-SCHLAGGAR[2], B. ADEYEMO[1], A. DWORETSKY[3], B. T. KRAUS[5], S. E. PETERSEN[1,3,4,8,9], C. GRATTON[5,6,7];
[1]Neurol., [2]Psychiatry, [3]Radiology, [4]Neurosci., Washington Univ. Sch. of Med., Saint Louis, MO; [5]Psychology, [6]Neurol., [7]Interdepartmental Neurosci. Program, Northwestern Univ., Evanston, IL; [8]Biomed. Engin., [9]Psychological and Brain Sci., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Many recent research efforts have focused on uncovering individual differences in functional brain networks measured with fMRI data. We have recently demonstrated the presence of stable localized regions of the brain where individuals differ from the typical group-average network organization - regions we call network variants. Evidence suggests that network variants are systematically organized; they appear in characteristic regions of the brain and tend to associate with particular functional networks. Moreover, the distribution of network variants across individuals clusters into at least two distinct clusters or subgroups in multiple datasets. Given these trait-like properties, here we investigated the heritability of network variants. We exploited the familial design of the Human Connectome Project, analyzing resting-state fMRI data from monozygotic (85 pairs) and dizygotic twins (46 pairs), non-twin siblings (64 pairs), and unrelated individuals (N = 362). We only included individuals with greater than 45 minutes of high-quality, low-motion data to ensure within-subject reliability of network variants (variant stability issues are discussed extensively in an accompanying abstract). From these data, we identified variant locations and functional network memberships by comparing individual-specific data to a group-average of 120 typical young-adults. Then, we clustered individuals into network variant sub-types on the basis of the distribution of their variants.
As has been described previously, overall network structure shows significant heritability.

Interestingly, network variant sub-types also showed significant heritability: monozygotic twin pairs were significantly more likely to have the same sub-type of variant distribution than any other relationship pairing. This may suggest that some network structure heritability derives from common patterns of individual differences.
Overall, our results suggest that network variant sub-types may be partially affected by genetic influences. In addition, the results point to strong environmental contributions to distributions of network variants.

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Poster

427. Personalized Brain Signatures

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Program #/Poster #: 427.13/CC23

Topic: H.02. Human Cognition and Behavior

Title: Stability of individual variations in functional connectivity across states

Authors: *B. KRAUS[1], B. A. SEITZMAN[3], A. DWORETSKY[4], S. E. PETERSEN[5], C. GRATTON[2];
[1]Psychology, [2]Psychology, Neurology, Northwestern Univ. Interdepartmental Neurosci. Program, Northwestern Univ., Evanston, IL; [3]Neurol., [4]Radiology, [5]Neurology, Psychological and Brain Sciences, Radiology, Neuroscience, Biomed. Engin., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Precision imaging is a technique utilizing large amounts of data to obtain reliable functional connectivity (FC) MRI measures of large-scale networks at the individual level. With this method it is possible to examine brain regions where an individual's network patterns deviate from group averages, which we call network variants. Network variants may prove to be valuable substrates for understanding individual differences in brain organization and how they relate to individual differences in behavior. We recently demonstrated that network variants are consistent within resting-state sessions of an individual with >40 minutes of artifact free data. However, one disadvantage of this method is that many current datasets do not have enough resting-state FC data to reach high reliability. If network variants are trait-like markers of individual differences in brain organization, it is possible that they will also be identifiable during task periods as well as rest (i.e., exhibiting stability across states) - opening up additional datasets for variant analysis.
Here we test this hypothesis by asking whether network variants identified from task fMRI data show a high degree of similarity to those seen during resting-state. In order to quantify variant similarity across states, we measured network variants during both task and rest in the Midnight

Scan Club dataset and measured the similarity in their locations via Dice overlap. As a comparison, we also examined the degree of variant overlap across individuals as well as within a given state (task or rest) across independent runs. We found that network variants showed a high degree of overlap across states (Dice = 0.62). This overlap was much higher than that seen across participants ($t(8) = 25.7$, $p < .001$, $d = 8.58$). However, network variant overlap across-states was still significantly lower than overlap within states (Dice = 0.73; $t(8) = 8.5$, $p < .001$, $d = 2.85$), although the effect was smaller. Overall, these findings indicate that network variants are fairly stable across task and rest states within participants relative to what would be expected across participants. However, a systematic difference is still present across states.
These findings suggest that variants are relatively more trait-like in their stability within individuals, but still carry some state-sensitivity. Our results suggest that it is feasible to measure individual differences, in the form of network variants, in a task-state, with the caveat that there are some systematic differences with resting-state FC. Future work will examine interactions between task state and individual differences on FC to better understand their sources.

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Poster

427. Personalized Brain Signatures

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Program #/Poster #: 427.14/CC24

Topic: H.02. Human Cognition and Behavior

Support: McDonnell Center for Systems Neuroscience Grant 22-3920-36239P

Title: Probabilistic mapping of human functional brain networks identifies regions of high inter-subject consensus

Authors: *A. DWORETSKY[1], B. A. SEITZMAN[2], B. ADEYEMO[2], M. NETA[6], R. S. COALSON[1,2], S. E. PETERSEN[1,2,3,4,5], C. GRATTON[7,8,9];
[1]Radiology, [2]Neurol., [3]Psychological and Brain Sci., [4]Neurosci., [5]Biomed. Engin., Washington Univ. Sch. of Med., St. Louis, MO; [6]Psychology, Univ. of Nebraska – Lincoln, Lincoln, NE; [7]Psychology, [8]Neurol., [9]Interdepartmental Neurosci. Program, Northwestern Univ., Evanston, IL

Abstract: While considerable headway has been made in advancing our understanding of functional network organization in the human brain, most studies have focused on representing data averaged across groups of individuals. However, recent work has demonstrated that individual differences in functional network organization are common and widespread, and that averaging across subjects may conceal such differences. These findings, therefore, warrant a re-examination of past group studies in light of individual variation. To this end, we produce a

probabilistic representation of 14 canonical brain networks that leverages information on both group consensus and individual specificity. Further, we present a set of publicly available tools derived from this representation that will enable researchers to apply information about individual variability and consistency across a variety of experimental contexts.
Specifically, voxelwise templates were created for each of 14 canonical brain networks. These templates were applied to each of 69 high-data individual subjects, producing 69 individual network maps. These maps were then overlaid to produce a voxelwise representation of network probability across subjects for each cortical location, both in volume and on the cortical surface. The resulting map shows separable "core" regions of very high reliability (consistent network assignments across >90% of individuals), and regions of high variability between the cores. Individual networks vary in the size of their core and in the extent of their surrounding higher variability components. Further, we show that regions with high variability validate previous findings on the distribution of regions with high inter-subject network variability and with a high occurrence of topological network variants.
Along with a nuanced exploration of reliability and variability, this probabilistic representation allows for novel methods of exploring other functional datasets and experiments. First, a set of high-probability network sensitive regions of interest (ROIs) are generated, which can be applied to group datasets to analyze the most consistent core regions of each network. Second, a voxelwise point-and-click probability tool is created which allows researchers using Connectome Workbench to explore the network membership probability of individual locations in their own data in task, structural, or resting-state studies.

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Poster

427. Personalized Brain Signatures

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Program #/Poster #: 427.15/CC25

Topic: A.09. Adolescent Development

Support: ERC Advanced Grant (AdG), SH4, ERC-2015-AdG

Title: Neural oscillations and nursery rhymes (an EEG study into individual differences in infant language development)

Authors: *A. ATTAHERI[1], A. NI CHOISDEALBHA[1], P. BRUSINI[1,2], S. ROCHA-THOMAS[1], S. FLANAGAN[1], N. MEAD[1], P. BOUTRIS[1], S. GIBBON[1], H. SCOTT[1], H. AHMED[1], I. WILLIAMS[1], U. GOSWAMI[1];
[1]Ctr. for Neurosci. in Educ., Univ. of Cambridge, Cambridge, United Kingdom; [2]Univ. of Liverpool, Liverpool, United Kingdom

Abstract: The synchronisation of cortical oscillations with different patterns of temporal information in the speech signal may be key in how the brain processes language. Our current research focuses on individual differences between infants in the entrainment of low frequency neural oscillations (delta band, ~2Hz, and theta band, ~5 Hz) in early development to both speech and non-speech inputs. In spoken language, a 2Hz rhythm reflects the production of stressed syllables, which are produced approximately twice a second across languages. Modulation energy in the delta band (0.9 - 2.5 Hz) is increased in infant directed speech (IDS) compared to adult directed speech (ADS), and across languages lullabies and nursery rhymes also converge on a 2 Hz beat rate. Our hypothesis is that accurate entrainment to a 2 Hz "beat" may provide a foundational entrainment rate across languages, offering a rhythmic skeleton upon which other language units (words, syllables, rhyme, phonemes) may be scaffolded. To test this, we are conducting a longitudinal assessment of infants from 2 months to 2.5 years of age. Our primary measures use EEG to measure oscillatory entrainment to different inputs (simple drum beat, rhythmic repetition of syllable 'ta' and phrasal prosody via nursery rhymes). We focus on the entrainment of these low frequency EEG oscillations to features from the language stimulus, across the first year of life (2-11 months). Infants are followed up between 12 and 30 months, using a range of phonological, morphological and vocabulary assessments. We report preliminary findings suggesting individual differences in the EEG responses to audio visually presented nursery rhymes at 4, 7 and 11 months. We first report individual differences in stimulus envelope reconstruction using a linear modeling technique (mTRF toolbox), with early indications that this entrainment correlates to language comprehension by infants. Secondly, we show phase amplitude coupling (PAC) in response to nursery rhymes and how this changes longitudinally during the first year of life. By tracking longitudinal measures through infant development, these results show the potential importance of individual differences in neural entrainment in predicting later language outcomes.

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Poster

427. Personalized Brain Signatures

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Program #/Poster #: 427.16/CC26

Topic: I.02. Systems Biology and Bioinformatics

Title: Local DTI patterns predict fMRI language task activation with group-level precision

Authors: *D. G. ELLIS, M. R. AIZENBERG;
Neurosurg., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: A fundamental assumption of neuroscience is that brain function is determined by brain structure. Even in healthy populations, both brain structure and brain function vary between individuals and groups. We hypothesize that individual and group differences in functional brain activation can be predicted by localized structural patterns of diffusivity. To this aim, we trained a convolutional neural network (CNN) to predict language task-fMRI (tfMRI) activation maps using only the maps of local mean diffusivity and fractional anisotropy as measured by the diffusion tensor model. The model reads in a 64mm x 64mm x 64mm image centered on a vertex on the pial surface. The model then predicts the language activation at that vertex. After training on 595 subjects, the model was used to predict the language activation maps for a validation group of 300 (128 male) subjects. To assess the model's ability to accurately predict variations between groups of subjects, a linear support vector machine (SVM) was trained to predict gender based on the parcellated actual fMRI language responses of the training group. The SVM was then used to predict the gender of the validation group using only the CNN predicted fMRI language data. The predicted fMRI data was able to predict gender with an area under (AUC) the receiver operating characteristic (ROC) curve of 0.69, which is significantly greater than chance ($p < 0.01$). This study demonstrates that local structural patterns are predictive of group-level variations in language task activation. Future research could utilize local DTI patterns in combination with fMRI data to provide functional mappings that are more robust to noise. From a clinical perspective, DTI patterns could be used to estimate functional activation in patients that have not undergone functional mapping.

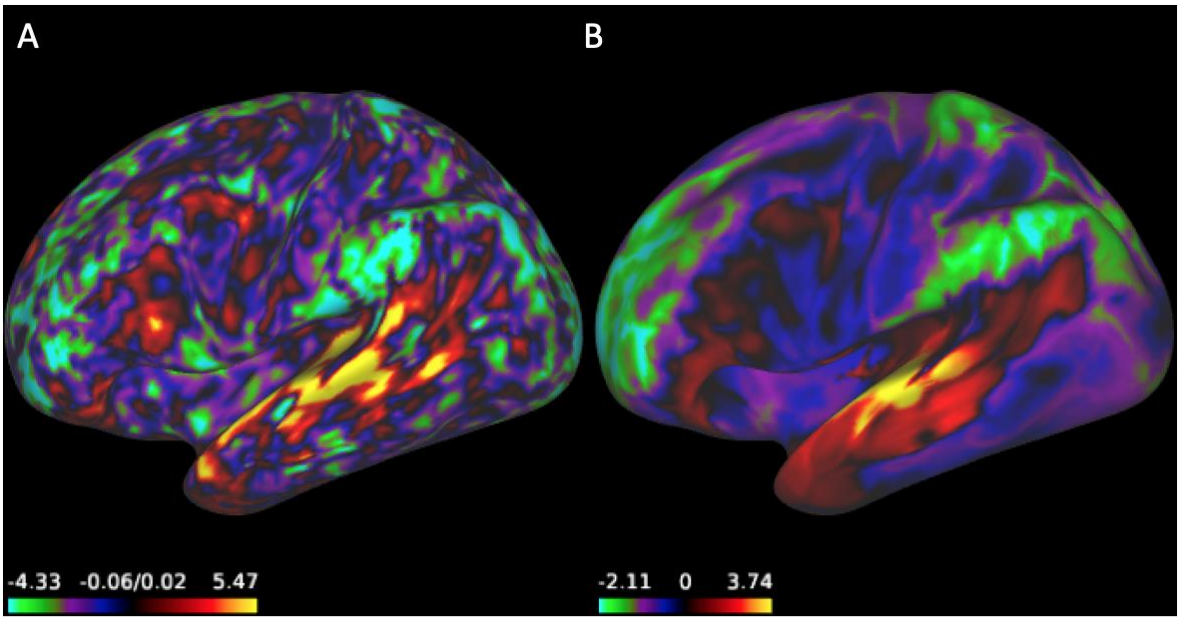

Figure 1 – (A) Single subject fMRI language "story" task activation; (B) predicted language "story" activation based on local DTI patterns.

Disclosures: D.G. Ellis: None. **M.R. Aizenberg:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.17/CC27

Topic: I.07. Data Analysis and Statistics

Title: Cortical networks associated with the human anterior medial temporal lobe estimated within the individual using intrinsic functional connectivity

Authors: *D. REZNIK[1], L. M. DINICOLA[1], R. M. BRAGA[1,2], P. ANGELI[1], R. L. BUCKNER[1,3,4];
[1]Harvard Univ., Cambridge, MA; [2]Stanford Univ., Stanford, CA; [3]Massachusetts Gen. Hosp., Boston, MA; [4]Harvard Med. Sch., Boston, MA

Abstract: Monkey anatomical tract-tracing studies indicate entorhinal, perirhinal and parahippocampal cortices are connected with multiple cortical regions in a manner that is characterized by both distinct and similar topography. Human functional connectivity studies have recapitulated several of these features (Greicius et al., 2004 *PNAS*; Vincent et al., 2006 *JNeurophys*; Kahn et al., 2008 *JNeurophys*). Recently, human explorations have revealed that canonical distributed networks defined using group-averaged data are divisible into two or more spatially distinct networks when estimated with individuals. More specifically, it was demonstrated that the canonical default network (DN) can be divided into at least two distinct networks, DN-A and DN-B, and that DN-A (but not DN-B) includes the posterior MTL (parahippocampal cortex; Braga & Buckner, 2017 *Neuron;* Braga et al., 2019 *JNeurophys*). Although imaging the anterior MTL is a challenge due to its sensitivity to magnetic susceptibility artifacts, here we examined this region in detail and explored the hypothesis that anterior portions of the MTL might associate with distinct cortical networks that have been hidden in prior analyses. We estimated the organizational properties of networks associated with entorhinal, perirhinal and parahippocampal cortices from resting-state data in eight densely sampled individuals (fixation data collected during 54.6 to 430.5 minutes). Consistent with previous findings, whole-brain correlation maps with seed regions placed in posterior parahippocampal cortex pointed to DN-A. Whole-brain correlation maps with seed regions placed in entorhinal and perirhinal cortices provided initial evidence for distinct distributed networks. Interestingly, inspired by these preliminary findings, examination of publicly available resting-state fMRI data from 4181 individuals released by the UK Biobank (a large-scale population based study) with seed regions placed in the group entorhinal and perirhinal cortices pointed to networks that reflect the topography we observed in individual subjects. These results provide further insight into the neuroanatomical organization of the human MTL.

Disclosures: **D. Reznik:** None. **L.M. DiNicola:** None. **R.M. Braga:** None. **P. Angeli:** None. **R.L. Buckner:** F. Consulting Fees (e.g., advisory boards); Roche.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.18/CC28

Topic: I.07. Data Analysis and Statistics

Support: NIH Pathway to Independence Award K99MH117226

Title: Fast temporal characterization of distributed association networks within the individual using intracranial recording and repeated sampling functional MRI

Authors: *R. M. BRAGA[1], C. A. SAVA-SEGAL[1], R. A. POLDRACK[2], J. PARVIZI[1]; [1]Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA; [2]Psychology, 450 Serra Mall Bldg. 420, Stanford, CA

Abstract: The cerebral association cortex is organized into networks that are distributed across frontal, parietal, temporal and midline association zones. Analysis of functional MRI (fMRI) data from repeatedly sampled individuals reveals that canonical networks, such as the default network, comprise two or more networks when defined within the individual (Braga and Buckner 2017). The distinction between networks has been replicated across multiple individuals and analysis methods (Braga et al. 2019). However, questions remain regarding to what extent the network distinctions correspond to differences in local neural activity, as well as long-distance coupling at faster frequencies. Further, it is unclear whether neighboring networks engage in cross-talk over short timescales and/or at frequencies that are not detectable by conventional fMRI.

To address these questions, we delineated the distributed networks with precision in an individual with intractable epilepsy who was scheduled for intracranial monitoring with stereo-electroencephalography as part of their routine presurgical evaluation. A primary aim was to establish that repeated sampling fMRI could in principle be performed, and the previously observed network distinctions be reproduced, in this population.

In a presurgical MR session, fMRI data (2.4 mm, 1.0 sec TR) were acquired during three runs of a fixation task, each lasting 7 min 2 sec. The patient was compliant, though complained of sleepiness at the end of the session. Several steps were taken to minimize movement, and the patient provided good quality data (mean and maximum framewise displacement range: 0.122–0.142 mm and 0.543–0.586 mm). Data were processed using the 'iProc' pipeline (Braga et al. 2019), which is optimized for individual-subject registration, and includes upsampling to 1mm resolution. Data were smoothed at 3 mm FWHM.

Initial analyses confirmed that the default network could be separated into two parallel networks

within this patient as previously reported in non-clinical subjects (Braga and Buckner 2017). Registration of the MRI data to the patient's CT image, containing the location of implanted electrodes, was performed using the iELVis pipeline. In the posteromedial and dorsal prefrontal cortices, side-by-side regions belonging to the two networks overlapped with implanted electrodes, suggesting that neighboring and distant regions of the two networks were being sampled. This work provides proof-of-principle that precision mapping is viable in presurgical epilepsy patients. Ongoing work is characterizing the networks at high temporal frequency and optimizing the data collection protocol for future patients.

Disclosures: R.M. Braga: None. **C.A. Sava-Segal:** None. **R.A. Poldrack:** None. **J. Parvizi:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.19/CC29

Topic: I.07. Data Analysis and Statistics

Title: Proof-of-concept longitudinal tracking of brain change within individual patients using repeated acquisitions of rapid structural scans

Authors: *L. C. HANFORD[1], J. A. NIELSEN[2], R. W. MAIR[1,3], J. A. COLLINS[3,4], M. QUIMBY[3,4], M. C. ELDAIEF[3,4], B. C. DICKERSON[3,4], R. L. BUCKNER[1,3,4];
[1]Harvard Univ., Cambridge, MA; [2]Brigham Young Univ., Provo, UT; [3]Massachusetts Gen. Hosp., Charlestown, MA; [4]Harvard Med. Sch., Boston, MA

Abstract: A major limitation of translational neuroimaging is reliability and sensitivity to change within the individual. To overcome this limitation, we explored estimating longitudinal change in structural brain images by collecting numerous repetitions of rapid acquisitions at each timepoint (Nielsen et al., 2019, *bioRxiv*). Individuals with expected increases in brain volumes (patients with Major Depressive Disorder receiving treatment) and expected decreases in brain volumes (patients with neurodegenerative disease including semantic dementia and posterior cortical atrophy) were enrolled. At each timepoint, a total of 4 sessions of 8 or 9 Multi-Echo MPRAGE T1-weighted images were acquired with vNAV to track within-acquisition motion (2.2mins, resolution:1.2mm, see Mair et al., 2012, *ISMRM*; Holmes et al., 2015, *Sci Data;* Nielsen et al. 2019). Sessions were spread out across two separate days. Subjects were tracked across 3 timepoints over 6 months, totaling 96 to 108 independent scans per subject. Despite the large number of scans, participants found this burden low and manageable, largely owing to the time in the scanner being brief. Images were processed using Freesurfer v6.0. Metrics were motion- and scan-order adjusted. Patients with MDD following TMS treatment showed increased total brain volume (0.49%, 1.54% annualized increase), and maintained or increased

hippocampal volume (0.01%, 0.68%). Patients with neurodegenerative disease showed decreased total brain volume (-2.46% to -1.11% annualized decrease), as well as hippocampal volume reductions (-4.37% to -2.46%). Estimated total intracranial volume, used as a control measure given its stability, changed minimally. Measurement error, estimated as the absolute difference in measurements for acquisitions across days within the same timepoint, was below 0.2% for eTIV and below or near 1.0% for cortical and hippocampal measures. These results provide support for adopting rapid, repeat scanning methods to achieve measurement stability within individuals in longitudinal designs. We discuss these results as well as ways to further improve sensitivity and reduce measurement error. Scan time can be further reduced by adopting even faster wave-CAIPI or compressed-sensing MPRAGE variants (able to achieve 1.0 mm scans in a minute, or sub-mm scans in a few min). Through repeated sampling, we can further interrogate sources of noise such as head position, and local B0 inhomogeneities. The goal of this work is to provide tools to measure clinically-relevant brain change in individuals or small groups as would be enrolled in targeted clinical research studies or early-stage clinical trials.

Disclosures: L.C. Hanford: None. **J.A. Nielsen:** None. **R.W. Mair:** A. Employment/Salary (full or part-time):; spouse affiliation, Takeda Pharmaceutical Company Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); spouse affiliation, Takeda Pharmaceutical Company Ltd. **J.A. Collins:** None. **M. Quimby:** None. **M.C. Eldaief:** A. Employment/Salary (full or part-time):; spouse affiliation, Takeda Pharmaceutical Company Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); spouse affiliation, Takeda Pharmaceutical Company Ltd. **B.C. Dickerson:** F. Consulting Fees (e.g., advisory boards); Biogen, Alector, Wave LifeSciences. **R.L. Buckner:** F. Consulting Fees (e.g., advisory boards); Roche.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.20/CC30

Topic: I.07. Data Analysis and Statistics

Title: Human structural connectomes are heritable

Authors: J. CHUNG, B. D. PEDIGO, C. PRIEBE, *J. T. VOGELSTEIN;
Johns Hopkins Univ., Baltimore, MD

Abstract: Understanding heritability, or the extent to which genes and environment determines human brain connectivity and structure, is important for improving our understanding of brain function and diseases. Current methodologies study connectomes estimated from neuroimaging data, such as diffusion and functional MRI, by computing graphs features, such as small

worldness, modularity, etc, which are difficult to interpret. This study leverages a statistically principled procedure for studying a population of connectomes, and show that the structural connectomes are heritable and strongly related to familial relationship.
Using the Human Connectome Project Young Adult S1200 dataset (n=985, age=22-37), structural connectomes are obtained from diffusion MRI (dMRI) via deterministic diffusion tractography. Flood-filled Desikan-Killiany atlas (83 region of interest (ROIs)) was used to parcellate the brain. Each connectome is embedded into *d* dimensions using adjacency spectral embedding (ASE) to obtain latent variables for each ROI. Distances between connectomes are obtained by computing the Procrustes distance of a pair of embeddings for all pairs of monozygotic (MZ, n=125 pairs), dizygotic (DZ, n=130 pairs), siblings (SIB, n=712 pairs), and unrelated (UN, n=1000 pairs) individuals. Unrelated pairs, defined as two individuals not sharing a mother or father, were sampled such that the distribution of difference in age were identical to that of SIB in order to control for age effects.
Distances between MZ connectomes were stochastically smaller than those of DZ, SIB, and UN, while the distances between UN connectomes were stochastically larger than those of MZ, DZ, and UN. The distances of DZ were stochastically larger than that of MZ, but smaller than that of SIB. This stochastic ordering suggest that the distance between a pair of connectomes is highly related to the genetic similarity between the individuals. Furthermore, order of DZ and SIB suggest age or environmental influences on brain structure since they share similar genetic variance. This claim is validated via two-sample Kolmogrov-Smirnoff (KS) test of distributions. The null hypothesis state that a pair of distance distributions are the same, and the alternate hypothesis is that one distribution stochastically larger than the other. Alternate hypothesis were formed for all six pairs of distributions while maintaining the stochastic order. (MZ < DZ, MZ < SIB, MZ < UN, DZ < SIB, DZ < UN, SIB < UN). Using a significance level of 0.05, KS tests find that there is a statistically significant difference in distributions for all tests.

Disclosures: **J. Chung:** None. **B.D. Pedigo:** None. **C. Priebe:** None. **J.T. Vogelstein:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.21/CC31

Topic: B.09. Network interactions

Support: CPS-18-01-KIST
NRF 2017R1A2B3012659
CRC-15-04-KIST

Title: cBRAIN: Tracking all individuals and marking their brain activity for the study of collective brain research

Authors: *C. KIM[1,2], J. KIM[1,3], W. YOUM[4], S. LEE[4], J. CHOI[1,2];
[1]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]Univ. of Sci. and Technol., Seoul, Korea, Republic of; [3]Korea Univ., Seoul, Korea, Republic of; [4]Electronics and Telecommunications Res. Inst., Daejeon, Korea, Republic of

Abstract: Understanding the neural mechanisms underlying the collective behaviors in a group is limited by the ability to track each individual and monitor its neural activity at the same time. Traditional neural recording systems send information over wires restraining the animals' habitat or behavior, whilst recently introduced innovative approach using telemetry still has limited capacity in simultaneous measurement of multiple individuals in a group. Here, we describe a novel method, cBRAIN (collective brain research aided by instant neurodisplay), which was developed for the purpose of tracking the position and certain neural activity of all naturally behaving individuals in a group. Basically, the cBRAIN is composed of its hardware and algorithm. Firstly, the cBRAIN hardware includes a headstage, CCD camera, and data logger. The 3.5-g weighted headstage is a telemetry featuring recording and data transmission, and blue and red LED lights indicating the position and the occurrence of user-set neural activity, respectively. Also, the headstage contains an internal counter to sync between headstages and a programmable log array for LED trigger working in a recognition scheme to detect the moment when the neural activity of interests occurs. The data logger records raw data up to eight headstages with sampling rate which is tunable up to 20 kHz. The CCD camera records the optical signals with 10~170 Hz sampling rate and a resolution of 2048 x 1088. Secondly, the cBRAIN algorithm has three main purposes: (i) tracking the position of individual mouse, (ii) detecting the moment of red LED on and merging it into the position data, and (iii) statistical mapping such as space occupancy and location map of neural activities of interest, and presentation of inter-agent proximity for the study of collective motion of a group. Finally, we demonstrate a study of social hierarchy in a group using cBRAIN. A classification algorithm for identifying social behaviors such as fighting, chasing, sniffing, and huddling, built based on the tracking data, will be introduced. Lastly, the cBRAIN system therefore provides a promising ground for studying dynamic interplay between social and neural activities from naturally behaving individuals living in a group, and further associating the neural activities with collective behaviors of a group of mice.

Disclosures: C. Kim: None. **J. Kim:** None. **W. Youm:** None. **S. Lee:** None. **J. Choi:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.22/CC32

Topic: I.07. Data Analysis and Statistics

Support: DFG BI 195/77-1
BMBF 16SV7701 CoMiCon
LUMINOUS-H2020-FETOPEN-2014-2015-RIA (686764)
Wyss Center for Bio and Neuroengineering

Title: Resting state EEG in patient with completely locked in syndrome

Authors: *M. KHALILI ARDALI[1], U. CHAUDHARY[1], N. BIRBAUMER[2];
[1]Inst. For Med. Psychology and Behavioural N, Tuebingen, Germany; [2]Wyss Ctr. for Bio and Neuroengineering, Geneve, Switzerland

Abstract: Different studies have reported conflicting results on the cognitive capacity of Individuals in locked-In state (LIS), due to amyotrophic lateral sclerosis (ALS), ranging from degrading to intact cognitive capacity. While on the other hand, there is no information available on the cognitive capacity of Individuals in completely locked-In state (CLIS), due to amyotrophic lateral sclerosis (ALS), who are left without any means of communication. There has been a significant advancement in determining the capacity of consciousness of patients with disorders of consciousness (DoC) by employing information integration theory (IIT) based indexes and global workspace network (GWN). Despite these advances it is still both difficult and challenging to apply such methods to estimate the level of consciousness for Individuals in CLIS. Even before we could determine the level of consciousness of Individuals in CLIS, in this study we propose to investigate the basic neurophysiological properties using electroencephalogram (EEG) acquired from CLIS patients during the resting state. High density EEG signal acquired from 5 individuals in CLIS will be investigated to elucidate the time domain and spectral features, as well as connectivity metrics of EEG signal of individuals in CLIS, which will be compared which healthy population. Initial analysis of EEG of individuals in CLIS shows shift in the spectral features towards the lower frequency bands immediately after the transition to CLIS as well as significant decrease in the amplitude. In depth analysis as proposed in this study will be performed to explicate the neurophysiological properties underlying the resting state brain activity of individuals in CLIS. Such an analysis might prove to be the first step towards the goal to estimate the metrics for the capacity of consciousness of Individuals in CLIS.

Disclosures: M. Khalili Ardali: None. **U. Chaudhary:** None. **N. Birbaumer:** None.

Poster

427. Personalized Brain Signatures

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 427.23/CC33

Topic: I.07. Data Analysis and Statistics

Support: NSF IIS-1636893
NSF BCS-1734853
NIH NIMH ULTTR001108
NIH NIMH U01MH097435
NIH NIMH 5 T32 MH103213
NIH NCATS UL1TR002529
Microsoft Research Award

Title: Human age is predicted by a strong multidimensional association of brain networks and behavioral factors

Authors: *B. MCPHERSON[1], F. PESTILLI[2];
[1]Psychological and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN; [2]Psychology, Neuroscience, Cognitive Sci. and Network Sci., Indiana Univ., Bloomington, IN

Abstract: Human behavior and cognitive performance change across the lifespan. Behavioral and cognitive performance improves in many domains during early development and peaks shortly after adolescence. Brain network connectivity also peaks shortly after adolescence. The degree to which these two lifespan trajectories map onto each other is unclear. Further, researchers have only recently attempted to link brain network connectivity to specific behavioral and cognitive domains with human chronological age. We used a multivariate approach to simultaneously model multiple behavioral and brain network factors. Our analyses demonstrate a strong association between the brain network properties and performance in a variety of behavioral domains. To test the association between brain networks, behavioral domains, and age (CCA; Smith et al., 2015). We estimated the association between measures of performance from 334 behavioral tasks across seven domains with a 376 node network estimate of brain connectivity measured in 594 human individuals across the lifespan (18-88 years). We processed the raw data and generated multiple brain network measures using diffusion-weighted and anatomical MRI data from the Cambridge Center for Aging Neuroscience (Taylor et al., 2014) and the cloud computing platform brainlife.io (Avesani et al., in press). Brain networks were built using multi-shell diffusion models, probabilistic tractography to trace candidate fibers between brain regions (Takemura et al., 2016; Smith et al., 2014), and brain atlas (Glasser et al., 2017). We found that a single dimension of covariation strongly associates (r=0.86) measures of performance across seven behavioral domains. This single dimension of covariation predicted the held-out age of the individuals in the sample (r=0.66). This is the first study to report a single, strong association between performance across behaviors and brain networks predicting age. The results suggest that human age can be predicted by the synchronous degradation of structural brain networks and behavioral performance.

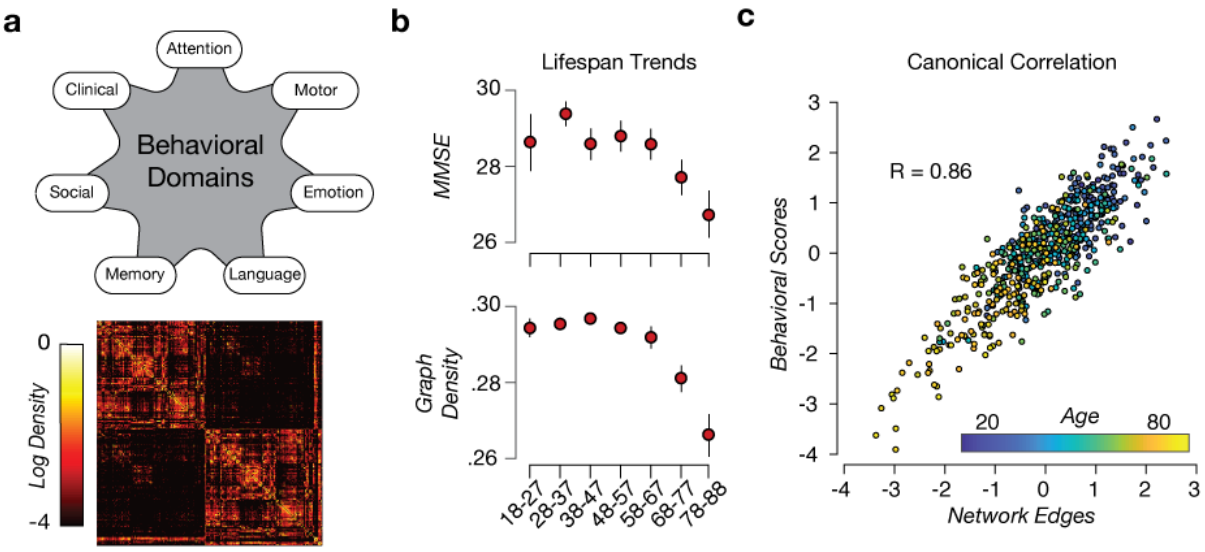

Figure 1. Covariation of behavior and brain networks across the lifespan. (a) *(top)* A diagram showing the behavioral domains used in the analysis. *(bottom)* One representative structural brain network reconstructed using tractography methods on brainlife.io. **(b)** *(top)* A representative example of the established lifespan trends reproduced in the Can-CAM dataset for one behavioral domain. The Mini-Mental State Examination (MMSE) shows declining cognitive ability across the lifespan. **(b)** *(bottom)* A representative example of the established lifespan trends observed in a brain network property. **(c)** Positive-negative single association axis linking behavior and brain networks using a canonical correlation analysis (R=0.86). Individual points in the plot are color-coded using each subject's age. The correlation between each subject's location in the association axis and the held-out age of the individual in the plot is high (R=0.66, not shown).

Disclosures: B. McPherson: None. **F. Pestilli:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.01/CC34

Topic: H.03. Schizophrenia

Support: CONACYT (Mexico) Grant 252808
CONACYT (Mexico) Grant 481316
MINECO (Spain) Grant SAF2016-75500-R

Title: Neurotrophic and antioxidant effects of risperidone improve dendritic spine dynamics in the prefrontal cortex of rats with neonatal ventral hippocampus lesion

Authors: *H. TENDILLA[1,2,3,4], R. A. VAZQUEZ-ROQUE[2], A. J. VÁZQUEZ-HERNÁNDEZ[2], D. MARTÍN-HERNÁNDEZ[3,4,5,6], K. MACDOWELL[3,4,5,6], L. GARCÉS-RAMÍREZ[1], J. LEZA[3,4,5,6], G. FLORES[2];
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Dept. de Farmacología y Toxicología, [4]Iuin, Univ. Complutense de Madrid, Madrid, Spain; [5]Ctr. de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Madrid, Spain; [6]Inst. de Investigación Sanitaria Hosp. 12 de Octubre, Madrid, Spain

Abstract: Schizophrenia is a mental disorder whose etiology and mechanisms underlie neurodevelopmental disruptions. Psychosis, social isolation and cognitive deficits observed in schizophrenia has been related to prefrontal cortex (PFC) dysfunction, since gray matter loss and lack of dendritic spines has been described in this brain region. Second generation antipsychotics are the drugs of choice for schizophrenia. Apart from their dopamine/serotonin antagonism activity, some of these substances ameliorate the symptoms by other mechanisms such as anti-inflammatory, antioxidant, neurotrophic and neuroprotective effects. Neonatal ventral hippocampus lesion (NVHL) in the rat is considered a developmental schizophrenia-related model. Adult rats NVHL had decreased spine density and a reduction of mature spines in the PFC, associated with the disruption of the brain-derived neurotrophic factor/tyrosine kinase B (BDNF/TrkB) pathway. Also, reduced brain levels of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which promotes the synthesis of antioxidant enzymes, as well as increased protein levels of cyclooxygenase-2 (COX-2), a potent pro-inflammatory mediator, in the brain and periphery was observed in NVHL animals. The abovementioned settles oxidative/nitrosative stress in the PFC in the NVHL, that was evidenced by the increased lipid peroxidation. The atypical antipsychotic risperidone (RISP) restored the BDNF/TrkB pathway and reduced the oxidative/nitrosative stress in the PFC and periphery of NVHL rats, that is associated with the enhancement of the mature spine population. Thus, our data show the neurotrophic and antioxidant effect of RISP and its impact on dendritic spine remodeling in this rodent model, which highlight the link between brain development and immune response in order to understand mechanisms related to the schizophrenia pathophysiology.

Disclosures: H. Tendilla: None. **R.A. Vazquez-Roque:** None. **A.J. Vázquez-Hernández:** None. **D. Martín-Hernández:** None. **K. MacDowell:** None. **J. Leza:** None. **G. Flores:** None. **L. Garcés-Ramírez:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.02/CC35

Topic: H.03. Schizophrenia

Support: MOST 106-2410-H-002-101
MOST 107-2410-H-002-117

Title: The effect of neonatal GSK3 inhibition on the regulation of behavioral deficits and neuromorphological alterations in AKT1 mutant mouse model of schizophrenia

Authors: *S.-C. WAN[1], T.-Y. JEN[2], W.-S. LAI[1,3,4];
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Abstract: Schizophrenia is considered as a neurodevelopmental disorder that leads to behavioral, neuronal, and biochemical abnormalities. Accumulating evidence suggests that *AKT1* (protein kinase Bα) contributes to susceptibility to schizophrenia and the AKT-GSK3 signaling pathway is involved in the pathogenesis of schizophrenia. GSK3, a key kinase modulating the development of the nervous system, is largely regulated by one of its upstream targets- AKT. Convergent evidence indicates a decrease in AKT1 protein levels and levels of phosphorylation of GSK3β in the peripheral lymphocytes and brains of individuals with schizophrenia. Furthermore, it has been found that direct inhibition of active GSK3 by GSK3 inhibitors alleviated the schizophrenia-like behaviors in adult *Akt1*$^{-/-}$ mice. Given the involvement of AKT-GSK3 signaling pathway in schizophrenia, here, we aim to investigate whether direct inhibition of GSK3 during early developmental period could rescue the behavioral deficits and neuromorphological alterations in adult *Akt1*-deficient mice. SB216763 (GSK3 inhibitor) was selected and administrated to both male and female *Akt1*$^{+/-}$ mice and their wild-type littermates every other day during postnatal day 7 to 27 (P7-27). Compared to vehicle controls, the administration of SB216763 resulted in a significant enhancement of phosphorylated GSK3α/β in the prefrontal cortex and hippocampus of *Akt1*$^{+/-}$ mice at P27. At 3 months of age, a battery of behavioral tasks was performed in these mice to evaluate their behavioral phenotypes, including the acoustic prepulse inhibition (for sensorimotor gating function), puzzle box (for problem solving), and fear conditioning (for associative learning and memory). Consistent with previous findings, our preliminary results indicate that *Akt1*$^{+/-}$ female mice displayed significant abnormality of prepulse inhibition, and *Akt1*$^{+/-}$ male mice exhibited potentially impaired performance on contextual fear conditioning. Both sexes of *Akt1*$^{+/-}$ mice also revealed different types of behavioral impairments in the puzzle box. Intriguingly, the neonatal administration of SB216763 seemed to somehow rescue observed behavioral deficits in these Akt1 mutant mice. Based on behavioral findings, we further analyze neuromorphological alterations in the prefrontal cortex and hippocampus using Thy1-GFP-M transgenic mice. The morphometric analysis is still in progress. Collectively, our findings suggest the involvement of AKT1-GSK3 signaling pathway in the pathogenesis of schizophrenia-related phenotypes and the early developmental inhibition of GSK3 in the treatment of schizophrenia, especially during neonatal stage.

Disclosures: S. Wan: None. **T. Jen:** None. **W. Lai:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.03/CC36

Topic: H.03. Schizophrenia

Support: BBM011208/1

Title: Investigating the role of the pre and postnatal maternal environments on offspring behavioural deficits in a rodent model of maternal immune activation

Authors: *H. G. POTTER, G. REVILL, R. M. WOODS, J. D. GLAZIER, J. C. NEILL, R. HAGER;
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Abstract: Converging evidence from epidemiological studies and animal models has implicated maternal immune activation (mIA) as a risk factor for neurodevelopmental disorders such as schizophrenia. The transient increase in maternal pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), elicited following mIA is thought to perturb fetal neurodevelopment and results in cognitive deficits. Here, we employed a split-litter cross-fostering design to investigate how the prenatal (maternal cytokine response) and postnatal (maternal care) environments interact to predict offspring cognitive deficits in a rodent model of mIA. 22 female Wistar rats were timed-mated and treated with a single intraperitoneal injection of 10mg/kg bodyweight polyinosinic-polycytidylic acid (poly I:C; low-molecular weight, InvivoGen) or vehicle (endotoxin-free 0.9% saline) on GD15. A tail vein blood sample was taken 3h post-treatment to measure maternal cytokine concentration. Offspring were culled to 10 pups/litter on postnatal day 1 (PD1) and either crossed to a dam in the opposite treatment group or remained in their home litter. Offspring ultrasonic vocalisations (USVs) were recorded on PD6, 10, and 14 and analysed using the open-source MATLAB script MUPET. Offspring were tested on the attentional set-shifting task (ASST) in adulthood. Statistical analysis was carried out in SPSS using General Linear Models (GLM) and General Linear Mixed Models (GLMM). Prenatal exposure to poly I:C, independent of postnatal maternal effects, significantly increased the number of syllables emitted by female ($F_{(1,295)}$=16.65, $p<0.001$) and male ($F_{(1,298)}$=13.05, $p<0.001$) pups across all time-points. Cross-fostering had no effect on the number of syllables emitted by female ($F_{(1,295)}$=0.23, p=0.631) or male ($F_{(1,293)}$=0.91, p=0.341) pups. In adult female offspring, poly I:C caused an increase in the number of trials taken in the ASST ($F_{(1,11)}$=10.14, p=0.009). Furthermore, the concentration of the maternal TNF-α response predicted a more severe set-shifting deficit ($F_{(1,11)}$=5.70, p=0.036). Poly I:C induced a rise in maternal TNF-α concentration. Neonatal and adult offspring from poly I:C treated dams exhibited a range of behavioural and cognitive deficits. Prenatal exposure to maternal pro-inflammatory cytokines,

but not the postnatal maternal environment, affected USVs in male and female offspring. Our data suggests that the prenatal environment, specifically the maternal inflammatory cytokine response, is an important predictor of later life cognitive deficits and contributes to the pathogenesis of cognitive dysfunction.

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Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.04/CC37

Topic: H.03. Schizophrenia

Support: NIH grant R01MH112168

Title: MicroRNA-206 signaling in cerebellar Purkinje cells regulates avoidance behaviors and sensorimotor gating

Authors: *M. P. HEYER[1], M. ISHIKAWA[1], G. FENG[2], P. J. KENNY[1];
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Abstract: The cerebellum is primarily known for its roles in motor learning and coordination. Emerging evidence suggests that the cerebellum also regulates more complex behaviors related to cognition, affect, and reward, and dysfunction in this brain structure may contribute to schizophrenia and other neurodevelopment disorders. The cerebellum shares extensive reciprocal connectivity with the neocortex, basal ganglia and hindbrain nuclei, and is thought to convey sensory experiences that shape cortical development and function. Nevertheless, the genes, cells, and circuits that govern cerebellar interactions with higher-order brain systems are poorly understood. Using brain-wide *in situ* hybridization, we find that the schizophrenia-associated microRNA, miR-206, is specifically enriched in cerebellar Purkinje cells. Mice with a targeted deletion of miR-206 exhibit impaired pre-pulse inhibition, an endophenotype of schizophrenia, increased innate fear-related and avoidance behaviors, and sex-dependent cognitive deficits. Pre-pulse inhibition impairments were recapitulated by conditional deletion of miR-206 in parvalbumin-expressing cells, including Purkinje cells, suggesting that altered cerebellar output may contribute to these behavioral abnormalities. Consistent with this possibility, the spontaneous firing frequency of Purkinje cells was increased in miR-206 null mice. Together,

these findings suggest that miR-206 regulates the function of cerebellar Purkinje cells and that this action controls cognitive, affective, and reward-related behaviors that are relevant to schizophrenia.

Disclosures: M.P. Heyer: None. **M. Ishikawa:** None. **G. Feng:** None. **P.J. Kenny:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

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Program #/Poster #: 428.05/CC38

Topic: H.03. Schizophrenia

Support: 2016R1D1A1B03931619

Title: Prenatal exposure to environmental factors affects protein expression of rat brain and increases risk of developing schizophrenia

Authors: H. LEE, H.-K. KIM, J.-T. KWON, *H.-J. KIM;
Clin. Pharmacol., Soonchunhyang University, Col. of Med., Cheonan / Chungcheongnam-Do, Korea, Republic of

Abstract: Exposing a pregnant female of early life to some environmental components (heavy metal and stress) may contribute to development of schizophrenia in adult offspring. We constructed an animal model of schizophrenia, which was induced by two environmental factor, low level of lead (lead acetate, 0.2% in drinking water) during pregnancy and lactation periods and a repeated-variable stress paradigm during the last week of gestation. Subsequently, our study aimed to investigate whether prenatally-stressed with lead (PNS-L) could affect behavioral impairment and expression level of protein associated with neurodevelopment. Behavioral and proteomic analyses were conducted in PNS-L adult offspring and controls. Many parameters changed in the offspring rats with environmental changes compared to those in non-exposed offspring in open-field, social interaction, and prepulse inhibition tests. Changes in the proteome of a brain tissue were detected by two-dimensional electrophoresis, and the 13 proteins exhibiting differential expression were identified by matrix-assisted laser desorption/ionizationetime-of-flight mass spectrometry. Down-regulation of two of these proteins, Pebp1 (phosphatidylethanolamine binding protein 1) and Prdx2 (peroxiredoxin 2), in PNS-L rat was confirmed by western blotting analyses. Our results suggest that Pebp1 and Prdx2 might be associated with environmental changes of early life and, thus, might be involved in the pathophysiology of schizophrenia.

Disclosures: H. Lee: None. **H. Kim:** None. **J. Kwon:** None. **H. Kim:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.06/CC39

Topic: H.03. Schizophrenia

Support: Division of Intramural Research Program, NIMH
University of Maryland Biophysics Graduate Program

Title: Neuronal avalanches in a developmental mouse model for schizophrenia

Authors: *S. R. MILLER[1,2], S. SENGUPTA[2], D. PLENZ[2];
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Abstract: The healthy neural state in mammalian cortex is characterized by scale-invariant dynamics balanced between excitatory (glutamatergic) and inhibitory (GABAergic) contributions to cortical activity. Recent research has pointed to NMDA and GABAergic signaling dysfunction as causes of positive and negative symptoms in schizophrenia. However, the specific population-level manifestations of pathological cortical activity patterns are unknown. A common method of eliciting schizophrenia-like symptoms in rodents, perinatal exposure to the NMDA receptor antagonist phencyclidine (PCP), has revealed altered neuronal avalanche characteristics in adolescent rats. We hypothesize that changes in neural ensemble dynamics in excitatory and inhibitory cell populations in cortex may explain behavioral deficits in PCP-treated mice. By leveraging transgenic mouse lines and two-photon calcium imaging, we confirm our previous results in rats (Seshadri et al., 2018) which show power laws that fulfill scaling criteria in both normal and PCP treated groups.

Disclosures: S.R. Miller: None. **S. Sengupta:** None. **D. Plenz:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.07/CC40

Topic: H.03. Schizophrenia

Support: NHMRC Project Grant APP1109283

Title: Impact of maternal immune activation and adolescent cannabinoid exposure on auditory mismatch responses in awake, freely-moving rats

Authors: A. DUNN[1], J. JALEWA[1], J. TODD[1], D. HODGSON[1], P. T. MICHIE[1], ***L. R. HARMS**[2];
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Abstract: Mismatch Negativity (MMN) is an auditory change detection response that is consistently found to be reduced in schizophrenia. This MMN impairment correlates with functional impairment in diagnosed individuals and is related to the cognitive impairments observed in the disorder, also of marked functional significance. For the past 10 years, strong evidence has emerged than mismatch responses, similar to human MMN, can be observed in rodents, particularly in rats. In this study, we aim to use mismatch responses as an outcome measure to examine a "two-hit" animal model of schizophrenia. We examined repetition suppression and prediction error, two elements of the mismatch response, believed to reflect simple neuronal adaptation processes and more complex predictive coding, respectively, in male and female rats exposed to maternal immune activation (MIA; 5mg/kg Poly I:C at gestational day 19), adolescent cannabinoid exposure (ACE; 0.075-0.1mg/kg/day HU-210 from postnatal day 35-48), neither exposure or both exposures. We also investigated whether these rats exhibited expected human-like attenuation and facilitation of the mismatch response in response to changing contingencies, such as increased difference between the expected and surprising auditory stimuli, and reduced probability of the surprising stimulus. Rats exposed to the two schizophrenia risk factors exhibited schizophrenia-like reductions in the mismatch response relative to controls and those exposed to either factor alone, which were due to reduced repetition suppression. All rats exhibited expected changes in mismatch response amplitude in line with shifts in experimental contingencies. These data indicate that rodent measures of MMN in general, and mismatch responses in our "two-hit" animal model, specifically, may provide a good platform for future preclinical drug development studies.

Disclosures: L.R. Harms: None. **A. Dunn:** None. **J. Jalewa:** None. **J. Todd:** None. **D. Hodgson:** None. **P.T. Michie:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.08/CC41

Topic: H.03. Schizophrenia

Support: Western BrainSCAN

Title: Blocking PirB to grow new dendritic spines in the prefrontal cortex; a potential novel treatment for the cognitive deficits in schizophrenia

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[1]Neurosci., [3]Anat. and Cell Biol., [2]Univ. of Western Ontario, London, ON, Canada; [4]Anat. & Cell Biol., Univ. Western Ontario, London, ON, Canada

Abstract: Schizophrenia is a psychotic disorder consisting of positive, negative and cognitive symptoms. While the positive and negative symptoms of schizophrenia have relatively high treatment effectiveness, the cognitive deficits remain largely untreated, causing difficulties for many schizophrenia patients. The quantity of spines present within a set area along a dendrite (spine density) in the prefrontal cortex (PFC) is thought to underlie the complex neuronal structure of cognition. A marked reduction of dendritic spines on layer II/III pyramidal neurons in the PFC is a consistent pathological indicator of schizophrenia. Due to the role of the PFC in executive function, this loss of dendritic spines in schizophrenia may be associated with the well-established decline in cognition.
Studies have identified a role of a receptor called PirB in inhibiting spine growth in adulthood. PirB has been manipulated in a variety of animal models both by genetic deletion and by pharmacological intervention. Blocking the activity of PirB in the visual cortex past the critical period of cortical plasticity by introducing a PirB ectodomain has been shown to increase dendritic spine density and functional recovery from long-term monocular deprivation. Furthermore, blocking PirB in aged mice has shown to increase cognitive and motor functions typically associated with aging. Taken together, these results provide exciting insight into the ability to re-open the critical period of cortical plasticity by blocking PirB in adulthood.
In this study, PirB will be blocked in the PFC of an adult rodent model of schizophrenia with the aim of recovering dendritic spine density and in turn cognitive function. All experiments will be conducted on 4 groups of animals. Two groups of animals will possess the schizophrenia symptoms while the other two groups will receive not. One group of animals from each of these two groups will receive infusion of a PirB ectodomain with a TAT sequence (for cellular penetration) while the other group from each of the surgery conditions will receive a control infusion. If the predicted results are obtained, this research will provide a next step towards developing a successful treatment for the cognitive deficits of schizophrenia.

Disclosures: H. MacNeil: None. **W. Inoue:** None. **B.L. Allman:** None. **R. Rajakumar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder.

Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.09/CC42

Topic: H.03. Schizophrenia

Support: NICHD Intramural Support to CJM, TJP, RD; NINDS support to MD

Title: Developmental NMDA receptor ablation in a subset of interneurons confers circuit-wide aberrant gene expression and schizophrenia-like impairments

Authors: *V. MAHADEVAN[1], R. CHITTAJALLU[1], A. PELTEKIAN[1], K. A. PELKEY[1], C. ESNAULT[2], Y. ZHANG[3], M. DRAGAN[4], X. YUAN[1], S. HUNT[1], D. ABEBE[1], R. DALE[2], T. J. PETROS[3], C. J. MCBAIN[1];
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Abstract: Medial ganglionic eminence (MGE)-derived forebrain GABAergic interneurons comprise several classes of interneurons namely, the parvalbumin, somatostatin, and neurogliaform / ivy cells that critically regulate cortical circuit maturation and refinement. NMDA-receptor (NMDAR) complexes within MGE interneurons mediate their development, physiology, and recruitment during network activity and plasticity. NMDARs provide a crucial source of calcium entry into neurons, and their signaling is a dominant convergence point for cell-intrinsic excitation-transcription (E-T) coupling. However, a comprehensive understanding of NMDAR-mediated E-T coupling in distinct interneuron classes is currently lacking, although interneuronal NMDAR-hypofunction is well known to underlie network and cognitive abnormalities in neurodevelopmental disorders, notably schizophrenia (SCZ). Here, we conditionally ablated the requisite GRIN1 subunit of NMDARs in the MGE-derived interneurons throughout development and examined its impact on cell-type specific gene expression, and behavioral abnormalities. Combined single-cell RNA sequencing and MGE-specific translating RNA sequencing analyses revealed a dramatic reduction in the expression of the neuron-specific inducible-transcription factor NPAS4 and its target BTG2 in the GRIN1-ablated cortical interneurons. Moreover, we observed a robust increase in the expressions of synapse regulators NTNG1, NORBIN, and KCNH2 in GRIN1-ablated hippocampal interneurons. Surprisingly, we observed non- cell-autonomous gene expression changes in pyramidal neurons and non-neurons, indirectly as a result of GRIN1-ablated MGE-interneuron impairment. In particular, we observed a robust increase in the pyramidal-neuron expressed immediate early genes, NPTX2, BDNF, and ARC, indicating aberrant pyramidal neuron hyperactivity; and a marked decrease in the microglia-expressed CX3CR1, IGF2. Importantly, several aberrantly expressed genes we identified are established human schizophrenia risk genes. Moreover, these animals exhibit novelty-induced hyperlocomotion, social anxiety and motor abnormalities reminiscent of SCZ-like impairments. Together, our study establishes that developmental NMDAR dysregulation initiated in a subset of GABAergic interneurons promotes circuit-wide gene expression abnormalities resembling those associated with SCZ etiology.

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Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.10/CC43

Topic: H.03. Schizophrenia

Support: NSERC Discovery Grant

Title: Role of delayed maturation of $GABA_A$ receptor function in schizophrenia

Authors: *G. LEE[1], N. RAJAKUMAR[2], W. INOUE[3];
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Abstract: Unlike positive and negative symptoms of schizophrenia, cognitive abnormalities of this disorder are manifested very early and are present in the prodromal period in preadolescent children. An imbalance in excitatory/inhibitory transmission in the prefrontal cortex (PFC) unrelated to synaptic pruning may underlie this preadolescent manifestation of cognitive deficits. In the normal brain, GABA binding to $GABA_A$ receptors cause depolarization during early development and then becomes hyperpolarizing owing to the changes in KCC2 and NKCC1 expression and function. Interestingly, premature ablation of the subplate (SP) layer of the developing visual cortex resulted in delayed upregulation of KCC2 causing a continued depolarizing effect of GABA in the cerebral cortex. Our laboratory described that neonatal ablation of the SP layer of the PFC results in multiple behavioral and neuropathological abnormalities of schizophrenia. In order to study the effects of a delayed GABA functional switch on cognitive function and PFC pathology of schizophrenia, we sought to ablate the SP layer of the developing PFC in postnatal day 1-old mice. Lesioned and sham-operated pups were sacrificed at different time points between P2 and P14, and coronal sections through the PFC were processed for immunohistochemical labeling of GAD67, VGluT2, KCC2, NKCC1, and $GABA_A$ receptor subunits. Immunohistochemical findings will be correlated to future patch-clamp electrophysiological investigations.

Disclosures: G. Lee: None. **N. Rajakumar:** None. **W. Inoue:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

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Topic: H.03. Schizophrenia

Support: DFG grant SFB 936 project B4 to D.K.
Federal State of Hamburg grant "Molekulare Mechanismen der Netzwerkmodifizierung" to D.K. and O.O.
Schaller-Nikolich Foundation grant to D.K. and O.O.
DFG grant KN556/11-2 (FOR 2419) to M. K.

Title: Consequences of Arc/Arg3.1 deletion during development on schizophrenia-endophenotypes in the adult mouse

Authors: *X. GAO[1], J. GRENDEL[1], M. MUHIA[2], S. CASTRO-GOMEZ[1], D. ISBRANDT[3], M. KNEUSSEL[2], D. KUHL[1], O. OHANA[1];
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Abstract: Arc/Arg3.1, an activity regulated immediate early gene, is essential for learning and memory and for consolidation of synaptic plasticity. Expression of Arc/Arg3.1 during early postnatal development is essential for proper development of spatial cognition and hippocampal activity, whereas aberrant Arc/Arg3.1 expression had been linked to neurodevelopmental disorders such as autism and Fragile X syndrome. Recent studies on schizophrenia patients implicated Arc/Arg3.1 as a molecular hub in synaptic gene-networks whose abnormalities possibly play a significant role in the pathogenesis of schizophrenia. However, whether Arc/Arg3.1 deletion or misregulation by itself can lead to schizophrenia-like phenotypes remains questionable. To address this question, we generated constitutive and conditional Arc/Arg3.1 KO mice in which the Arc/Arg3.1 gene was deleted at different time points of development. We used behavioral, biochemical and *in vivo* electrophysiological methods to investigate the presence of schizophrenia endophenotypes in adult mice. Our data shows that loss of Arc/Arg3.1 during early development does not cause schizophrenia-like behavior, but can alter network activity in schizophrenia-relevant brain regions. Our results differ from previous reports and highlight a novel mechanism of Arc/Arg3.1 in neurodevelopmental disease.

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Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.12/CC45

Topic: H.03. Schizophrenia

Support: NIEHS grant ES006189-24

Title: Chronic early-life lead exposure disrupts behavior and neuronal systems relevant to mental disorders and substance abuse

Authors: *D. ALBORES-GARCIA[1], J. L. MCGLOTHAN DZIEDZIC[1], D. R. BROOKS[1], K. H. STANSFIELD[2], T. R. GUILARTE[1];
[1]Envrn. Hlth. Sci., Florida Intl. Univ., Miami, FL; [2]Envrn. Hlth. Sci., Columbia Univ., New York, NY

Abstract: Environmental factors have been associated with psychiatric disorders and recent epidemiological studies suggest an association between prenatal lead (Pb^{2+}) exposure and schizophrenia (SZ). Preclinical studies show that developmental Pb^{2+} exposure recapitulates specific neuropathological and dopaminergic system changes present in SZ. Given the high comorbidity of substance abuse in SZ, we hypothesized that early life Pb^{2+}-exposure could sensitize neuronal systems relevant to both conditions. Male and female rats were developmentally exposed to Pb^{2+} using a validated experimental paradigm. This exposure paradigm results in blood lead levels averaging 0.6 ± 0.1 (control) or 22 ± 0.07 μg/dL (Pb^{2+}). Using this model, we investigated sensorimotor gating using pre-pulse inhibition of the acoustic startle (PPI) in early adolescence (postnatal day 28, PN28), late adolescence (PN50) or at adulthood (PN120). Chronic Pb^{2+} exposure did not have an effect in PN28 male rats, however, PPI deficits were present in PN50 male rats and were more pronounced in male adult rats (PN120), consistent with the age trajectory of PPI deficits in SZ. We found no effect of Pb^{2+} exposure on PPI in female rats at any age. To study sensitivity to psychostimulants, rats were administered saline, 5 or 15 mg/kg of cocaine-HCl before being placed in activity chambers. Cocaine locomotor activation was 78% higher in PN28 male Pb^{2+} rats ($p=0.027$) and 55% higher in PN28 female Pb^{2+} rats compared to controls ($p=0.008$). In PN50 rats, only Pb^{2+} males had increased cocaine-induced locomotor activity ($p=0.007$). These effects were abrogated by a D1-dopamine receptor (D1R) antagonist. We then measured D1R and mu opioid receptor (MOR) levels in relevant brain regions using autoradiography. In PN28 Pb^{2+} male rats, we found an increase of D1R in the striatum (STR), nucleus accumbens (NAC) and olfactory tubercle (OT) of rats relative to controls, the same in PN28 females, except for NAC. In PN50 Pb^{2+} male, but not female rats, there was an increase in D1R levels in OT only. MOR levels were increased in the STR, NAC, basolateral amygdala and several areas in the thalamus of male and female PN28

Pb^{2+} rats. Ongoing studies are determining MOR levels in PN 50 and PN120 animals and cocaine-induced locomotor activity and D1R at PN120. These studies show that chronic early life Pb^{2+}-exposure results in PPI inhibition, an endophenotype for SZ and produces changes in the dopaminergic and opioid systems that could sensitize and predispose the animal to substance abuse in an age and sex-dependent fashion. Our findings suggest the role of environmental factors, such as Pb^{2+}, as a risk factor for mental disorders and substance abuse.

Disclosures: D. Albores-Garcia: None. **J.L. McGlothan Dziedzic:** None. **D.R. Brooks:** None. **K.H. Stansfield:** None. **T.R. Guilarte:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.13/CC46

Topic: H.03. Schizophrenia

Support: NIH Grant R01 MH110681
Dr. Jim Woodgett

Title: Glycogen synthase kinase 3 (GSK3) inhibition alleviates deficits in *in vivo* spike synchrony, gamma oscillation and cognitive impairment in a schizophrenia mouse model

Authors: *K. NAKAO[1,2], R. HUNTER[1], K. NAKAZAWA[1,2];
[1]Southern Res., Birmingham, AL; [2]Dept. of Psychiatry and Behavioral Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Auditory steady-state responses (ASSRs), tone-evoked EEG oscillations at 40-Hz, is known to be compromised in patients with schizophrenia, although its underlying mechanism and functional outcome is poorly understood. Forty-Hz click train-evoked LFP (local field potential) activity in primary auditory (A1) cortex is also defective in a schizophrenia mutant model (Nakao and Nakazawa, 2014), in which Grin1 gene encoding the indispensable NMDA receptor subunit is deleted in ~50% off cortical and hippocampal GABA neurons in early postnatal development (Ppp1r2cre/Grin1 KO mice). Furthermore, we recently found that immunoreactivity (IR) against phospho-GSK3 (at Y216 in GSK3β), an auto-activated form of GSK3α/β, is augmented in the PV neurons (presumably Grin1-deleted), but not pyramidal neurons, of the mutant mPFC. The IR of GSK3β protein, but not GSK3α, in the PV neurons was also higher in Grin1 mutants. To assess the impact of predictive GSK3β over-activity on the *in vivo* action potential (AP) spike synchrony of cortical pyramidal neurons and *in vivo* tone-evoked LFP gamma oscillation, we took a pharmacological and genetic approach. First, we found that systemic pretreatment of TDZD-8 (nonselective GSK3 inhibitor, 2.5 mg/kg, IP) alleviates in vivo AP spike synchrony deficits (21 pairs, $p = 0.0004$, paired student's t-test) and diminished

tone-evoked gamma oscillations in the Grin1 mutant mice (6 channels, p=0.018). To determine which isoform of GSK3, GSK3α or GSK3β, elicits the impairment via over-activity, we used the GSK3β-paralog selective inhibitor, BRD3731 (30 mg/kg IP). BRD3731 alleviated the defective gamma oscillation in the Grin1 mutant mice (6 channels, p=0.009). To address whether GSK3β-specific inhibition in GABA neurons is sufficient to rescue the mutants' gamma oscillation deficits, we crossed a floxed-GSK3β strain (kindly provided by Dr. J. Woodgett) to the Ppp1r2cre/Grin1 KO mutant mice to generate the GABA neuron-selective GSK3β haploinsufficiency. Preliminary data showed that GSK3β haploinsufficiency reverses the *in vivo* AP spike synchrony deficits (P = 0.001, Grin1 KO (24 pairs) vs GSK3β haploinsufficiency (31 pairs), student's t-test). Finally, we assessed whether GSK3 inhibition restored the cognitive dysfunction in the Grin1 mutant mice. TDZD-8 restored the spontaneous alternation in Spatial Y-maze (n=6, $p < 0.05$) and paired-pulse inhibition (PPI) of the Grin1 mutant mice (n=12, p=0.05, two-way ANOVA). These results suggest GSK3β inhibition acting on cortical GABA neurons may ameliorate the cognitive dysfunction in schizophrenia presumably by the restoration of gamma synchronous activity.

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Poster

428. Animal Models of Risk Factors for Schizophrenia

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.14/CC47

Topic: H.03. Schizophrenia

Support: NIMHANS

Title: Neonatal ventral hippocampal lesions causes deficits in value-based decision making in adult rats

Authors: *N. PREM[1], L. T. RAO[1], J. P. JOHN[2], B. M. KUTTY[1];
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[2]Dept. of Psychiatry, Natl. Inst. of Mental Hlth. & Neurosciences, Bangalore, India

Abstract: Cognitive deficits observed in schizophrenia patients have been recognized as the core feature of the disorder. Impairments in executive functions such as decision making is one of the cognitive domains that is affected. The neonatal ventral hippocampal lesion (NVHL) rodent model is a well-established neurodevelopmental model of schizophrenia. In the present study, we investigated the performance of NVHL animals in value-based decision-making task. Adult NVHL rats were subjected to test battery that consisted of the open field test (OFT), the direct interaction test (DIT) and the three-chambered social interaction test (SIT). NVHL animals exhibited hyperactivity in OFT, reduced social interaction and aggressive behavior in DIT and

impairment in social memory and deficits in preference for novel experiences when compared to control animals in SIT. Further, all animals were subsequently subjected to value-based decision-making task in the operant chamber using light cues. NVHL animals demonstrated impaired cue discrimination as they made more response error demonstrating inability to acquire stimulus-response associations. When subjected to unequal reward outcomes, NVHL animals developed preference for big reward more slowly. Cognitive flexibility was assessed by reversing the task rules and NVHL animals exhibited impairment in reversal learning as they were unable to track changes and made more incorrect responses when compared to control animals. Neonatal ventral hippocampal lesion leads to the disruption of normal development of the hippocampus and its efferents such as the prefrontal cortex. Therefore, the findings observed in this study encompass the notion that the cognitive deficits observed may be due to the compromised anatomical and functional integrity of the prefrontal cortex as a consequence of early developmental insult to ventral hippocampus.

Disclosures: **N. Prem:** None. **L.T. Rao:** None. **J.P. John:** None. **B.M. Kutty:** None.

Poster

428. Animal Models of Risk Factors for Schizophrenia

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 428.15/CC48

Topic: H.03. Schizophrenia

Support: BrainsCAN Accelerator Grant, Canada First Research Excellence Fund (CFREF)

Title: Interleukin-15/natural killer cell deficiency alters the effect of poly IC maternal immune activation on the offspring

Authors: *F. HADDAD[1], S. PATEL[2], C. DE OLIVEIRA[3], K. WIECZERZAK[1], B. ALLMAN[3], S. RENAUD[3], S. SCHMID[3];
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Abstract: Maternal infection and its associated immune response during pregnancy are known risk factors for neurodevelopmental disorders such as autism spectrum disorder (ASD) and schizophrenia in the offspring. In rodents, maternal immune activation (MIA) by pathogen-free immune stimulants in pregnant mothers produces brain and behavioral deficits in the offspring. The contribution of different maternal immune cells to these deficits is still unclear. Natural Killer (NK) cells are innate immune cells whose maturation is dependent on Interleukin-15 (IL-15) and are involved in the response to infections, and previous studies have shown changes in peripheral NK cell phenotype in individuals with ASD and schizophrenia. Distinct uterine NK populations are also involved in establishing fetal blood supply and may be able to modulate the

immune signals that reach the fetus, and hence modulate the impact of MIA. We induced MIA using the viral mimic polyinosinic: polycytidylic (poly I:C) at gestation day 9.5 in homozygously bred wild type (+/+) or IL-15 knockout (-/-) rats and tested offspring in adolescence and adulthood. Our results indicate that IL-15 deficiency reduces offspring hearing threshold and signal latency in the auditory brainstem response and increases startle reactivity in adolescence regardless of exposure to MIA. On the other hand, poly I:C MIA increased male offspring body weight regardless of age or genotype and reduced open field exploration in both +/+ and -/- offspring. Moreover, poly I:C differentially affected offspring prepulse inhibition (PPI), startle reactivity and open field exploration in an age and genotype-dependent manner. For example, poly I:C MIA reduced baseline startle reactivity in adult +/+ but not -/- offspring. In contrast, poly I:C MIA reduced PPI in adolescent -/- but not +/+ offspring. Experiments in progress will use immunohistochemistry to search for molecular changes associated with the observed deficits. Our results point towards a role for IL15/NK cells in development of the auditory brainstem response and modulating MIA's effects on brain development. Further work will attempt to elucidate the contribution of fetal and uterine NK populations in this model.

Disclosures: **F. Haddad:** None. **S. Patel:** None. **C. De Oliveira:** None. **K. Wieczerzak:** None. **B. Allman:** None. **S. Renaud:** None. **S. Schmid:** None.

Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.01/CC49

Topic: I.03. Anatomical Methods

Support: The Beijing Municipal Science & Technology Commission (Z181100001318002)
The National Basic Research Program of China (973 Program; grant 2015CB856402)
The General Program Natural Science Foundation of China (project 31671118)
The General Program Natural Science Foundation of China (project 31371442)
NIH brain initiative grant NS103558

Title: Development of genetically encoded multiplex barcodes for cell labeling under electron microscopy

Authors: *R. SUN[1,2], Y. YANG[1,2], Y. HU[1,2], Z. ZHANG[1,2], Y. LI[1,2,3,4];
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Abstract: The human brain consists of billions of neurons, with one neuron forming more than 1000 synaptic connections with others. One crucial step to understand brain function is to construct neuronal connectomics at the synaptic resolution with cell-type specificity. Electron microscopy (EM), because of its unique nanometer resolution and excellent structural preservation, provides a powerful tool to build 3D reconstruction for neuronal connection network with increasing scale. However, conventional EM alone renders only gray scale images, lacking critical cell-type information most of the time. Genetically-encoded EM tags, such as APEX and miniSOG, could mark a few cell types a time, constraining our understanding of rules that may govern information flow from distinct cell-types. Here, we develop a series of new genetically-encoded EM tags by combining proteins that target distinct organelles with APEX2, which together creates different patterns under EM. We also exploit APEX with different activity or different copy number, to achieve different strength of electron density. Lastly, we also develop methods to control the sparseness of organelle tags. Importantly, these three labeling strategies are orthogonal to each other, thereby offering opportunities to combine them for generating combinatorial EM barcodes to uniquely label distinct cell types. We have validate the applicability of such multiplex EM tags in cultured cells and plan to apply them in more intact nervous systems, such as in flies or in mouse brains.

Disclosures: **R. Sun:** None. **Y. Yang:** None. **Y. Hu:** None. **Z. Zhang:** None. **Y. Li:** None.

Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.02/CC50

Topic: I.03. Anatomical Methods

Support: Microscopy Innovations, LLC

Title: Rapid automated serial block face SEM preparation of brain tissue

Authors: *S. L. GOODMAN[1,2], J. M. CAMPBELL[3], P. H. SMITH[3], E. K. BENSON[4], G. J. KIDD[4];
[1]Neurolog. Surgery & Pathobiological Sci., Univ. of Wisconsin, Madison, WI; [2]Microscopy Innovations, LLC, Marshfield, WI; [3]Dept. of Neurosci., Madison, WI; [4]Renovo Neural, Inc., Cleveland, OH

Abstract: Serial block-face electron microscopy (SBEM) provides powerful 3D insight into neurological structures for mapping and quantification. While image acquisition is now computer-controlled, SBEM sample preparation typically requires tedious circa week-long manual exchanges of toxic reagents.
We report herein an SBEM protocol for neurological tissue where all reagent exchanges are

automatically performed in one day using the programable mPrep ASP-1000 Automated Specimen Processor (ASP). The ASP enables high-speed preparation of biological tissues with rapid and repeated fluid exchanges that accelerate reagent diffusion into specimens, as shown for transmission electron microscopy (TEM) [1]. For SBEM, a circa 4-day manual preparative protocol [2] was reduced to 7.5 hours of automated processing, followed by overnight resin curing.
Adult rats were perfusion-fixed with cacodylate-buffered glutaraldehyde-paraformaldehyde. Cortex and corpus callosum specimens (1-3 mm) were oriented in mPrep/s capsules, loaded onto the ASP, and all preparative reagents were aliquoted into microwell plates on the ASP stage. The ASP executed the protocol by aspirating successive reagents into each capsule for the programmed time with agitation provided by gentle flow reversals every few seconds. Epoxy infiltrated specimens in the capsules were then removed from the ASP and cured overnight at 60C. An FEI Teneo VolumeScope imaged blocks at 2.0 kV, 0.1 nA under high vacuum with the T1 BSE detector. Imaged volumes were ~60 x 60 um by 20 um from 70 nm sections.
Specimens prepared with the 7.5 hour ASP protocol (plus resin curing) and with the 4-day manual protocol were compared from the same animal: Even myelin staining in both indicated good reagent penetration since lipid-rich myelin membranes are a dense target for metal staining and a barrier to diffusion. Synaptic vesicle clarity and discrimination of mitochondrial cristae against the dark mitochondrial matrix were also comparable. Neither ASP or manually-prepared specimens exhibited deficits characteristic of poor staining, dehydration, or infiltration. The reproducibility of automation, programmable control to optimize protocols for different tissue samples, and the potential inclusion of immuno-labeling, as demonstrated for TEM [3], are all benefits of programmable automated processing. Finally, since the ASP reduces reagent handling, and as it vents into or is contained in a fume hood, experimenter exposure to noxious reagents may also be reduced.
[1] Strader TE, et al, Microsc. Microanal. 24 (2018) 1284. [2] Deerinck, TJ, et al, Microscopy, (2010), 6. [2] Marques P, et al, Microsc. Microanal. 24 (2018) 1300.

Disclosures: **S.L. Goodman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Microscopy Innovations, LLC. **J.M. Campbell:** None. **P.H. Smith:** None. **E.K. Benson:** None. **G.J. Kidd:** None.

Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.03/CC51

Topic: I.03. Anatomical Methods

Support: ERC: European Research Council

Title: Real consequences of improved histocompatibility: A TEM study of flexible organics vs standard rigid implants in mice and the effect on seizure onset

Authors: *S. SAFIEDDINE[1], E. RUSINA[1], R. POULKOURAS[1], F. MISSEY[1], B. BOTZANOWSKI[1], E. ACERBO[1], A. SLEIZA[1], M. DONAHUE[2], M. CARRÈRE[1], A. WILLIAMSON[1];
[1]Inst. de Neurosciences des Systèmes, Marseille, France; [2]Dept. of Bioelectronics, Ecole Nationale Supérieure des Mines, Gardanne, France

Abstract: Stimulating and recording the electrical activity of neurons *in vivo* are critical functions in contemporary biomedical research and in the treatment of patients with neurological disorders such as Epilepsy, Alzheimer and Parkinson Disease. The current widely used stimulation implants are inorganic and tend to exhibit shorter effective lifespans. This is due to the degradation of the signal transmission resulting from the tissue response at the electrode-brain interface, and the tissue damage from the limited charge storage capacity (CSC) of the electrodes due to the generation of Reactive Oxygen Species (ROS). To overcome these limitations, new electrodes are designed to minimize tissue response and to increase the charge storage capacity. Conducting polymers such as PEDOT:PSS (poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) allow a high CSC, avoiding the use of high voltages that could cause tissue damage. However, *in vivo* studies using such new materials for implants have been conducted on a limited sample size. The main focus was not on the histocompatibility of the devices tested, but on the aspect of the material. Here, we present the first detailed histocompatibility study of flexible organic implants and demonstrate the effect of implant type on the threshold for seizure onset. We report it on the cellular level and more particularly the mitochondrial size and number in the cells surrounding the inorganic rigid and organic flexible electrode. These were done in the hippocampus of mouse-model for one, four, seven or fifteen days using Transmission Electron Microscopy. At the same time, we focus on the evolution of the response of microglia, astrocytes and oligodentrocytes to the implants. In this poster we show that mitochondrial fission and number in the cells surrounding the flexible organic electrode are lower than the ones in a standard rigid electrode. Our results show negligible immune reaction of the flexible implants compared to standard rigid stimulators where the threshold for seizure onset is dramatically reduced.

Disclosures: S. Safieddine: None. **E. Rusina:** None. **R. Poulkouras:** None. **F. Missey:** None. **B. Botzanowski:** None. **E. Acerbo:** None. **A. Sleiza:** None. **M. Donahue:** None. **M. Carrère:** None. **A. Williamson:** None.

Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.04/CC52

Topic: I.03. Anatomical Methods

Title: Whole brain staining for electron microscopy

Authors: G. WILDENBERG[1], ***A. M. SOROKINA**[2], N. B. KASTHURI[3];
[1]Univ. of Chicago/Argonne Natl. Lab., Chicago, IL; [3]Neurobio., [2]Univ. of Chicago, Chicago, IL

Abstract: The ultimate realization of connectomics is to map the wiring of a whole brain but we lack reliable and scalable methods for staining whole brains for electron microscopy. We and others have found previously published methods for staining whole mouse brains to be difficult to reproduce prompting us to develop an alternative protocol. Here we describe a perfusion-based method for preparing whole brains for electron microscopy where, following aldehyde fixation, osmium tetroxide is transcardially delivered to the whole brain. We compare different osmium staining methods and show reduced osmium provides complete staining and ultrastructure preservation. We show that post-staining ultrathin sections with uranyl acetate and lead nitrate provide sufficient contrast from such perfusion approaches for algorithmic automatic segmentation. This method solves a major bottleneck in preparing whole brains for serial electron microscopy, staining entire brains, paving the way for large volume serial electron microscopy.

Disclosures: G. Wildenberg: None. **A.M. Sorokina:** None. **N.B. Kasthuri:** None.

Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.05/CC53

Topic: I.03. Anatomical Methods

Support: Department of Education P382A150041

Title: Towards a partial connectome of hair and skin

Authors: *E. A. OLAPO[1], N. B. KASTHURI[3], J. AUSTIN, II[4], A. MASELLI[2];
[2]Dept. of Biol. Sci., [1]Chicago State Univ., Chicago, IL; [3]Dept. of Neurobio., [4]Advanced Electron Microscopy Facility, Univ. of Chicago, Chicago, IL

Abstract: The skin is the body’s largest sensory organ receiving tactile stimuli and transmitting pain. Despite it's obvious importance in normal brain functioning and its role in human pathology, little is known about how nerves innervate the skin. A three dimensional nanoscale model of skin and its innervation would significantly advance our understanding. We report here efforts to use automated serial electron microscopy -connectomics- using the ATUM (Automated Tape Collecting Ultramicrotome) to build a three dimensional model of the sensory apparatus

surrounding a hair follicle in mouse skin. We are optimizing sample preparation for electron microscopy including fixation with aldehydes, heavy metal staining, and plastic embedding tailored for nanoscale reconstructions of skin and hair. Initial results suggest that skin samples can be prepared with sufficient signal for large volume serial electron microscopy and long runs of serial sections can be collected with the ATUM. We will use our connectomic reconstructions of wild type skin from the mouse to compare across skin regions without hair follicles, across species, and in pathology.

Disclosures: **E.A. Olapo:** None. **N.B. Kasthuri:** None. **J. Austin:** None. **A. Maselli:** None.

Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.06/CC54

Topic: I.03. Anatomical Methods

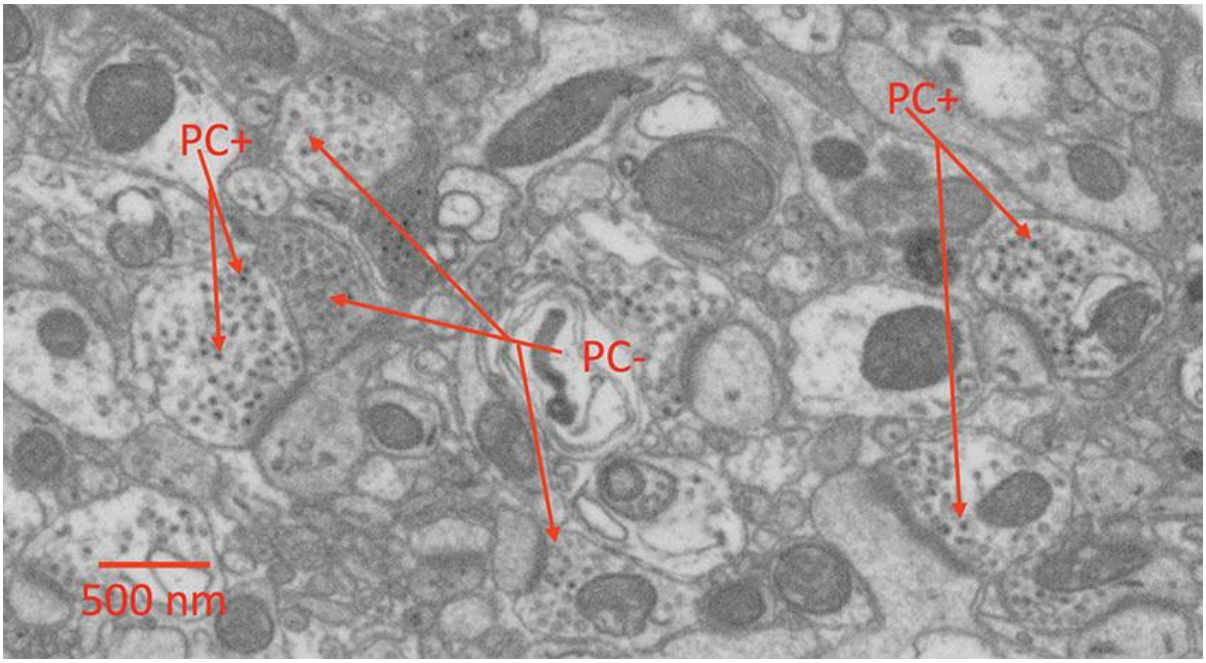
Support: Wellcome Trust PRF 201225
ERC AdG 695709

Title: A quantitative ultrastructural readout of *in vivo* presynaptic activity for connectomics

Authors: *A. SIMON[1], A. ROTH[1], M. FISEK[1], V. MARRA[2], C. RACCA[3], K. STARAS[4], M. HAUSSER[1];
[1]Univ. Col. London, London, United Kingdom; [2]Univ. of Leicester, Leicester, United Kingdom; [3]Newcastle Univ., Newcastle-Upon-Tyne, United Kingdom; [4]Univ. of Sussex, Brighton, United Kingdom

Abstract: The patterns of synaptic input received by cortical neurons during behavior are not known. Ideally, they should be measured in the context of the wiring diagram of the circuit. How to quantitatively assess synaptic weights and synaptic activity is also an open problem for current connectomics approaches. Here we describe a strategy for measuring presynaptic activity and release probability during circuit function along with circuit connectivity at nanoscale resolution. The method relies on FM 1-43FX labelling and dye photoconversion as a marker of functionally recycled vesicles *in vivo*, permitting active terminals to be visualized using electron microscopy-based connectomics approaches.
FM 1-43FX was injected via a patch pipette into L2/3 of V1 in awake head-fixed mice running on treadmill. The FM bolus was monitored by 2-photon imaging while mice were shown a visual stimulus for 10 min. After transcardial perfusion fixation, the brain was postfixed overnight. Vibratome sections containing the dye-loaded presynaptic boutons were photoconverted and processed for EM. FIBSEM was chosen to acquire high-resolution 3D data (6.2 x 6.2 x 9.3 nm^3 voxels), allowing each synaptic vesicle to appear on 5-6 consecutive images. Two distinct

vesicle populations were found in presynaptic terminals (Fig. 1): i) dye-loaded, photoconverted (PC+) active vesicles with a dark lumen, ii) unloaded (PC-) vesicles with a clear lumen. Voxel classification using ilastik readily distinguished the two vesicle types.
A large majority of excitatory and inhibitory boutons contained PC+ vesicles, suggesting that most were active during visual stimulation. In active boutons, the fraction of vesicles classified as PC+ varied widely, indicating broad distributions of activity levels and presynaptic release probabilities. In summary, our method provides a powerful readout of presynaptic activity with ultrastructural context, enabling the organization of active synapses *in vivo* to be revealed for the first time. We anticipate our approach will provide a valuable new strategy for functional connectomics.



Disclosures: **A. Simon:** None. **A. Roth:** None. **M. Fisek:** None. **V. Marra:** None. **C. Racca:** None. **K. Staras:** None. **M. Hausser:** None.

Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.07/CC55

Topic: I.03. Anatomical Methods

Support: NIDDK P30DK042086

Title: The influence of the microbiome on the 'connectome' of the enteric nervous system

Authors: V. SAMPATHKUMAR[1], ***M. E. FRITH**[2,3], A. SHAHBAZI[5], N. PATEL[4], V. LEONE[3], E. B. CHANG[3], N. B. KASTHURI[1];
[1]Neurobio., [2]Interdisciplinary Scientist Training Program, [3]Med., [4]Univ. of Chicago, Chicago, IL; [5]Natl. Inst. of Mental Hlth. (NIMH), Natl. Inst. of Hlth. (NIH), Gaithersburg, MD

Abstract: The enteric nervous system (ENS) is a semi-autonomous collection of neurons and enteric glial cells critical for gastrointestinal function. Despite its importance, little is known about the circuitry of the ENS and how that circuitry is shaped by the nearby microbiome. In order to address this gap, we have developed a multi-scale, multi-modal imaging approach that combines synchrotron source x-ray microscopy (μXCT; 1-micron resolution over cm^3), automated large volume serial electron microscopy using the ATUM (EM; 10 nm resolution over mm^3), stimulated emission depletion (STED) and standard confocal microscopy, to map differences in the cellular, vascular, and the connectivity of the enteric neurons between conventionally raised (specific pathogen-free, SPF) and germ-free (GF) wildtype mice. We report that ENS neurons in both SPF and GF mice have less elaborated dendritic arbors compared to CNS neurons and receive approximately 40 inputs per cell, distributed over the soma and dendrites. ENS neurons in a specific ganglion receive the majority of their connections from neurons in other ganglia, and the same axons make multiple connections with single post-synaptic targets. In GF animals, we find large scale structural differences including altered composition of the gut wall musculature, decreased branching of the vasculature, and potential reductions in the number of synapses per enteric neuron. We report, for the first time, the basic principles of connectivity in the ENS and how connectivity changes in the absence of the microbiome.

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Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.08/CC56

Topic: I.03. Anatomical Methods

Title: Segmentation of murine cervical vagus nerve fibers from transmission electron microscopy images using domain randomization and deep convolutional neural networks

Authors: *V. TÓTH, T. TSAAVA, E. H. CHANG, A. BORCA-TASCIUC, T. P. ZANOS; Inst. of Bioelectronic Med., Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Selective modulation specific fibers within peripheral nerves is a major goal of emerging bioelectronic medicine efforts. To achieve this goal, we need to understand the axon

distributions and features of the nerves that we record and stimulate. The vagus nerve (VN) is a primary interface target various bioelectronic medicine devices, however, the fiber composition within this nerve is not well understood. In transmission electron microscopy (TEM) images of this nerve, unmyelinated C-fibers that carry nociceptive signals are challenging to quantify due to their small diameter and lack of a myelin sheath. Manual segmentation labeling of C-fibers on TEM images is time-consuming while training state-of-the-art deep learning methods to segment and subsequently count the fibers demands a substantial amount of labeled images. One way to address these issues is to generate synthetic pre-labeled images that resemble real TEM images. These images can be subsequently used to train deep neural networks to segment and count fibers in TEM images. To this end, ultrathin cross-sections of the mouse cervical VN were prepared following glutaraldehyde-paraformaldehyde fixation, 1% osmium tetroxide, and uranyl acetate staining. Samples were acquired on a transmission electron microscope with the Gatan UltraScan 4000 imaging system. TEM images were acquired at 1200X magnification to assemble the real dataset. To further enrich the data beyond image augmentations of rotation and stretching, we applied domain randomization by automatically generating labeled image sets of neural fibers using a 2D physics engine. By assembling axons in physics simulations, we spawned diverse, morphed forms of both myelinated and unmyelinated fibers resembling the ones on the TEM images. The simulation engine provided exact labels of axons, myelin sheath and background pixels for the acquired dataset. A deep convolutional neural network was first trained to segment the automatically generated, randomized electron microscope images, then the same network was fine-tuned using labeled real images retrieved from the VN of mice. A control convolutional network was only trained on the real images. The model pre-trained on the synthetic images significantly outperformed the control neural network, demonstrating the benefit of using domain randomization for axon segmentation. Our developed methodology can be translated to fiber counting of nerves other than the cervical VN, where the distribution, size, and form of myelinated and unmyelinated fibers are different.

Disclosures: **V. Tóth:** None. **T. Tsaava:** None. **E.H. Chang:** None. **T.P. Zanos:** None.

Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.09/CC57

Topic: I.03. Anatomical Methods

Support: NIH R21NS085320
NIH RF1MH114047
Bertarelli Program in Translational Neuroscience and Neuroengineering
Edward R. and Anne G. Lefler Center
Stanley and Theodora Feldberg Fund

IARPA DoI/IBC D16PC00004
NIH T32MH20017

Title: Reconstruction of motor control circuits in adult *Drosophila* with automated transmission electron microscopy

Authors: *D. G. C. HILDEBRAND[1], J. T. MANIATES-SELVIN[1], B. J. GRAHAM[1], A. T. KUAN[1], L. A. THOMAS[1], B. L. SHANNY[1], W.-C. A. LEE[1,2];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]F.M. Kirby Neurobio. Ctr., Boston Children's Hosp., Boston, MA

Abstract: Animal behavior is ultimately controlled by populations of motor neurons integrating diverse synaptic inputs in the nervous system and innervating muscles in the periphery. Understanding networks and computations that orchestrate movement has been challenging due to the inaccessibility and complexity of the underlying circuitry. Here, we present reconstructions of the neuronal circuits supporting motor control in an adult female *Drosophila* ventral nerve cord (VNC) using a tape-based, reel-to-reel, large-scale electron microscopy (EM) pipeline. This method, called GridTape, combines automated serial sectioning with automated, high-throughput transmission EM to provide nanometer-resolution imaging with consistent sample collection, which accelerates imaging and analysis throughput for a fraction of the cost of alternative approaches. The resulting dataset affords the opportunity for comprehensive, unbiased mapping of circuits that coordinate walking and flight. To examine how the nervous system controls locomotor behavior, we reconstructed all the motor neurons innervating the front legs. To facilitate combined structural, functional, and genetic analyses, we registered the EM dataset with a light microscopy-based reference VNC atlas. Our analysis demonstrates functionally specific sensory feedback onto motor neurons and considerable bilateral symmetry. We provide open-access to the dataset, reconstructions, and GridTape instrumentation designs to fuel further discovery of network motifs supporting motor codes and to make EM-based connectomics more broadly accessible.

Disclosures: D.G.C. Hildebrand: None. **J.T. Maniates-Selvin:** None. **B.J. Graham:** None. **A.T. Kuan:** None. **L.A. Thomas:** None. **B.L. Shanny:** None. **W.A. Lee:** None.

Poster

429. Anatomic Methods: Electron Microscopy

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 429.10/CC58

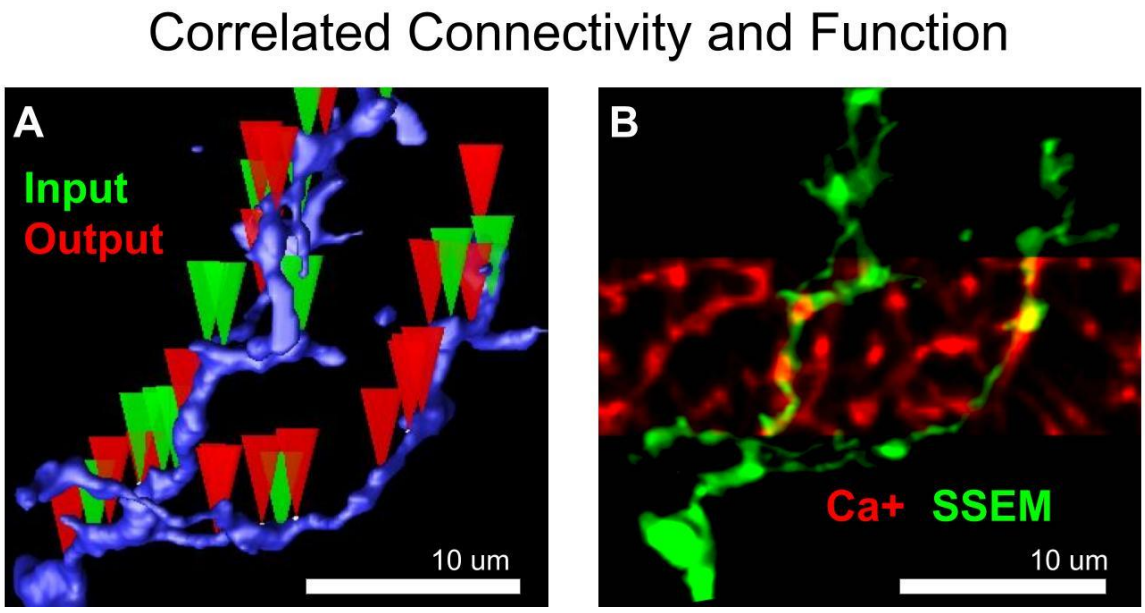
Topic: I.03. Anatomical Methods

Support: Research to Prevent Blindness Career Development Award

Title: Combined light and electron microscopy characterize microcircuits in an excitatory retinal amacrine cell

Authors: *K. FRIEDRICHSEN, J.-C. HSIANG, D. KERSCHENSTEINER, J. L. MORGAN; Ophthalmology & Visual Sci., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: The Vesicular Glutamate 3-Expressing Amacrine Cell (VG3-AC) is a retinal amacrine cell which participates in multiple retinal circuits simultaneously. It receives input from both the ON and OFF visual pathways and provides ON, OFF, or ON/OFF output to multiple cell types using either excitatory (glutamatergic) or inhibitory (glycinergic) synapses. Calcium imaging has shown that light responses in the VG3-AC arbor are heterogenous, with some areas exhibiting only ON or OFF responses and others a combination. Because input and output synapses are interspersed throughout the arbor, the response heterogeneities may represent many distinct input/output pathways within a single neuron.How the synaptic connectivity and morphology of the arbor selectively mix and segregate synaptic inputs and direct them to appropriate outputs is unclear. We are combining calcium imaging with serial section scanning electron microscopy (SS-SEM) to reconstruct the connectome and ultrastructure of a functionally characterized VG3-AC, allowing us to better understand how synaptic inputs are combined or segregated to produce observed light responses and how this information is targeted to appropriate downstream neurons.We use calcium imaging to capture the responses to light stimuli of an excised live mouse retina expressing GcAMP-6F in VG3-ACs in a Cre-dependent manner. We then use an Automated Tape-Collecting Ultramicrotome (ATUM) to collect thousands of serial sections representing the full thickness of the retina. These are imaged at multiple resolutions, aligned, and annotated to reconstruct the structure and connectome (fig 1A) of the same VG3-ACs characterized by calcium imaging. Using this method, we can directly correlate the structure and connectivity of an arbor region with its functional responses to light stimuli (fig 1B).With this combined data set, we have begun identifying synaptic motifs in the connectivity of VG3-AC segments and correlating these microcircuit domains with their functional response patterns.



Disclosures: **K. Friedrichsen:** None. **J. Hsiang:** None. **D. Kerschensteiner:** None. **J.L. Morgan:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.01/CC59

Topic: I.04. Physiological Methods

Support: University of Wisconsin-Whitewater Undergraduate Research Grant Program

Title: Building and testing a system for neural ensemble recording from a subcortical bundled electrode

Authors: C. AYON-GARCIA, J. HARRINGTON, N. KUHLMANN, M. TCHERNOOKOV, ***M. A. WARACZYNSKI**, O. YAVUZCETIN, D. ZAMZOW;
Univ. of Wisconsin Whitewater, Whitewater, WI

Abstract: Neural ensemble recording affords a wealth of data that relate the activity of cell groups to behavioral phenomena observed in conscious, unrestrained animals. However, relatively little of this type of research has involved recording from neural groups located substantially below the cortex, given the difficulty of accurately implanting fine wire electrodes at depth. Here we present our work on developing a system for doing so by bundling electrodes and implanting them within a confining guide cannula. Bundling the electrodes presents an additional challenge: The KlustaKwick open source spike sorting software requires that the user know the bundle's adjacency graph, i.e., the position of each electrode tip relative to all others in the bundle. The user must also determine the amplifier channel input to which each electrode is connected. This adjacency information is lost as the wire bundle is twisted, a step necessary to give the bundle sufficient resilience to survive the implant process intact. We present novel software for determining that adjacency information based on delivering a known AC signal to each electrode in turn, and recording the received signal at each other electrode. These recordings allow us to generate a matrix of Fourier components corresponding to the frequency of the supplied signal. Based on the relative size of these components the software then iteratively assigns neighbors to the stimulated electrodes, producing a binary adjacency matrix. We present proof of concept data obtained by creating an adjacency graph for an electrode bundle that is not twisted, i.e., with known adjacency. We also present data testing the validity of our adjacency determination software, obtained by delivering known, artificial neural-like signals to an electrode bundle and comparing KlustaKwik's outputs to these known inputs. These data indicate that our system validly collects neural ensemble activity data.

Disclosures: **M.A. Waraczynski:** None. **C. Ayon-Garcia:** None. **J. Harrington:** None. **N. Kuhlmann:** None. **M. Tchernookov:** None. **O. Yavuzcetin:** None. **D. Zamzow:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.02/CC60

Topic: I.04. Physiological Methods

Support: University of Michigan MCubed Project ID 8488
University of Michigan Neuroscience Scholars

Title: Dura penetration, deep insertion, and electrophysiological recording with cellular-scale microelectrodes using 3D-printed custom skull cap

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Abstract: Electrophysiological methods are a mature methodology for recording brain dynamics at millisecond timescales in either local or large spatial scales. Microelectrode arrays (MEAs) are a well-established tool for chronic recording of electrophysiological signals and have the advantage of minimal brain damage if constructed from microelectrodes with cellular-scale diameters (25um or less). However, further development of large cellular-scale MEAs is necessary for wide use by neuroscientists, especially for deep insertion cases. Efforts to reduce the size of microelectrodes and make them more flexible bring collateral difficulties due to buckling during penetration through membranes (dura/pia) and consequent inability to implant deeply into the brain. In this study, we investigated the insertion force of cellular-scale microelectrodes through pia and dura maters using a newly-developed cantilever-beam based force measurement system with flexible range (mN level) and resolution (sub-μN level). We also developed a custom skull cap with precision guide holes to stabilize the brain and membranes, provide sufficient support to microelectrode along the insertion path, and minimize the unsupported length of microelectrodes during dura/pia penetration and deeper insertion. Insertion force measurements found that both diameter reduction and tip sharpening reduce the rupture force during membrane penetration. Due to the decreasing unsupported length of the microelectrode and slow increase of friction force, buckling is less likely after membrane rupture for cellular-scale microelectrodes. Insertion tests through the skull cap showed that a 25 μm diameter tungsten microwire penetrated through the dura mater and was inserted over 10 mm into the brain without buckling and could be used for electrophysiological recordings. In comparison, without the cap or precision guide, insertion of the same microwire caused over 2

mm dimpling of the dura without penetration and finally led to electrode buckling. This study showed that with proper supporting mechanism above the membrane, it is feasible to insert cellular-scale microelectrodes deep into the brain through the dura mater without buckling. We are conducting in-vivo brain recording tests in freely behaving rats with cellular-scale microelectrodes through the skull cap to verify the feasibility and durability for both acute and chronic electrophysiological recordings.

Disclosures: L. Chen: None. **J. Hartner:** None. **T. Dong:** None. **P.R. Patel:** None. **J. Richie:** None. **A. Shih:** None. **C.A. Chestek:** None. **B.O. Watson:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.03/CC61

Topic: I.04. Physiological Methods

Support: Shaw Scientist Grant
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Title: MRI compatible, 3D printed microdrive for chronic or acute neurophysiological recordings

Authors: R. SWADER[1], E. BAEG[2], H. LEE[3], A. ROSENBERG[4], ***B. KIM**[4];
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Abstract: We present an MRI compatible 3D printed microdrive for conducting chronic or acute neurophysiological recordings. Testing results are presented using recordings from macaque monkeys. Major innovations 1. Construction of a microdrive with high resolution 3D printing. A microdrive requires miniature moving parts to carry various types of electrodes with micrometer accuracy. Cutting-edge 3D printing enables the printing of materials with such high resolution. Moreover, with 3D printing, the construction and modification of microdrive components are highly convenient and more cost effective than with conventional machining. 2. Novel and innovative design of a recording grid system that enables micro-drilling for introducing electrodes into the brain that eliminates the need for large craniotomies and debridement of dura matter. 3. Full MRI compatibility. All parts of the microdrive are constructed from MRI compatible plastics, except for the electrode actuating threads which are made from MRI compatible brass. The actuating threads are distal from the brain and introduce negligible MRI distortion. Since no craniotomy is performed, the system does not create an air-filled space on top of the dura that can introduce imaging artifacts. 4. Versatile microdrive for chronic or acute

neurophysiological recordings. Since all of the moving parts and recording grid are enclosed in the microdrive body, the system is naturally isolated from the external environment. The system further allows electrodes to be individually replaced without reinstalling the microdrive body. This design makes it suitable for both long-term chronic recordings as well as daily acute recordings with minimal maintenance.

Disclosures: **R. Swader:** None. **E. Baeg:** None. **H. Lee:** None. **A. Rosenberg:** None. **B. kim:** None.

Poster

430. Techniques: Microelectrodes I

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Topic: I.04. Physiological Methods

Support: 5R01MH105397-03
AWS preseed fund

Title: An open-source, interactive neuronavigation system for planning, simulating and performing surgeries

Authors: *S. SCHAFFELHOFER[1,2], W. ZARCO[1], R. PRÜCKL[2], W. FREIWALD[1];
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Abstract: Accessing cortical and subcortical areas accurately and safely is crucial for most disciplines in neuroscience and medicine. Traditional navigation methods rely on operations in stereotaxic coordinates for which mechanical frames and arms are positioned to reach targets in the brain. The manual adjustment of these parts can cause cumulative positioning errors and lack in error-detection. Here, we report a low-cost neuronavigation system (NNS), which measures the position of surgical instruments directly via infrared cameras and transforms and visualizes their location into the medical images of a surgical subject at run-time and sub-voxel resolution. Preoperative, the introduced NNS can merge the medical scans of a subject, such as CT, MRI, and fMRI. Based on scan intensities, 3D iso-surfaces of multiple tissues, such as cortex, blood-vessels, and skull can be computed and merged in a multimodal image. Additionally, CAD files can be imported and merged with the biological structures. This way, implants, such as electrodes can be set virtually to optimize their position relative to the biological target and to define a surgical plan.
Intraoperative, the NNS connects to infrared cameras (NaturalPoint) to detect the position and orientation of surgical instruments via triangulation (6 degrees of freedom). At the beginning of a procedure, surgeons use a pointer to measure the location of multiple fiducial markers on the

subject's head in order to register them to their corresponding location in the medical images (point-set registration algorithm). These transforms are acquired once and can be afterwards accessed to transfer the instruments into the medical imaging space at sampling rates >60Hz. Thus, surgeons can see their instruments relative to the subject's anatomy on a computer screen to follow the preoperatively defined plan.
Our NNS was evaluated in-vitro (fiducial point detection on 3D printed head) and in-vivo (recording chamber in macaques) and reached positioning accuracies with errors of 430±202 µm (mean±sd, n=72) and 348µm respectively (mri resolutions =0.5mm isotropic). Additionally, six microelectrode arrays (Microprobes) were implanted successfully at sub-millimeter precision and provided functional spiking activity.
Together, our navigation platform leads to safe, fast and accurate interventions. The prospects in many other scenarios are significant not only for research, but also for eventual clinical translation. The software is provided open-source under the MIT-license: http://www.cortEXplore.com/Software/NeuronavigationSfN2019.zip

Disclosures: **S. Schaffelhofer:** A. Employment/Salary (full or part-time):; cortEXplore GmbH. **W. Zarco:** None. **R. Prückl:** A. Employment/Salary (full or part-time):; cortEXplore GmbH. **W. Freiwald:** None.

Poster

430. Techniques: Microelectrodes I

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.05/CC63

Topic: I.04. Physiological Methods

Title: Accelerated aging test of organic and inorganic packaging materials for implantable neural interface devices

Authors: *Y. GONG, W. YANG, W. LI, Q. FAN;
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Abstract: Implantable neural interface devices have been widely used in neuroscience and clinical research to understand brain functions. Many implantable devices target long-term (>1 year) recording period in the host body. During chronic applications, the stability of an insulation layer is a critical factor to ensure the reliable performance of implantable neural devices. Typically an insulation layer is utilized to isolate unwanted noise, prevent cross-talking, protect electronics and metallic elements, as well as provide biocompatibility. Although Parylene-C has been widely used as a substrate and insulation material for many FDA-approved implantable devices, pinholes in Parylene-C thin films greatly limit their barrier performance. Inorganic thin films enable hermetic packaging performance but they are more mechanically rigid than polymers. To facilitate the design and development of packaging materials for neural implants,

we studied and compared the barrier performance of four different organic and inorganic thin films: Parylene-C, SiO_2, Si_3N_4, Al_2O_3. To prepare samples, tungsten microwires were coated with single- or multi-layer thin films of different materials under various thicknesses. The coating at the tip of the wire was precisely removed to expose electrode sites. The samples were aged in three solutions: phosphate-buffered saline (PBS), 30 mM and 150 mM H_2O_2 mixed with PBS, to mimic acute post-surgery inflammatory reaction. To accelerate the aging test, the solutions were heated at high temperatures of up to 60 °C. Electrochemical impedance spectroscopy (EIS) was used to monitor the changes in impedance over the course of soak testing, as an indication of degradation of the insulation coating. Mean-time-to-failure (MTTF) at body temperature of 37 °C was extrapolated using $Age_{37℃} = (Age_{60℃})*Q^{|Taa-Trt|/10}$, where Q_{10} is a 10 ℃ increase rate of the chemical reaction, T_{AA} is accelerated aging temperature and T_{RT} is recommended shelf temperature-body temperature. Scanning electron microscope (SEM) was used to identify signs of physical damages of the insulation coating and tungsten electrodes.

Disclosures: Y. Gong: None. **W. Yang:** None. **W. Li:** None. **Q. Fan:** None.

Poster

430. Techniques: Microelectrodes I

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.06/CC64

Topic: I.04. Physiological Methods

Support: St. Olaf College Psychology Innovation Fund

Title: Chronic *in-vivo* recording of rodent neural systems utilizing an updated affordable and open-sourced microdrive

Authors: A. RAMACHANDRAN, A. ALAVI, A. EL BANNA, ***G. M. MUIR**;
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Abstract: Among the many challenges of chronically recording neuronal activity from multiple cells is the cost of purchasing or producing drives that fit the functional and spatial requirements needed for individual recordings. These challenges are even greater when employing such technologies in the instruction of undergraduates or during research at under-funded institutions. We have created an updated customizable microdrive array that allows for the independent driving of 8 tetrodes (32 electrodes) with a drive body that is entirely 3D-printable using a standard 3D printer. This is assembled using cheap and open source materials, and it is compatible with the open-ephys system. The quick turn-around time and cheap cost of our microdrive allows for basic in-vivo electrophysiology to be feasible for undergraduate or teaching laboratories.

Disclosures: **A. Ramachandran:** None. **A. Alavi:** None. **A. El Banna:** None. **G.M. Muir:** None.

Poster

430. Techniques: Microelectrodes I

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Program #/Poster #: 430.07/CC65

Topic: I.04. Physiological Methods

Support: NSF INSPIRE CBET-1343193
NIH U01 NS099703-01

Title: Developing a 3D parylene-based multi-electrode array for large-scale recordings from the rat brain

Authors: *W. JIANG, H. XU, X. WANG, E. MENG, D. SONG;
USC, Los Angeles, CA

Abstract: Large-scale monitoring of resolvable neuronal activities from multiple brain regions of behaving animals provides valuable insights into complex brain mechanism at the cellular level. However, current state-of-the-art probes such as microwire or silicon electrodes are orders of magnitude stiffer than brain tissue, which largely increases the chance of inflammation and damage caused by chemical and mechanical interactions, and therefore makes difficult to consistently record high-quality signals over long period of time. Due to its biocompatibility and flexibility, parylene C is a promising alternative structural and insulation material for building neural probes with reduced mechanical mismatch between tissue and implants. In our previous work, a 64-channel parylene-based multi-electrode array with 8 shanks was developed and applied to chronic recording of multiple hippocampal sub-regions in behaving rats. In our current work, a novel 3D parylene-based probe consisting of 24 (3x8) shanks with a 250um spacing was developed. Each shank contains 8 platinum electrodes and thereby realizes a total of 192 electrodes for large-scale recordings of multiple brain regions in rat. To aid insertion, dissolvable polyethylene glycol (PEG) brace was employed to temporarily support the flexible probe, shorten the effective length of the shanks, and increase the critical buckling force. The PEG block is designed in a wedged shape to avoid unbalanced dissolving rates on the two sides. In the preliminary studies, after craniotomy and removal of dura mater and blood vessels, a sham 3D parylene array was successfully implanted to the full depth of 5.14mm in vivo. The depth of implantation was confirmed with micro-CT imaging. This result demonstrated the feasibility of implanting 3D flexible polymer probe to deep brain structures. In future, corresponding packages and data transmission system for functional 3D parylene array will be developed; the large-scale, high-density 3D parylene probe will be tested both acutely and chronically for recording from multiple brain regions in behaving rats.

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Poster

430. Techniques: Microelectrodes I

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Topic: I.04. Physiological Methods

Support: MNDRIVE RSAM
UMN MRSEC Grant DMR-1420013
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NSF NNCI

Title: Transparent inkjet-printed ECoG electrode arrays integrated into polymer skulls for simultaneous whole cortex electro- and opto- physiological monitoring

Authors: ***P. DONALDSON**[1], Z. S. NAVABI[2], M. RYNES[2], L. GHANBARI[2], S. B. KODANDARAMAIAH[2,3], S. L. SWISHER[1];
[1]Electrical and Computer Engin., [2]Mechanical Engin., [3]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Considering the separate, yet complementary origins of electrophysiological and optophysiological (e.g. fluorescence from genetically encoded calcium indicators) recording techniques, a powerful approach to studying cortical dynamics would be to utilize a platform that combines high spatial resolution optical imaging with high temporal resolution electrical recording across a large swathe of the cortex. Recently, we have developed transparent polymer skulls consisting of a flexible and highly transparent PET film bonded into a 3D-printed frame. Here we demonstrate the integration of transparent and reconfigurable electrocorticography (ECoG) electrode arrays into these transparent skulls by patterning a conductive and transparent polymer (PEDOT:PSS) directly onto the PET film using an LP50 inkjet materials printer (Meyer-Burger Technologies). Outside of the imaging region, PEDOT:PSS traces connect to inkjet-printed silver traces that route signals to printed bond pads. The electrode arrays are encapsulated in the transparent insulating polymer Parylene, with openings over the electrodes and bond pads. The bond pads are bonded to a conventional PCB to interface the array with external electronics. Finally, the electrode arrays are bonded to 3D-printed frames and implanted, along with a titanium headplate which provides support for the PCB and locations to head-fix the mouse for imaging experiments. Printed PEDOT:PSS features show negligible optical absorption (less than 3% throughout the visible and NIR wavelengths). PEDOT:PSS electrodes with 300 μm diameters exhibit channel impedances of less than 10 kΩ at 100 Hz for up to 3 weeks in 37°C PBS. ECoG-integrated transparent skulls have been chronically implanted on C57/BL6 mice for over 6 weeks. Impedances of functional channels were found to be 203 ± 176 kΩ at 100 Hz for

the 6 week period. We are currently refining the implant design and performing experiments to measure stimulus evoked electrode potentials, and compatibility with epifluorescence and 2-Photon imaging of calcium activity. We believe this platform will enable novel neuroscience studies that can utilize the complementary benefits of electrical and optical neural sensing modalities to unravel the intricacies of cortical neurophysiology.

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Poster

430. Techniques: Microelectrodes I

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Topic: I.04. Physiological Methods

Support: TÜBİTAK grant no: 117F481 under European Union's FLAG-ERA JTC 2107 project GRAFIN
H2020 Graphene Flagship Core 2

Title: Functional characterization of graphene microelectrodes on rat sensorimotor cortex

Authors: *F. T. DUVAN[1], D. VIANA[2], S. WALSTON[2], J. A. GARRIDO[2], B. GÜÇLÜ[1]; [1]Inst. of Biomed. Engin., Boğaziçi Univ., İstanbul, Turkey; [2]Catalan Inst. of Nanoscience and Nanotechnology – ICN2, Barcelona, Spain

Abstract: Neuroprostheses based on cortical implants are promising to provide partial sensorimotor function in severe neurological conditions such as spinal cord injuries and amyotrophic lateral sclerosis. One of the key components of these systems is the microelectrode array, which is used for recording brain activity to control a robotic limb and/or for stimulation to induce somatosensory feedback. Due to its unique electrical, mechanical, and biochemical properties, graphene exhibits a great potential as electrode material. We tested 64-channel flexible surface neural probes (site diameter: 25 μm) based on reduced graphene oxide thin film technology for functional characterization on rat sensorimotor cortex (SI). Evoked local field potentials were recorded epidurally at the hindpaw representation of SI in anesthetized Wistar albino rats. The vibrotactile stimuli were bursts of sinusoidal (5, 40, and 250 Hz) displacements (duration: 0.5 s, amplitude range: 19-270 μm) applied on the glabrous skin. Consistent with our previous results from platinum surface electrodes, robust evoked potentials could be observed shortly after the onset of contralateral stimuli. At low stimulus intensity, the evoked potential mostly lasted about 0.1-0.2 s, but frequency-following entrainment could be obtained for 5 Hz at higher intensities. Additionally, the stimulus onset caused large coherence changes among the channels. Ipsilateral vibrotactile stimuli usually did not produce evoked potentials, but they

sometimes changed the ECoG rhythm especially in the gamma and higher (30-150 Hz) bands due to interhemispheric networks. At different filter settings, small-medium amplitude (<80 μV) multi-unit spike activity, and sometimes single-unit spikes could be recorded. On the other hand, electrical stimulation (10 biphasic current pulses, 0.25 ms phase duration at 100 Hz) of the cortical surface resulted in twitch responses mostly at neck, face, and trunk muscles only at high current thresholds (~0.7 mA) above material and Shannon limits. Although generating a motor output at the lower leg seems to be not feasible by surface stimulation under anesthesia, graphene electrodes with small sites may be used to induce artificial sensation for somatosensory feedback during chronic experiments, in which much smaller currents (<0.1 mA) are applied.

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Poster

430. Techniques: Microelectrodes I

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Topic: I.04. Physiological Methods

Support: BMBF Grant FKZ: 01ZX1503

Title: Simultaneous *in vivo* measurement of neural activity, extracellular glutamate and GABA concentrations using an MRI-compatible implantable microelectrode array

Authors: E. MITRICHEVA, R. KIMURA, L. PASCUAL MANSILLA, E. KRAMPE, A. OELTERMANN, N. K. LOGOTHETIS, ***H. R. NOORI**;
Max Planck Inst. For Biol. Cybernetics, Tuebingen, Germany

Abstract: Glutamate and γ-aminobutyric acid (GABA) are the most common neurotransmitters in the central nervous system, activating both ionotropic and metabotropic (modulatory) receptors. By exciting, inhibiting, and even modulating neural elements and microcircuits, these chemicals critically regulate brain information processing and energy metabolism at different spatiotemporal scales. Although a great deal of work has been done at the biophysical and cellular level, the exact relationship between the extracellular concentration of these molecules and emergence of specific patterns in neuronal ensemble activity remains elusive. Partly this is due to the fact that recording of the mean extracellular field potentials (mEFP) concurrently with a quantitative assessment of alterations in the concentration of such neurochemicals are currently unavailable. Here, we present a silicon-based implantable ultrafine microelectrode array (35 μm diameter and 50 μm thickness) composed of several iridium-stabilized electrochemical and electrophysiological contacts. The distance between each electrode channel is 250 μm. The electrophysiological electrodes have an average impedance of 0.5 MΩ at 1 kHz. The

amperometric electrochemical channels are divided into two groups of glutamate- and GABA-responsive electrodes with the former showing a sensitivity of 0.39 nA μM^{-1} for glutamate, while the adjacent channel has a sensitivity of 0.38 nA μM^{-1} for GABA. Both have a detection limit of 0.2 µM. This novel multimodal microelectrode was used to simultaneously monitor extracellular glutamate and GABA concentrations, spikes, multi-unit neuronal activity (MUA) and local field potentials (LFP) in the lateral geniculate nucleus (LGN) of anaesthetized, adult Wistar rats (n=5). Retinal stimulation with flickering monochromatic light, emphasizing the simplest form of feedforward processing in thalamus, induced neuronal response patterns in LGN that were highly correlated with the temporal alterations in glutamate concentrations. GABA responses, while similar in profile to MUA and LFP recordings, were found to be event-selective and uncorrelated with the overall range of neuronal activity, suggesting the involvement of network processes that require further investigation. Our findings suggest that this multimodal method may greatly contribute into our understanding of microcircuit organization, by reducing the inherent ambiguity in the mEFP through neurotransmitter-release-tracking. Understanding microcircuits and their interactions is the only hope to develop neural networks models that my underly the brain's function and dysfunction.

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Poster

430. Techniques: Microelectrodes I

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Topic: I.04. Physiological Methods

Support: This work was supported by BrainLinks-BrainTools, Cluster of Excellence funded by the German Research Foundation (DFG, Grant number EXC 1086)

Title: A multisite active neuro-technology array for high density recordings and stimulation

Authors: M. VOMERO[1,2], C. BOEHLER[1,2], R. LILJEMALM[1], T. STIEGLITZ[1,2], ***M. ASPLUND**[1,2];
[1]Dept. of Microsystems Engin., [2]BrainLinks-BrainTools, Univ. of Freiburg, Freiburg, Germany

Abstract: In the field of neurotechnology, there has been a general trend towards miniaturization of implantable probes. Smaller probes allow for better integration with the surrounding tissue and ultimately promote improved longevity for chronic applications. Given the large amount of neurons in the brain (~86 billion), it lies close at hand that selective communication with individual neurons demands smaller electrode contacts for adequately resolving single unit activity. While miniaturization for stiff silicon probes has substantially advanced, there is still a

lack of flexible probes providing stimulation and recording at high density. We here present a Multisite Active Neuro-Technology Array (MANTArray) with 32 recording/stimulation sites at a pitch of 24 µm on polyimide (PI) to advance this trend also to polymer-based neural probes. The MANTArrays were fabricated with standard cleanroom lithographic technologies in a design employing multiple metal layers to increase packing density of recording and/or stimulating sites at a minimal device footprint: they are, in fact, only 78 µm wide and 12 µm thick. Connection tracks were embedded below the electrode sites and spread over four metal planes, separated by PI layers. The electrode contacts were coated with Iridium Oxide (IrOx), nanostructured platinum (nanoPt) or PEDOT/PSS to ensure low impedance for the microsized electrode contacts ($15x15\mu m^2$) and efficient charge injection. An impedance of $45.06 \pm 1.66k\Omega$ (at 1kHz) could be reached for the $15x15\mu m^2$ sites, suggesting excellent recording and stimulation properties. With the presented arrays, it is now possible to place all microelectrodes within e.g. the same layer of one cortical column and allow for neural recordings at exceptional spatial resolution. A large number of densely spaced single neurons can be followed with the MANTArrays even over long implantation times. The implementation of three-dimensional electrode materials, such as nanoPt and PEDOT/PSS, in addition, promotes high signal-to-noise recordings and makes MANTArrays suitable also for microstimulation.

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Poster

430. Techniques: Microelectrodes I

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Program #/Poster #: 430.12/CC70

Topic: I.04. Physiological Methods

Support: NIH Grant 1U01NS094190-01

Title: Dense cortical and sub-cortical recording of brain activity in different animal models using SiNAPS active probes

Authors: ***F. BOI**[1], A. LECOMTE[1], A. CASILE[2], G. MANDELBAUM[5], B. ZAAIMI[6], N. PERENTOS[8], G. SCHWESIG[8], J. ASSAD[5,3], B. L. SABATINI[9], A. M. SIROTA[10], A. JACKSON[7], G. N. ANGOTZI[3], L. BERDONDINI[4];
[1]NBT, Fondazione Inst. Italiano di Tecnologia, Genova, Italy; [2]Fondazione Inst. Italiano di Tecnologia, Ferrara, Italy; [4]Neurosci. and Brain Technologies, [3]Fondazione Inst. Italiano di Tecnologia, Genova, Italy; [5]neurobiology, Harvard Med. Sch., Boston, MA; [6]Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [7]Newcastle Univ., Newcastle-upon-Tyne, United Kingdom; [8]Ludwig-Maximilians-Universität München, Munich, Germany; [9]Neurobio., Harvard

Med. Sch. Dept. of Neurobio., Boston, MA; [10]Ludwig-Maximilians Univ. München, Planegg-Martinsried, Germany

Abstract: Simultaneous Neural recording Active Pixel Sensor technology (SiNAPS) is a modular circuit solution (Angotzi et al., 2019) to devise implantable active multielectrode array probes for simultaneous recording from hundreds to thousands of densely packed electrodes. Exploiting the modularity of SiNAPS technology, we were able to rapidly design and experimentally validate two different probe configurations, a single-shank and a four-shank probe. The former integrates 512 electrode-pixels (size 21x21µm, pitch 26µm in x direction, 28µm in z direction) arranged in 3 columns along a narrow (120 µm) and elongated (6.2mm) single shank; the latter integrates 1024 electrode-pixels (size 15x15µm, pitch 28µm) arranged on four distinct shanks (80µm wide x 5mm long; incorporating 256 contacts/shank covering 3.6mm; shank separation 560µm). Here we report the recording capabilities of these SiNAPS probes as evaluated *in vivo* in multiple labs and different animal models, including recordings from awake head-fixed mice, anesthetized rats, and anesthetized monkeys. Our SiNAPS probes acquired broad-band neural signals simultaneously from up to 1024 channels, with low noise, 7.5μV_{RMS} and 24.5 μV_{RMS} in the action potential and local field potential (LFP) bands, respectively. Noise levels measured in saline and *in vivo* were comparable, and in line with simulation results. The use of SiNAPS probes in rodents allowed us (1) to map LFPs across all layers of the cortex and down to the deeper hippocampal and thalamic regions (2) to track the propagation of neuronal activations across close-by electrodes and adjacent shanks and (3) to monitor the spiking activity of single units. In the monkey model, single shank probes were able to record the activity of the motor cortex with high spatiotemporal accuracy.

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Poster

430. Techniques: Microelectrodes I

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Topic: I.04. Physiological Methods

Support: INOPRO (BMBF, 16SV7656)

Title: A multi-channel capacitive coupled implantable system to restore natural sensory feedback

Authors: *P. KIELE, C. F. PASLUOSTA, T. STIEGLITZ;
Lab. for Biomed. Microtechnology, IMTEK-Dept Microsystems Eng, Univ. of Freiburg, Freiburg im Breisgau, Germany

Abstract: The loss of a limb severely restrains even simple activities of daily life. Prosthetic devices provide functional replacement of the missing limb but still lack of somatosensory perception, which is essential to restore proper object manipulation, the senses of embodiment and agency. Previous studies have demonstrated that natural sensory restoration is possible by electrically stimulating afferent nerves via implanted intrafascicular electrodes. However, assessing the remaining nerves after a limb amputation is usually difficult. Targeted Muscle Reinnervation (TMR) is a surgical intervention to re-route the remaining nerves from the stump to other part of the human body where they become more accessible. Thus, TMR presents as an excellent opportunity for restoring somatosensory feedback via electrical stimulation since sensory nerves also spread out while reinnervating the host muscles. This is specified as Targeted Sensory Reinnervation (TSR). We propose to take advantage of the natural branching of the reinnervated fibers to stimulate somatosensory pathways via an implanted system. Implanted stimulation electrodes are connected to a transcutaneous wireless coupling unit for the provision of energy and signals. A multichannel capacitive coupling approach is proposed for the transcutaneous coupling unit, consisting of extracorporeal electrodes placed on top of the skin and a subcutaneous counterpart. This approach unifies the beauty of eliminating failure due to complex implanted electronics and permanent skin trauma. Flexible and very thin implanted electrode arrays minimize the invasiveness of such a system and therefore foreign body reactions can be reduced. We evaluated the electrical crosstalk behavior of a ten channel coupling unit in an *ex vivo* study using healthy explanted human skin. Two different transfer strategies with and without direct contact of the metal part of the electrode and the skin tissue were evaluated. While the first provides additional resistive paths through the skin, the latter blocks these with a polymeric insulation layer. To integrate such system with lasting wearable devices (e.g., prostheses' socket), dry and reusable extracorporeal electrode arrays are required. Requirements on the implanted pickup electrodes are long-term stability and biocompatibility, which comprises thin and flexible arrays. The implication on resorting a selective natural sensory feedback with the proposed implantable system are further discussed.

Disclosures: P. Kiele: A. Employment/Salary (full or part-time):; University of Freiburg. **C.F. Pasluosta:** A. Employment/Salary (full or part-time):; University of Freiburg. **T. Stieglitz:** A. Employment/Salary (full or part-time):; University of Freiburg.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.14/CC72

Topic: I.04. Physiological Methods

Title: 3D-printed implants that facilitate chronic recordings and reuse of neuropixels probes in freely behaving rodents

Authors: *F. MICHON, C. AYDIN, R. VAN DAAL, H. DEN BAKKER, J.-J. SUN, S. HAESLER, F. KLOOSTERMAN;
NeuroElectronics Res. Flanders, Leuven, Belgium

Abstract: Studying the relationship between the activity of neuronal populations and behavior is critical to advance our understanding of brain function. Assays in which animals are unrestrained that promote naturalistic behaviors are better suited for the study of cognitive functions such as navigation or social interactions. Extracellular electrophysiological recordings provide the high temporal resolution to monitor single cells spiking activity as well as local field potentials. Recent technological advances have enhanced the sampling capabilities of neural probes. A single Neuropixels probe[1] enables recording from over 380 switchable electrodes distributed along the 10mm long probe shank. It offers the possibility to simultaneously record from several hundred cells from multiple brain regions in awake animals. Neuropixels probes have been successfully used in chronic preparations, with stable recordings over time and high cluster fidelity. However, at present little to no technical support exists to use these devices chronically in unrestrained behaving animals. In particular, current designs for chronic implants lack an efficient mechanism to recover the probes and do not support the integration of multiple probes in a single implant.
We have developed a 3D-printed implants for Neuropixels probes that facilitates the reliable use, and re-use, of probes chronically implanted in freely behaving rodents. The implants meet three key criteria: 1) it is lightweight and can be easily carried by rats and mice, 2) it enables chronic insertion of several Neuropixels probes at flexible coordinates, and 3) the neural probes are easily recovered and reused with high success rate. The 3D-printed implant has been successfully used to record from populations of cells over days in our lab. Overall, the system has the potential to facilitate and broaden the use of Neuropixels probes, and potentially similar devices, in chronic preparations for freely behaving animals.
[1] Jun J.J. 2017 Fully integrated silicon probes for high-density recording of neural activity, Nature 551, 232-236

Disclosures: **F. Michon:** None. **C. Aydin:** None. **R. Van Daal:** A. Employment/Salary (full or part-time):; Atlas Neuroengineering. **H. den Bakker:** None. **J. Sun:** None. **S. Haesler:** None. **F. Kloosterman:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.15/CC73

Topic: I.04. Physiological Methods

Support: NIH R01NS102917
NIH R21NS102964
W81XWH-16-10580
NIH K25HL140153

Title: Continuous long-term tracking of neuronal clusters with ultraflexible oversampling electrode array

Authors: *H. ZHU[1], X. LI[1], Z. ZHAO[1], F. HE[1], X. WEI[1], L. LUAN[1], N. TRAN[2], C. XIE[1];
[1]Biomed. Engin., [2]Mathematics, The Univ. of Texas At Austin, Austin, TX

Abstract: The brain is formed by massively interconnected and constantly evolving networks of neurons that communicate in milliseconds. Therefore, the ability to reliably track gradual changes of local neural circuits with high temporal resolution longitudinally is crucial for the understanding brain functions. Conventional rigid extracellular electrode arrays provide simultaneous large scale neuronal recording. However, their capability to follow the same neurons over extended period of time remain elusive due to the gradual degradation of cell-electrode interface attributable to the chronic neuro-inflammatory response induced by the mechanical mismatch. Additionally, such mechanical mismatch under physiological micro-motions induces relative movements at the biotic-abiotic interface, which makes the same neurons difficult to track. Here we demonstrate semi-supervised mapping and tracking of neuronal ensembles over 30 days with ultraflexible electrode arrays [1] featuring oversampling contact densities. Ultraflexible electrode arrays with oversampling detection range were fabricated using our published method [1]. 32 channel probes were implanted in the mouse fore-limb sensory cortex. After two months of surgery recovery, tethered continuous recording was performed for 32 days. Recordings were segmented into overlapping one hour periods and sorted individually with MountainSort [2]. We sorted an average of 50.4580 ±5.9217 (n=1356) single units per time segment, or 2138.2 ± 150.3 (n=32) when pooled over all sessions per day. Units identified in each segment were compared against those detected on nearby sessions by waveform similarity and linked according to a binary split structured mutual nearest neighbor principle. After this step, units were grouped into 112.1250 ± 11.1782 distinct neurons. Within the same day, support vector machine based supervised tracking further merged the same units, which yielded 71.7813 ± 3.4895 uniquely tracked units per day. Across multiple days, similar semi-automatic procedure yielded 212 identified neurons, which could be tracked for 10.8349

±11.6153 days. Among these tracked neurons, 30 were identifiable every day. They moved, over this 32-day period, 13.8661 ± 10.1473 μm. We finally examined the waveforms of the 212 automatically tracked neurons visually and merged them into 90 final units. With ultraflexible electrode arrays, we demonstrate their capacity to reliably detect the same neurons longitudinally. References: [1] Luan, Lan (L.L.) Sci Adv, 2017, Vol 3, e1601966. [2] Jason E. Chung (J.C.) Neuron, 2017,1381-1394.

Disclosures: H. Zhu: None. **X. Li:** None. **Z. Zhao:** None. **F. He:** None. **X. Wei:** None. **L. Luan:** None. **N. Tran:** None. **C. Xie:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.16/CC74

Topic: I.04. Physiological Methods

Title: Using EEG as a translatable pharmacodynamic biomarker in non-human primates: Test case and reliability

Authors: *P. GARCES[1], M. POULIOT[2], S. AUTHIER[2], J. F. HIPP[1];
[1]Roche Pharma Res. Early Development. Neurosci. and Rare Diseases., Roche Innovation Ctr. Basel, Basel, Switzerland; [2]Citoxlab North America, Laval, QC, Canada

Abstract: Robust and reliable pharmacodynamic (PD) biomarkers are crucial for first in human studies. Modulation in the intensity of brain rhythms as measured with EEG (electroencephalography) can be used for this purpose, as shown in the benzodiazepines literature. However, identifying EEG biomarkers for clinical trials from preclinical experiments is challenging: EEG effects may not be translatable due to differences between species and experimental conditions. Here, we highlight the value of non-human primate EEG to inform clinical studies. We present a test case with diazepam and scopolamine in n=13 freely moving cynomolgus with EEG/EOG/EMG telemetry recordings (L04 transmitters, DSI). We opted for a freely moving telemetry setup rather than a constrained recording (with e.g. primate chair) which could be formally closer to a human resting state paradigm in order to allow for an extended EEG and behavioural evaluation (24h) while ensuring subjects' welfare and minimizing stress and emotional interference. Power spectra and power at classical frequency bands: delta (2-4Hz), theta (4-8Hz), alpha (8-13Hz), low beta (13-20Hz) and high beta (20-30Hz) were estimated with Morlet wavelet transforms for two EEG derivations: fronto-occipital (Fz-Oz) and frontal (Fz-F3). Only data without artifacts (visual and automated scoring) was used. Awake and asleep intervals (expert scoring) were considered separately. The statistical significance of drug effects relative to vehicle treatment was determined with linear mixed effects models. Significant modulations were observed for both drugs: decrease in theta power and increase in beta power with diazepam

(4 mg/kg p.o.) and increase in low frequency power with scopolamine (0.03 mg/kg i.m., all $p<0.001$). These patterns match the outcomes of previous human experiments. Notably, awake and asleep modulations, although qualitatively similar, differed in strength and variability. Additionally, the test-retest reliability of EEG power was estimated from two separate vehicle recordings (test-retest interval: 2-5 weeks) and was found excellent for all frequency bands (intraclass correlation coefficient > 0.9). Overall, the translatability of the EEG PD signatures for diazepam and scopolamine, along with an excellent test-retest reliability support the further use of this NHP EEG design to inform clinical studies

Disclosures: **P. Garces:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **M. Pouliot:** A. Employment/Salary (full or part-time):; Citoxlab North America. **S. Authier:** A. Employment/Salary (full or part-time):; Citoxlab North America. **J.F. Hipp:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.17/CC75

Topic: I.04. Physiological Methods

Support: NINDS 1U01NS094375
NINDS 1UF1NS107659
NSF 1707316
MiBRAIN
NIMH R01MH110932
NIDA R01DA045783
NIDA R01DA039952

Title: Improved carbon fiber electrode array targeting, density, and fabrication throughput for the detection of electrophysiological and dopaminergic activity

Authors: ***P. R. PATEL**[1], B. D. LUMA[1], E. J. WELLE[1], A. VEGA-MEDINA[1], J. RICHIE[1], T. DONG[1], D. EGERT[2], L. CHEN[1], A. J. SHIH[1], J. D. BERKE[2], D. CAI[1], J. B. BECKER[1], C. A. CHESTEK[1];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: Our group has developed carbon fiber electrode arrays that can record both electrophysiological and dopaminergic activity. These arrays can be implanted in a variety of brain regions at different depths. Previously, we used a glass cannula as an insertion guide to reach more ventral depths, but we noted that this would occasionally lead to heavy bleeding. To eliminate this problem the array was re-designed with carbon fibers secured within individual

silicon tines, or supports, that allow for a cannula-free insertion, while still maintaining functionality. The supports can be made to any desired length which has allowed us to record chronic dopamine (DA) in rat dorsal striatum as well as electrophysiology from rat motor and pre-frontal cortex (PFC). In three separate rats we have successfully implanted 8 channel arrays (pitch=160um), optimized for dopamine sensing, into dorsal striatum. In the first 2 rats, DA was detected intraoperatively on at least 6 of the 8 implanted channels after stimulation of the medial forebrain bundle (MFB). In a third rat, with a chronically implanted array, DA was detected on 6 of 7 active channels at day 12 after MFB stimulation. At day 16 in the same animal, DA was detected on all 7 active channels after an intraperitoneal (IP) injection of raclopride which came 20 minutes after an IP injection of cocaine.
To validate the ability of these array to detect electrophysiology we targeted two brain regions, motor cortex and PFC. In one animal we chronically implanted a 16 channel array (pitch=80um), functionalized with poly-3,4-ethylenedioxythiophene, into rat motor cortex. In this animal we detected unit activity out to 3 months. In a separate group of rats (n=9) we implanted an 8 channel variant (pitch=160um) into PFC and acutely detected activity from both excitatory and inhibitory neurons. In the latter group of animals we cemented the probes in place, perfused, and used our slice in place technique to isolate the probe tips within the tissue. Briefly, the skull, brain, and electrodes remain intact and soak in a solution that decalcifies the bone, which allows us to cryosection through all three at the same time. We can then identify cell types adjacent to our recording tips using antibody staining.
Taken together, these results point to the viability of this new platform to sense both electrophysiological and dopaminergic activity in various brain regions. We are developing automated fabrication methods to mechanically deposit and cut the carbon fibers to length to improve our device throughput and channel count. Increasing the electrode density and count will continue to provide more information about neural population dynamics.

Disclosures: **P.R. Patel:** None. **B.D. Luma:** None. **E.J. Welle:** None. **A. Vega-Medina:** None. **J. Richie:** None. **T. Dong:** None. **D. Egert:** None. **L. Chen:** None. **A.J. Shih:** None. **J.D. Berke:** None. **D. Cai:** None. **J.B. Becker:** None. **C.A. Chestek:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.18/CC76

Topic: I.04. Physiological Methods

Support: NSF Grant 1831962

Title: Fast functional imaging of neural networks with nanoelectrode arrays

Authors: *N. MUKUNDAN[1], H. YOON[2], A. KHAN[2], C. OWUSU-ANSAH[2];
[1]Neural Engin. Lab., Norfolk State Univ., Norfolk, VA; [2]Neural Engin. Lab., Norfolk State Univ., Yorktown, VA

Abstract: This research is to develop a fast electrical impedance tomography (fEIT) imaging system which can simultaneously image multiple areas of brain including deep brain structures on in behaving animals. This will enable chronic imaging of fast neural activity for deep brain structures and neuronal networks with high temporal resolution. This EIT imaging technology for fast neural activity could lead to radical advances in understanding brain function and enable quantitative mathematical modeling and analysis of neural systems. In this research, we fabricated vertically aligned IrOx/AuNW electrodes with low impedance values. For fEIT imaging of neural network, electrochemical properties of electrodes including impedance spectrum determines spatial and temporal resolution of imaging. We also developed an electronic system for effectively recording impedance changes during neuronal depolarization in the brain. Results from bench testing with various phantom samples to evaluate the EIT system functions and imaging algorithm will be presented.

Disclosures: **N. Mukundan:** None. **H. Yoon:** None. **A. Khan:** None. **C. Owusu-Ansah:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.19/CC77

Topic: I.04. Physiological Methods

Title: Highly flexible microelectrode arrays for intracortical single unit recording

Authors: *M. LEBER, A. HURLBUT, S. WIEBE, R. BHANDARI, S. NEGI;
Blackrock Microsystems, Salt Lake City, UT

Abstract: Implantable microelectrodes are indispensable in neuroscience research, offering the possibility to record and to stimulate extracellular potentials from single neurons, as well as multi-unit and local field potential recordings. Although rigid silicon based and metallic microwire electrode arrays have allowed intracortical recordings over the last decades, there has been continued interest to reduce the form factor of the microelectrodes. Smaller probe dimensions have the advantage being less damaging to the tissue, while flexible devices mitigate mechanical mismatch between the device and the surrounding tissue, leading to reduced foreign body response and therefore, improved signal quality and longevity of the device. Here we present MicroFlex Array (MFA), that unites all these favorable implant properties into a new robust and reliable microelectrode array solution. The MFA allows for a highly customizable layout of electrodes made of either platinum or iridium oxide. The electrodes are located on a 10

µm thin and 200 µm wide polyimide shaft, that can reach lengths of up to 80 mm (Figure 1 A). The MFA can be connected to a headstage through various connector types, such as Omnetics, pedestal, and Molex. Figure 1 B displays an assembly of 5 MFAs connected to a pedestal type connector. Using a minimally invasive process, the MFA can be implanted at various depths, avoiding the necessity of large craniotomies, or opening the dura during implantation. With a patent pending implantation technology, the MFA can record from single units during stereotaxic insertion. This allows the device to be implanted at the desired optimal depth in the tissue. We have achieved acute recording from hippocampus area with signal-to-noise ratios of over 20:1 and are currently pursuing our chronic experiments for several weeks. With the data recorded from the MFA up to date, the presented technology will mark a new milestone in electrophysiology and single unit recordings in neuroscientific research.

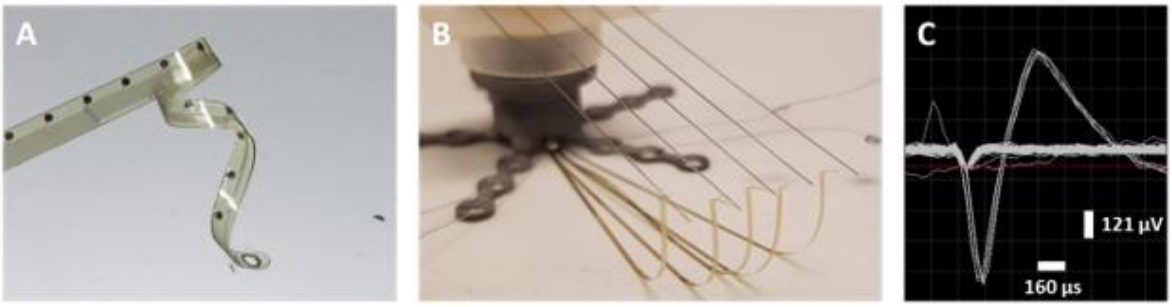

Figure 1: A) Flexible MFA shaft with electrodes. B) Assembly of 5 MFAs to pedestal with insertion needles. C) Recording of 2 single units in acute rodent experiment form same iridium oxide electrode (30 µm diameter).

Disclosures: **M. Leber:** A. Employment/Salary (full or part-time):; Blackrock Microsystems (full time). **A. Hurlbut:** None. **S. Wiebe:** None. **R. Bhandari:** A. Employment/Salary (full or part-time):; Blackrock Microsystems (full time). **S. Negi:** A. Employment/Salary (full or part-time):; Blackrock Microsystems (full time).

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.20/CC78

Topic: I.04. Physiological Methods

Title: Ultra-sensitive measurement of *in vivo* brain penetration with microscale probes and live imaging

Authors: A. M. OBAID[1], *Y.-W. WU[2], M.-E. HANNA[4], J. B. DING[5], N. MELOSH[3]; [1]Stanford Univ., Stanford, CA; [2]Stanford Univ., Palo Alto, CA; [3]Materials Sci., Stanford Univ.,

Stanford, CA; [4]Mina-Elraheb Hanna, Stanford University, CA; [5]Neurosurg., Stanford Univ. Dept. of Neurosurg., Palo Alto, CA

Abstract: While penetrating microelectrodes have been used effectively in experimental neuroscience for decades, important quantifications of the force and work exerted on the brain tissue during insertion are not well described. The most critical theme of these microwire electrodes is to have the wires inserted into the brain without buckling but also have them small enough so that they can insert and reduce physiological damage. Mechanical measurements of single wire insertion into the brain are important quantifications of the force and work exerted on the brain tissue during insertion and may help identify lower-damage microelectrode designs and surgical procedures.Many aspects of the mechanics of insertion remain unknown, such as the effects of wire diameter and tip shape. To address the limitations of typical force transducers, we have devised a high-resolution mechanical measurement system to probe the ultra-compliant properties of the brain at much higher force and temporal resolution than previous measurements. We perform a systematic study of the insertion mechanics into traditional brain mimics, freshly removed murine brains (ex-vivo), and in-vivo for a series of different microwire diameters (7.5 um to 125 um) and tip geometries (flat, angled, and electrosharpened).Surprisingly, both penetration force and tissue compression scaled linearly with wire diameter, rather than cross-sectional area. Linear brain compression with wire diameter strongly suggests smaller probes will cause less tissue damage upon insertion, though unexpectedly no statistical difference was observed between angled and flat-tipped probes. The data from brain insertion was strikingly different from those in traditional brain mimics and excised tissue and provide the first clear view of how insertion mechanics depend on wire geometry.These ultra-sensitive force measurements were coupled with live 2-photon microscopy and epifluorescence imaging, providing a unique visualization of the insertion process with simultaneous force measurement. We found no disruption of microvasculature with wire diameters of 25 um or less, while damage is clearly observed with diameters greater than or equal to 50 µm. These first of their kind measurements provide a mechanical framework for designing effective microprobe geometries while limiting mechanical damage.

Disclosures: Y. Wu: None. **A.M. Obaid:** None. **M. Hanna:** None. **J.B. Ding:** None. **N. Melosh:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.21/DD1

Topic: I.04. Physiological Methods

Support: NIH Grant R43 NS105500-01A1

Title: Improved insertion of floating microelectrode arrays in brain with an ultrasonic vibration insertion system

Authors: *R. S. CLEMENT, N. N. TIRKO, J. K. GREASER, R. B. BAGWELL, M. L. MULVIHILL;
Actuated Med. Inc., Bellefonte, PA

Abstract: Penetrating microelectrode arrays have been a valuable research tool for neuroscientists to interface with and study brain circuits with both high temporal and spatial resolution. A common challenge with these devices is how to successfully implant them to a pre-determined cortical depth without excessive dimpling and trauma. Sub-optimal insertion mechanics can result in non-uniform shank insertion, increased risk of trauma, bleeding and inflammation at the implant site, and may accentuate the chronic foreign body response (FBR) leading to neural cell death, glial scaring, and device failure. These issues have limited the use of penetrating arrays in preclinical studies as well as clinical translation of these devices to applications such as brain-machine interfacing and cortical visual prostheses. We have previously shown that ultrasonic vibration of fixed microwire electrode arrays during insertion improves insertion accuracy and reduces insertion force and tissue dimpling, without interfering with electrode integrity or performance. Adapting the approach to floating arrays (FAs)—designed to float with the brain to reduce relative micromotion at the brain-implant interface—offers specific challenges related to effective coupling and non-perturbing post-insertion release. Here we present on our efforts to adapt our ultrasonic vibration technology (www.neuralglider.com) to several FA types, including NeuroNexus (H-series, Matrix) and Microprobes floating microwire arrays. Our implant insertion system incorporates a piezoelectric ultrasonic transducer, operated in an axial resonant mode, which transmits high frequency micro-vibrations to the FA. We developed several reversible coupling approaches to efficiently couple vibration between the insertion system and the FA, allowing for non-perturbing release of the FA following insertion. These have included temporary adhesive (e.g. polyethylene glycol) or mechanical gripping-based approaches. Approaches were evaluated in benchtop insertion studies in agar and *ex vivo* tissue models, as well as *in vivo* insertion studies in acute and chronic rat and pig models. Results (insertion video analysis, neural recordings, histology) support the feasibility and potential of the insertion system for improving insertion mechanics of a wide range of implant types.

Disclosures: **R.S. Clement:** A. Employment/Salary (full or part-time):; Actuated Medical, Inc. **N.N. Tirko:** A. Employment/Salary (full or part-time):; Actuated Medical, Inc. **J.K. Greaser:** A. Employment/Salary (full or part-time):; Actuated Medical, Inc. **R.B. Bagwell:** A. Employment/Salary (full or part-time):; Actuated Medical, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actuated Medical, Inc. **M.L. Mulvihill:** A. Employment/Salary (full or part-time):; Actuated Medical, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actuated Medical, Inc..

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.22/DD2

Topic: I.04. Physiological Methods

Support: NIH NINDS NS054894
NIH NINDS NS072651

Title: Suture-like braided microprobes for peripheral nerve interfaces and multi-sites and combinatorics for higher yields in neural recording

Authors: *T. KIM[1], K. A. SCHMIDT[3], S. F. GISZTER[2];
[2]Dept Neurobiol & Anat, [1]Drexel Univ. Col. of Med., Philadelphia, PA; [3]Drexel Univ., Philadelphia, PA

Abstract: The braided multi-electrode probe (BMEP) is a bundle of tubularly braided ultrafine microwires used as a neural probe to record neural signals or stimulate neural tissue with electrical current for neural interfaces. A BMEP has potential empty space due to the intrinsic tubular structure. Any material can form the core and occupy the lumen. Surgical sutures are one core material. We call long BMEPs with sutures, or without a suture core 'suture-like BMEPs' - they could be used as sutures. Peripheral nerve interfaces require a smaller size and greater mechanical flexibility due to the long thin form and freely moving conditions of peripheral nerves. We show comparisons of BMEPs consisting of 6 x 9.6um wires, with and without 10-0 suture cores with the LIFE electrode. The LIFE has the smallest footprint (35um diameter) as an intrafascicular peripheral nerve interfaces. Our suture-like BMEP not only has higher mechanical flexibility (albeit bigger diameter), but also 6 recording/stimulation channels. We suggest that the suture-like BMEP is ideal for some peripheral nerve interfaces. In addition, suture-like BMEPs support increased neural yield per wire in single unit electrophysiology using combinatoric probe designs. Combining individual wire sites in different locations can provide unique wire combinations with polytrode pickup capabilities at each location. These help to localize and separate units along the length of the probe. We have performed computational simulations under the condition that 9 wire probes arranged as multisite wires (4 sites per wire) in an iterated ${}_3C_2$ pattern with 5 seconds of 40Hz Poisson distributed spike trains from 4 neurons with unique spike shapes at 30KHz sampling rate. To extract mixed signals, ICA (Independent Component Analysis), a blind source separation algorithm was used. From simulation results, we found good combinatoric signal extraction (ICA separated each spatial site's activity nearly perfectly, with correlation values between the original site activity without noise added, and activity reconstructed via ICs averaging 0.9981 ± 0.0007). This result shows that the potential neural yields with this technique could be increased by a factor of 4. This technique can be used with

modified braid geometry by our second generation microbraiding machine in near future. The 6 wire suture-like BMEP described above can be arranged as a $_3C_2$ pattern of paired wires assembled in groups of 4 under this strategy. Supported by NIH NINDS NS054894 and NS072651

Disclosures: T. Kim: None. **K.A. Schmidt:** None. **S.F. Giszter:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.23/DD3

Topic: I.04. Physiological Methods

Title: Leveraging MRI, CAD, CAM and 3D printing to implant five microelectrode arrays in three nodes of the macaque frontoparietal grasp network

Authors: *R. N. TIEN, A. B. SCHWARTZ;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Comprehensive study of the neural basis of complex behaviors such as grasping and object interaction requires simultaneous electrophysiological recording in multiple cortical brain areas, some of which are located deep in sulci. Here, we present a novel method in which MRI, CAD, CAM and 3D printing were used to enable rapid, accurate and stable implantation of multiple microelectrode arrays into three brain areas. We used structural MRI to non-invasively image the brain and skull anatomy. We then segmented these MRI images and imported them into a 3D CAD model to plan the placement of five electrode arrays (four Microprobes FMA electrodes and one Blackrock Utah array) totaling 224 channels. This model was then used to generate a bespoke 3D printed insertion guide. Use of this guide reduced total surgery duration and ensured precise electrode array placements, resulting in high unit yield. Traditional computer-aided manufacturing techniques were leveraged to fabricate a custom titanium pedestal to house the FMA connectors for robust long-term recording. A 3D printed model of the subject's skull allowed for intuitive, hands-on planning of the incision, craniotomy, bone strap and pedestal placements. This method could easily be extended to streamline the implantation of a multitude of different probes in cortical or subcortical sites.

Disclosures: R.N. Tien: None. **A.B. Schwartz:** None.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.24/DD4

Topic: I.04. Physiological Methods

Title: A Utah Array based acute microelectrode array for intra-operative recordings from human cortex

Authors: *G. PALIS[1], R. BHANDARI[1], V. NGO[1], R. FRANKLIN[1], M. GERHARDT[1], F. SOLZBACHER[2];
[1]Blackrock Microsystems, Salt Lake City, UT; [2]Univ. of Utah, Salt Lake City, UT

Abstract: Research Focus: Tools and methods
Rationale: An increasing number of neurosurgeries (DBS implantation, epilepsy monitoring with SEEG) are completed with burr hole incisions instead of traditional craniotomies due to reduced surgical complications. Vast amounts of data could be collected from micro-electrodes during these surgeries.
Objective: Characterize a re-designed Utah Microelectrode Array's ability to record single unit activity and local field potentials intra-operatively through a burr hole.
Abstract:
The NeuroPort Electrode, the FDA cleared Utah Multi-Electrode Array, (UMEA) has served as the only FDA cleared cortical microelectrode and has provided significant volume of data invaluable to the neuroscience community. The UMEA has been most impactful in the fields of brain computer interface and motor prosthetics, however it has played a critical role in the study of learning, memory, sensation, and visual processing/prosthetics.
The UMEA is an invasive cortical electrode that requires a craniotomy for implantation. The surgical risk associated with craniotomy limits the volume of the research efforts with the UMEA. Due to reduced surgical complications when using burr holes instead of craniotomies, more and more common neurosurgeries, such as epileptic monitoring and placement of deep brain stimulation (DBS) leads, are completed with a burr hole only. As DBS devices are implanted more frequently for treatment of the motor symptoms of Parkinson's disease and are shown to be safe and effective treatments for additional neurological disorders the number of neurosurgeries completed with burr holes will continue to increase.
A UMEA adapted for acute recordings through a burr hole would allow collection of significant amounts of otherwise inaccessible single unit activity and local field potential (LFP) data, advancing the field of neuroscience without posing significant additional risk to patients undergoing said procedures.
The goal of this poster is to characterize the adapted UMEA, dubbed the Right-Angle Array, to demonstrate suitability for single unit and LFP recordings from cortex through a burr hole >4 cm

diameter. This poster will also present surgical experience and data collected by the first neurosurgeons to use the Right-Angle Array in human studies.
Conclusion: The Right Angle Array is a modified UMEA proposed for acute recordings during neurosurgery procedures, such as DBS implantation, for collection of otherwise inaccessible single unit and LFP data without posing additional surgical risk to patients.

Disclosures: G. Palis: A. Employment/Salary (full or part-time):; Blackrock Microsystems (full time). **R. Bhandari:** A. Employment/Salary (full or part-time):; Blackrock Microsystems (full time). **V. Ngo:** A. Employment/Salary (full or part-time):; Blackrock Microsystems (full time). **R. Franklin:** A. Employment/Salary (full or part-time):; Blackrock Microsystems (full time). **M. Gerhardt:** A. Employment/Salary (full or part-time):; Blackrock Microsystems (full time). **F. Solzbacher:** A. Employment/Salary (full or part-time):; Blackrock Microsystems.

Poster

430. Techniques: Microelectrodes I

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 430.25/DD5

Topic: I.04. Physiological Methods

Support: Fapesp 2016/18314-0

Title: Tissue reaction to the neural probes implanted into the rat brain: A transcriptome analysis

Authors: *E. V. DIAS[1], J. D. MACHADO[1], R. PANEPUCCI[2], R. COVOLAN[1], F. CENDES[1], I. T. LOPES-CENDES[1], A. S. VIEIRA[1];
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Abstract: Brain probes are important tools for understanding the nervous system. They allow evaluating the electrical activity of single neurons and its relation with the subject behavior. Since neural probes need to be positioned within tens of micrometers of the neurons of interest, they are necessarily invasive devices. As it happens with any foreign material that is implanted into the body, the tissue reaction process starts with the attempt to clean up the site and eliminate the threat of the invader. If the threat persists, a chronic inflammatory process ensues, with the attempt to shield the affected area from the surrounding tissue. This shielding, which in the brain consists of a capsule formed predominantly by astrocytes, gradually decreases the quality of the recorded neuronal signals. This study evaluates tissue response to the neural probes designed and fabricated by the BRAINN research groups, comparing it with the tissue response to other recording devices implanted into the brain. Stereotaxic surgery for implantation of recording neural probes was performed in Fischer 344 male rats. Rats received recording neural probes developed in BRAINN projects, or commercial silicon probes, or stainless steel micro wires. Recording probes were implanted into the dentate gyrus of the hippocampus (AP -3.0; L ±2.0; V

-3.5). After a period of 2 or 28 days, rats were euthanized and the brains were removed. Laser microdissection of the regions proximal to probe implantation was carried out and the material was subjected to transcriptome analysis by RNA-seq using Illumina HiSeq platform. Neural tissue was also analyzed with immunofluorescence labeling for markers for foreign body reaction (GFAP and CD68), and for neuronal marker (NeuN). All procedures were approved by the Ethics Committee for Animal Research at the Unicamp (protocol 4438-1). Transcriptome analysis showed around 8000 differentially expressed genes among control and implanted groups. Between implanted groups, there are 748 differentially expressed genes in the tissue surrounding the BRAINN probe compared to the tissue surrounding the stainless steel micro wire. Two days after surgery, the tissue surrounding the probes is disorganized and immunostaining for CD68 around the probe sites reveals reactive microglia. Our preliminary data show that probes implanted into the brain lead to an acute reaction with drastic changes in gene expression. Furthermore, different probe materials induced different tissue responses.

Disclosures: E.V. Dias: None. **J.D. Machado:** None. **R. Panepucci:** None. **R. Covolan:** None. **F. Cendes:** None. **I.T. Lopes-Cendes:** None. **A.S. Vieira:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.01/DD6

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

Support: DARPA

Title: EXTRACT: Automated cell extraction for large-scale neural calcium imaging based on a framework of robust statistics

Authors: *H. INAN[1], T. TASCI[1], C. SCHMUCKERMAIR[1], B. AHANONU[2], O. HERNANDEZ[1], M. A. ERDOGDU[3], M. J. SCHNITZER[4];
[2]Dept. of Biol., [1]Stanford Univ., Stanford, CA; [3]Univ. of Toronto, Toronto, ON, Canada; [4]Depts. Biol. & Applied Physics, Stanford Univ. Dept. of Biol., Stanford, CA

Abstract: Fluorescence imaging of neural calcium activity enables studies that track the dynamics of large ensembles of individual cells in awake behaving animals. These experiments can extend across repeated imaging sessions, and due to recent advances in optical imaging technology, can sample thousands of cells per session. Thus, there is a major need for fast and accurate computational approaches that can extract the identities of individual neurons and their activity traces from large video datasets. Common computational challenges include nearby cells that share overlapping sets of video pixels, and non-stationary background fluorescence from neuropil activation. Past research has sought to address these challenges, but a general-purpose

framework that is applicable across a wide range of imaging modalities has not emerged. Here we present a computational platform, termed EXTRACT, for cell extraction based on the framework of robust statistical estimation. By testing our approach on simulated and experimental datasets, we found that this framework enables substantial improvements in the accuracy with which neural morphologies and activity traces can be estimated under realistic imaging conditions. EXTRACT is well suited to use on datasets exhibiting a broad range of signal and noise attributes, including those typical of recent one- and two-photon fluorescence imaging modalities for use in head-fixed and freely behaving animals. Efficient implementation of EXTRACT, including parallelization across multiple graphical processing units (GPUs), allows imaging datasets to be processed about ten-fold faster than the actual recording durations. We used EXTRACT to analyze data from several different calcium imaging experiments in behaving animals; the resulting neural activity traces enabled superior reconstructions of animal behavior than activity traces from other cell sorting algorithms. Overall, EXTRACT is a powerful, high-fidelity approach for automated analysis of large-scale calcium imaging datasets.

Disclosures: H. Inan: None. **T. Tasci:** None. **C. Schmuckermair:** None. **B. Ahanonu:** None. **O. Hernandez:** None. **M.A. Erdogdu:** None. **M.J. Schnitzer:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.02/DD7

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

Support: NIMH Grant R44-MH108053
NIMH Grant R44-MH105091

Title: Automatically delineating anatomic regions and mapping cell populations in mouse brain

Authors: *N. J. O'CONNOR[1], B. S. EASTWOOD[1], P. J. ANGSTMAN[1], N. D. LIESE[1], C. S. GERFEN[1], H. L. KESSLER[1], M. GULENKO[1], C. R. GERFEN[2], J. R. GLASER[1];
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Abstract: Advances in molecular neuroanatomical methods have expanded the ability to map connections of specific neuron subtypes in the context of behaviorally or pathologically driven patterns of neuronal activity. Analyses of connectivity and neuronal activity across whole mouse brains that have been registered to a reference atlas reveal details about the functional organization of brain circuits related to behavior and pathologies that can be compared across animals, experiments, and laboratories. Here we present novel advances to our previous work for automatically aligning and reconstructing images of mouse brain sections labeled with standard histochemical techniques into whole-brain 3D image volumes, registering these brain image

volumes to the Allen Mouse Brain Common Coordinate Framework (CCF), and subsequently automatically delineating anatomical brain regions in experimental tissue sections.
Sections containing brain regions with well-defined anatomic landmarks are registered to the CCF with a transform model that accounts for artifacts due to histological cutting, mounting, and fixation. This transform is propagated to other sections with linear and nonlinear refinements that preserve the histologic sectioning model. We compared atlas-based segmentation results using this method to the previous single-section approach using manual delineations of 15 anatomic regions.
Examples of whole brain analysis of connectivity using trans-synaptic rabies labeling of neurons providing inputs to Cre-expressing neuron subtypes and of analysis of behaviorally relevant brain circuits using fos labeling of activity in neurons correlated with specific behaviors. For analysis, coronal brain sections are reconstructed into a 3D volume, labeled neurons are automatically marked using a Laplacian of Gaussian algorithm, and brain sections and marked neurons are registered to the CCF. The number of labeled neurons in each of the 2500 brain structures in the CCF are tallied, allowing for comparative quantitative analysis between mice.

Disclosures: **N.J. O'Connor:** A. Employment/Salary (full or part-time):; MBF Bioscience. **B.S. Eastwood:** A. Employment/Salary (full or part-time):; MBF Bioscience. **P.J. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience. **N.D. Liese:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C.S. Gerfen:** A. Employment/Salary (full or part-time):; MBF Bioscience. **H.L. Kessler:** A. Employment/Salary (full or part-time):; MBF Bioscience. **M. Gulenko:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C.R. Gerfen:** None. **J.R. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.03/DD8

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

Title: Automatic segmentation and detection of neuronal cell types from serial-two photon obtained whole-brain mouse images using U-Net

Authors: *C. ELOWSKY[1], J. PALMER[2], K. UMADEVI VENKATARAJU[2];
[1]Cold Spring Harbor Lab., Cold Spring Harbor Laboratory, NY; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Since the advancement in automated high-throughput whole brain imaging, there has been a large effort to identify the existence of diverse neuronal cell types. Manually tracing these cells is a tedious and time consuming process, and automated segmentation methods, such as traditional convolutional neural networks, have proven to be sub-optimal. U-Net, a generic deep-

learning-based model, designed as a modified convolution network architecture, has proven to achieve more precise cell detection and segmentation. Using special data-augmentation strategies, U-Net allows for accurate segmentation of large amounts of biological images, with only minimal training data needed. We propose the use of U-Net in our computational pipeline for detection and segmentation of both cytoplasmic and neuronal signal from fluorescently labeled whole-brain mouse images obtained via serial two-photon tomography.

Disclosures: C. Elowsky: None. **J. Palmer:** None. **K. Umadevi Venkataraju:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

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Program #/Poster #: 431.04/DD9

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

Support: NIH Support R43MH119842

Title: A neuronal dynamic clamp system for improving action potential recording in human stem cell derived neurons

Authors: *M. W. NOWAK[1], B. K. PANAMA[2,1], B. FRANKS[1], L. KORBEL[1], R. L. RASMUSSON[2,1], G. C. L. BETT[2,1];
[1]Cytocybernetics Inc., North Tonawanda, NY; [2]SUNY Buffalo, Buffalo, NY

Abstract: Human stem-cell derived (hiPSC) neurons are a model system for studying the electrophysiological properties of native neurons. A significant limitation of the neuronal hiPSCs is the decreased/lack of expression of background K^+ currents (e.g. TASK, TWIK, TREK) resulting in depolarized resting membrane potentials (RMPs) and the inability to evoke stable action potentials (APs). We have overcome this limitation by using a neuronal dynamic clamp system to electronically express in real-time tunable K+ current-voltage relationships to hyperpolarize the RMP. iCell hiPSC GABANeurons (Fujifilm Cellular Dynamics, WI) were cultured according to the manufacturer's instructions and used 7-27 days after plating. Standard electrophysiology voltage clamp with the whole-cell ruptured configuration was used, and APs were triggered by stimulating with 0.5-1.0 nA pulses for 0.3-1.5 ms at 0.5 Hz. Background K^+ currents were electronically expressed using a real-time dynamic clamp system (Cytocybernetics, NY).
The resting membrane potentials (RMP) of the neuronal hiPSCs were highly depolarized (31±1 mV, n=72) with distorted evoked APs (Amp. = 60±3 mV, after-hyperpolarization (AHP) at -45±2 mV). We tuned the RMP to -65 mV via injection of a constant current (I_{const}) and the electronic expression of a background outwardly rectifying K^+ current (I_{GHK}). Injection of a constant current resulted in unstable RMPs (σ=2; n=8) while electronic expression of I_{GHK} had a

more stable RMP (σ=0.7 mV, n=10). There were differences in AP morphology. With expression of I_{GHK} the amplitude showed a decrease as compared to constant current injection (I_{GHK}: 112±4 mV, I_{const}=123±4 mV, n=8, $p<0.05$).
To examine the effects of background conductance on excitability we extended the electronic expression of I_{GHK} to allow tuning of RMP to hyperpolarized potentials (-55 to -65 mV) resulting in the recording of evoked APs with a characteristic after-hyperpolarization (for RMP= 55 mV: Amp.= 95±4 mV, AHP at 62±1 mV, n=25). In a subset of hiPSC neurons, we also observed RMP-dependent spontaneous firing.
Taken together, we have shown that electronic addition of background K+ currents during electrophysiological recording in hiPSC neurons allows the tuning of RMPs to physiological levels improving the measurement of functional responses (action potential morphology, phasic firing, post synaptic summation, after-hyperpolarization and long-term potentiation).

Disclosures: **M.W. Nowak:** A. Employment/Salary (full or part-time):; Cytocybernetics, Inc. **B.K. Panama:** A. Employment/Salary (full or part-time):; Cytocybernetics, Inc. **B. Franks:** A. Employment/Salary (full or part-time):; Cytocybernetics Inc. **L. Korbel:** A. Employment/Salary (full or part-time):; Cytocybernetics Inc. **R.L. Rasmusson:** 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.; Cytocybernetics Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cytocybernetics Inc. **G.C.L. Bett:** A. Employment/Salary (full or part-time):; Cytocybernetics Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cytocybernetics Inc..

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.05/DD10

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

Support: NIH U01 EB021921
NSF IIS

Title: Connectivity analysis of multielectrode data from simulated CPG and real neural systems using a stochastic framework

Authors: M. ABOLFATH-BEYGI[1], T. D. SANGER[2], ***S. F. GISZTER**[3];
[1]Dept. of Biomed. Engin., [2]Biomed. Engin., USC, Los Angeles, CA; [3]Neurobio., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: We have developed a new methodology for connectivity analysis using a stochastic model. This may also be combined with neural perturbation. The method is based on stochastic dynamic operators (SDO) used in order to quantify the peripheral effects in EMG and limb state and the internal inter-relations of neural dynamics. Our goal has been to develop a methodology for discovering structural connectivity maps with stimulation of fewer neurons in the network. The SDO framework quantifies uncertainty in the dynamics, and we aim to use this framework for discovering effects and connectivity in the presence of uncertainty and without any averaging or dimensionality reduction in the high-level network behavior. SDO allows for quantifying the dynamic effects of both individual and ensemble of neuronal populations on outputs and activity of other populations. By learning these dynamic effects, we can predict the output or bias control in the network. The framework goals are understanding neural connectivity, and developing predictive models suitable for neuroprostheses. The advantages of the SDO framework over current neural analysis and predictive models include: 1) ability to describe state-based probabilistic dynamic effects of neurons, 2) methodologies to embed nonlinear dynamics in a linear framework in the probability domain, and 3) capacity to extend pair-wise neural analysis to multi-neural analysis through linear superpositions in probability. In the SDO framework, each spike triggers a Markov operator mapping probability of current state of movement variables to the probability of next state. An SDO thus represents both sensory (current state) and motor (mapping to the next state) effects in the neuron. We use both real neural data collected in spinal frogs, and artificial data from a simulated spinal network as a 'ground truth' network of known connectivity and hierarchy, driven by Hodgkin-Huxley dynamics, as developed by Rybak, Shevtsova, and Markin for spinal cord simulation of populations of pattern generation neurons. The 'fictive' simulated network generates rhythms, deletions, and motor pool spiking which can be integrated to simulate electromyographic (EMG) recordings. Previous tests validated the use of SDO-based predictive models of EMG as well as interneuron firing activities. We have assembled the framework into two analysis packages in MATLAB which are now under beta testing by users before broader distribution. In conclusion, the SDO framework shows significant promise as a new mathematical model for understanding, interacting with and controlling neural activity in BMI and prosthetics.

Disclosures: M. Abolfath-Beygi: None. **T.D. Sanger:** None. **S.F. Giszter:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.06/DD11

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

Support: Wellcome Trust grant 205093 to MC and KDH.

Title: Rigbox: An open-source toolbox for probing neurons and behavior

Authors: *J. BHAGAT[1], M. J. WELLS[1], A. J. PETERS[1], K. D. HARRIS[1], M. CARANDINI[1], C. P. BURGESS[2];
[1]Univ. Col. London, London, United Kingdom; [2]Deepmind, London, United Kingdom

Abstract: Setting up a behavioral neuroscience experiment is a complex process that is often managed with ad hoc solutions. To streamline this process, we developed Rigbox, a high-performance, open-source software toolbox for managing experiments. Rigbox simplifies hardware/software interfacing, synchronizes data streams from multiple sources, manages experimental data via communication with a remote database, and creates an environment where experimental parameters can be easily monitored and manipulated. The toolbox's object-oriented paradigm facilitates a modular approach to designing experiments.
The main submodule of Rigbox, Signals, allows straightforward programming of behavioral experiments. Signals is built around the paradigm of functional reactive programming: an experiment is represented as a reactive network whose nodes represent experimental parameters which evolve over time through mutual interactions. The input nodes to this network represent time, experiment epochs, and hardware input sensors. This framework allows an experimenter to concisely describe an experiment as a set of transformations on the input nodes that update as the experiment unfolds.
Rigbox runs in MATLAB, with some Java components to handle network communication, and a C library to boost performance. Rigbox requires two computers, referred to as the "Master Computer" (MC) and "Stimulus/Slave Computer" (SC). MC is responsible for selecting, parameterizing, starting and monitoring an experiment via a MATLAB GUI. SC is responsible for running an experiment on a rig and interacting with that rig's hardware during runtime. MC can control multiple SCs simultaneously. MC and SC communicate during runtime via Java WebSockets using TCP/IP. Precise hardware requirements depend on the complexity and number of experiments run concurrently. For most experiments, typical modern desktop computers running Windows will suffice. Rigbox is available open-source at https://github.com/cortex-lab/Rigbox.
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Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.07/DD12

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

Support: NSF NeuroNex Award DBI-1707398
The Gatsby Charitable Foundation

Title: BehaveNet: Behavioral video embedding and neural analysis toolbox

Authors: E. BATTY[1], ***M. R. WHITEWAY**[1], S. SAXENA[1], D. BIDERMAN[1], T. ABE[1], S. MUSALL[2], W. GILLIS[3], J. E. MARKOWITZ[4], A. K. CHURCHLAND[2], S. R. DATTA[3], S. LINDERMAN[1], L. PANINSKI[1];
[1]Columbia Univ., New York, NY; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [4]Dept. of Neurobio., [3]Harvard Med. Sch., Boston, MA

Abstract: A fundamental goal of systems neuroscience is to understand the relationship between neural activity and behavior. Behavior has traditionally been characterized by task-related variables such as movement speed or response times. More recently, there has been a growing interest in analyzing video data collected during experiments. One approach is the supervised tracking of body parts [Mathis et al. 2018, Pereira et al., 2019, Graving et al. 2019], though this requires user effort to label training images and may not capture all of the useful information in the image. Fully-unsupervised linear dimensionality reduction methods have been used to represent behavioral videos directly [Musall et al. 2019, Stringer et al. 2019], but these require a large number of dimensions to represent behavioral videos, hampering downstream analyses. Here we introduce a probabilistic framework for the unsupervised analysis of behavioral video and neural activity, building upon previous work [Wiltschko et al. 2015, Johnson et al. 2016, Gao et al. 2016, Markowitz et al. 2018]. This framework provides tools for compression, segmentation, generation, and decoding of behavioral videos. Compression is performed using convolutional autoencoders (CAEs), which yield a low-dimensional, continuous representation of behavior that requires fewer dimensions than linear methods (e.g., PCA) to obtain the same video reconstruction error. We then use an autoregressive hidden Markov model (ARHMM) to segment the CAE representation into discrete "behavioral syllables" and find that many syllables correspond to interpretable behaviors (e.g. grooming, rearing). The resulting generative model can be used to simulate behavioral video data. Finally, we fit decoders which take in neural activity and output estimates of the full-resolution behavioral video. These analyses allow us to explore how behavior is represented across different brain regions.
We demonstrate the use of this framework using a wide range of experimental paradigms and behavioral and neural recording technologies: video and neuropixel recordings from freely behaving head-fixed mice [Steinmetz et al. 2018], video and widefield imaging from task-engaged head-fixed mice [Musall et al. 2019], and 3D depth imaging and microendoscopic calcium imaging from freely moving mice exploring an open field environment [Markowitz et al. 2018].

Disclosures: **E. Batty:** None. **M.R. Whiteway:** None. **S. Saxena:** None. **D. Biderman:** None. **T. Abe:** None. **S. Musall:** None. **W. Gillis:** None. **J.E. Markowitz:** None. **A.K. Churchland:** None. **S.R. Datta:** None. **S. Linderman:** None. **L. Paninski:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

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Program #/Poster #: 431.08/DD13

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

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Title: Localized semi-nonnegative matrix factorization (LocaNMF) for widefield calcium imaging data

Authors: *S. SAXENA[1], I. KINSELLA[1], S. MUSALL[5], J. MESZAROS[2], D. N. THIBODEAUX[3], S. H. KIM[2], J. P. CUNNINGHAM[1], E. M. HILLMAN[4], A. K. CHURCHLAND[5], L. M. PANINSKI[2];
[1]Columbia Univ., New York City, NY; [3]Biomed. Engin., [4]Biomed. Engineer, [2]Columbia Univ., New York, NY; [5]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Widefield calcium imaging is a high-throughput approach for recording large-scale neural activity across dorsal cortex. In order to examine the relationship of these neural signals to the resulting behavior, it is helpful to compress, denoise, and demix the videos into meaningful spatial and temporal components that can be decoded and compared across different behavioral sessions and experimental conditions. Mapping the inferred components onto well-defined brain regions provides an interpretable, stable decomposition. Although existing brain atlases define detailed maps of the cortical structures for an average mouse, no current tools satisfactorily extract the activity of the different brain regions in individual mice in a data-driven manner, while taking into account mouse-specific and preparation-specific differences.
Here, we introduce a variant of Nonnegative Matrix Factorization (NMF), termed Localized semi-NMF (LocaNMF), that efficiently decomposes widefield video data and allows us to directly compare activity across multiple mice by outputting mouse-specific localized functional regions. LocaNMF uses a fast low-rank version of Hierarchical Alternating Least Squares (HALS), and outputs components that are significantly more interpretable than more traditional NMF or SVD-based techniques. Specifically, while representing a user-defined fraction of the variance in denoised data (usually chosen to be 99%), it also outputs spatial components that are soft-assigned to atlas-defined regions, thus easily enabling comparison across animals.

Moreover, it provides a natural subspace to directly compare correlation maps and neural dynamics across different mice, and enables identification of task- and movement-related brain regions. In experimental data from mice expressing different calcium indicators and exhibiting a variety of behaviors, we find that (a) functional regions are consistent across different sessions in the same mouse, (b) correlation maps across these regions are consistent across different sessions in the same mouse and even across some mice, (c) the frontal areas of cortex are consistently useful in decoding the direction of licks in a spatial discrimination task, and (d) the parietal areas of cortex are useful in decoding the movements of the paws during the same task, as tracked using DeepLabCut. Thus, the LocaNMF decomposition provides a useful framework when analyzing widefield calcium imaging activity across mice.

Disclosures: **S. Saxena:** None. **I. Kinsella:** None. **S. Musall:** None. **J. Meszaros:** None. **D.N. Thibodeaux:** None. **S.H. Kim:** None. **J.P. Cunningham:** None. **E.M. Hillman:** None. **A.K. Churchland:** None. **L.M. Paninski:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

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Program #/Poster #: 431.09/DD14

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

Support: NIH EY R01EY022979
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Simons Collaboration on the Global Brain

Title: Cortex-wide activity during task learning reveals increasing engagement of parietal and frontal areas during decision-making

Authors: *S. MUSALL, S. GLUF, H. MOHAN, X. AN, Z. HUANG, A. K. CHURCHLAND; Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Multiple cortical areas are involved in learning and execution of complex behaviors. During learning, neural activity within local cortical circuits undergoes significant changes, such as enhanced representation of task-related sensory stimuli or decision variables. However, how cortex-wide activity patterns change with task-learning and how they relate to changes in animal behavior is unknown. Further, it is not known whether different types of excitatory, cortical neurons may represent distinct functional circuits that contribute differently to decision-making. To address these questions, we trained GCaMP6f-expressing, transgenic mice on a delayed, audio-tactile rate discrimination task and recorded neural activity across the dorsal cortex using widefield calcium imaging. Mice were imaged continuously over 4-5 months while learning

increasingly complex task stages. The total variance and dimensionality of cortical activity was similar across all sessions, indicating that large-scale cortical activity remains largely stable over longer time periods. In addition, we used video cameras and behavioral sensors to record animal movements and quantify long-term changes in animal behavior. Using a multivariate-regression model, we found that cortical activity in all sessions was mostly related to animal movements. Over several weeks of training, animal movements became less variable and their predictive power decreased while the importance of task variables increased. To isolate task-related activity, we then trained a logistic regression model to predict sensory stimuli and upcoming animal choices. Choices were well-predicted by activity in frontal cortical areas while sensory stimuli were most represented in primary sensory, parietal and secondary motor cortices. These results were confirmed by optogenetically inactivating parietal or frontal cortex. In both areas, inactivation strongly impaired task performance, with parietal effects being stronger during stimulus presentation and frontal effects being stronger during a subsequent delay. Lastly, we collected imaging data from transgenic mice that expressed GCaMP6f in excitatory neurons, either projecting to the striatum or other subcortical regions. Here, we found that corticostriatal projection neurons in parietal cortex reliably encoded auditory stimulus information while subcortical projection neurons in frontal cortex reflected animal's upcoming choices. Our results offer a detailed quantification of long-term changes in cortical activity and animal behavior and highlight the importance of parietal and frontal cortices for decision-making.

Disclosures: S. Musall: None. **S. Gluf:** None. **H. Mohan:** None. **X. An:** None. **Z. Huang:** None. **A.K. Churchland:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.10/DD15

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

Support: NVIDIA Academic Seeding Grant
NIH Grant U01GM104604
NIH Grant 9U01EB025830
ONR Grant N00014-13-1-0211

Title: From artificial to biological neural networks: How machine learning facilitates simulation of nonlinear synaptic dynamics in large-scale models

Authors: *D.-T. J. PHAM[1], J.-M. C. BOUTEILLER[2], G. J. YU[3], D. SONG[2], T. W. BERGER[2]; [1]Biomed. Engin., USC, Los Angeles California, CA; [2]Biomed. Engin., [3]USC, Los Angeles, CA

Abstract: Accurately capturing synaptic dynamics using computational models may prove important in identifying neuron- and network-level emergent properties. Existing detailed kinetic models can replicate complex synaptic nonlinear dynamics, but they are not practical in large-scale models due to the computational complexity associated with the number of differential equations that need to be solved at every timestep. This motivates the development of a more parsimonious model capable of maintaining biological accuracy while keeping computational load to a minimum. Previously, we have developed the Laguerre-Volterra Input-Output (LV-IO) Synapse model and its successor, the Laguerre-Volterra Network (LVN) as means for accurately predicting the synaptic dynamics while circumventing the complexity of the kinetic models. Using these models resulted in significant reduction in computational load. In the present study, we introduce two new models and associated methodologies. The first is the Laguerre-Volterra Network using Artificial Neural Networks (LVN-ANN) which implements the LVN model within the Keras deep learning framework. The second is the Laguerre-Volterra Network Look-Up Table (LVN-LUT) in which specific waveforms are stored in a pre-computed look-up table thereby replacing costly operations with computationally fast memory access. The results we obtained indicate that the LVN-ANN is capable of significantly reducing model training time by a factor of 40 when compared to the previous LVN model implementation, while also slightly improving accuracy. Additionally, the LVN-LUT model further reduces simulation time, offering over 10-fold speed increase over our previously fastest model, while yielding similar accuracy levels. Here, we present our results and demonstrate how using such methodologies enable us to extend simulations to large-scale network-level models that include a very large number of synapses, all while maintaining biologically realistic accuracy and efficient runtimes.

Disclosures: D.J. Pham: None. **J.C. Bouteiller:** None. **G.J. Yu:** None. **D. Song:** None. **T.W. Berger:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.11/DD16

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

Support: NIH Grant U01GM104604
NIH Grant 9U01EB025830
ONR Grant N00014-13-1-0211

Title: A numerical approach to calculating LFPs in multi-scale neural network models

Authors: C. S. BINGHAM[1], C. GIRARD[2], J. PAKNAHAD[3], D. SONG[1], ***J.-M. C. BOUTEILLER**[4], G. LAZZI[3], T. W. BERGER[1];

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Abstract: Significant progress has been made toward model-based prediction of hippocampal activation in response to extracellular electrical stimulation, but challenges remain in the accurate and efficient estimation of local field potentials (LFP). Analytical methods of estimating electric fields are a first-order approximation that may be suitable for model validation but they are computationally expensive and cannot accurately capture boundary conditions in heterogeneous tissue. While there exist many appropriate numerical methods of solving electric fields in neural tissue models, there isn't an established standard for mesh geometry nor a well-known rule for handling any mismatch in spatial resolution or alignment between current sources and mesh nodes in a finite-element or resistor-network method volume conduction model. Therefore, using a previously published and validated multi-scale model of the hippocampus, the authors have formulated an algorithm for bidirectional communication between Admittance Method (AM) volume conduction models and biologically detailed neural circuit models constructed in NEURON. Development of this algorithm required that we assess meshes of (i) unstructured tetrahedral and grid-based hexahedral geometries as well as (ii) differing approaches for managing the spatial misalignment of current sources and mesh nodes. The resulting algorithm is validated through the comparison of AM-predicted evoked potentials with analytically estimated LFPs. Establishing this method is a critical step toward closed-loop integration of the AM and NEURON models that could lead to substantial improvement of the predictive power of multi-scale models of cortical tissue. These models may be used to deepen our understanding of hippocampal pathologies and the identification of efficacious electroceutical treatments.

Disclosures: C.S. Bingham: None. **C. Girard:** None. **J. Paknahad:** None. **D. Song:** None. **J.C. Bouteiller:** None. **G. Lazzi:** None. **T.W. Berger:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.12/DD17

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

Support: NIH Grant U01 EB025830
ONR Grant N00014-13-1-0211

Title: Towards synaptic plasticity in a computational, large-scale network of hippocampus: An investigation of spike-timing dependent plasticity models

Authors: *Z. Z. CHOU[1], G. J. YU[1], T. W. BERGER[2];
[2]Biomed. Engin., [1]USC, Los Angeles, CA

Abstract: The function of the hippocampus is definitively implicated with memory and requires synaptic plasticity to achieve this function. However, the exact role of the hippocampus with respect to memory is still unknown. We have been developing a computational platform with which a large-scale, spiking neuronal network model of a hippocampus can be simulated, and previous work has focused on the construction of the topographic connectivity that would provide the neural architecture of a hippocampal network. This previous work did not include synaptic plasticity. In the present work, we compare different methods for implementing spike-timing dependent plasticity (STDP) in a large-scale CA3 network consisting of an associational system and entorhinal and dentate inputs. Three different STDP rules, developed by other groups, were investigated: additive, multiplicative, and log-normal STDP. Additive STDP uses an update rule in which the synaptic weight is updated with an addition or subtraction amount that is dependent on the timing difference between presynaptic and postsynaptic spikes. Multiplicative STDP modifies the update by multiplying the current synaptic weight by a factor. Finally, log-normal STDP implements a customized update function, based on the Fokker-Planck equation, that produces a long-tail distribution of synaptic weights, with a small number of synapses having very large weights. It has already been shown that these three different rules have trade-offs in the stability of weights and synaptic competition (Gilson and Fukai, 2011). Here, we investigate further by exploring how the evolution and convergence of synaptic weights under different rules affect the spatio-temporal dynamics of a large-scale network. In combination with the previously developed large-scale model of rat hippocampus, the present work with synaptic plasticity will result in a computational platform through which the effects of synaptic plasticity, and its interaction with other properties of the hippocampus, on learning and memory can be investigated.

Disclosures: Z.Z. Chou: None. **G.J. Yu:** None. **T.W. Berger:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

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Program #/Poster #: 431.13/DD18

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

Support: NIH Grant U01GM104604
NIH Grant 9U01EB025830
ONR Grant N00014-13-1-0211

Title: Computational modeling of somatic calcium release due to cholinergic modulation in CA1 pyramidal cells

Authors: *A. MERGENTHAL, J.-M. BOUTEILLER, T. BERGER;
Biomed. Engin., USC, Los Angeles, CA

Abstract: Cells throughout the hippocampus are subject to cholinergic modulation. Depending on the cell type, the result of this modulation can vary depending on the receptor types expressed. In the CA1, the pyramidal cells express M_1 and M_3 muscarinic acetylcholine receptors (mAChRs). The activation of M_1/M_3 mAChRs initiates two chain reactions which modulate cell excitability. These reactions include an inhibition of ion channels that depolarize the cell membrane and the release of calcium from intracellular stores. These effects are well documented, but how these translate into changes in overall network activity is still poorly understood. Yet, these network-level effects are important as they may shed some light on a variety of disorders associated with the widespread dysregulation of cholinergic modulation (e.g. Alzheimer's disease). To this effect, we have been working toward developing a network model of the CA1 region by implementing single cell models of multiple cell types. We herein discuss our efforts aimed at modeling the release of calcium from intracellular stores in the somatic region of pyramidal neurons. We detail the steps taken to tune this model and demonstrate the model's ability to faithfully replicate dynamics observed in in-vitro experiments. Among the behaviors replicated are the ability for acetylcholine to inhibit spiking due to activation of calcium-gated potassium (SK) channels, intracellular store depletion after repeated activation, and increased calcium concentrations following action potentials restocking intracellular stores. We discuss our results, list future steps, and elaborate on the overall perspectives of this effort.

Disclosures: A. Mergenthal: None. **J. Bouteiller:** None. **T. Berger:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.14/DD19

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

Support: NIH Grant U01 EB025830
ONR Grant N00014-13-1-0211

Title: A large-scale neuronal network model of the trisynaptic pathway of rat hippocampus

Authors: *G. J. YU, J.-M. C. BOUTEILLER, D. SONG, T. W. BERGER;
Biomed. Engin., USC, Los Angeles, CA

Abstract: The axonal projections between neural populations are often anatomically organized resulting in ordered topographic maps that determine the connectivity of the neural system. They are seldom, if at all, connected uniformly random or all-to-all. The hippocampus is a system that

clearly demonstrates a higher-level organization as described by the trisynaptic circuit in which activity predominantly propagates in a feedforward manner through the three hippocampal subfields. In addition, each projection exhibits its own distinct topography. However, there have been few studies that investigate how the individual topographies affect the dynamics of each subfield and the activity transformations that each subfield performs.
We have developed a computational platform for simulating the full-scale, in terms of numbers of neurons and synapses and the geometric volume, of a rat hippocampus. Using this platform, we have constructed a neuronal network using the NEURON simulation environment which incorporates the unique topographies of the perforant path projection, the mossy fiber projection, the CA3 associational system, and the Schaffer collaterals. In this work, we created a network composed of 112 000 entorhinal cortical cells, 120 000 granule cells, 25 000 CA3 pyramidal cells, and 38 000 CA1 pyramidal cells that include basket cells for each subfield. The geometric area for the axon terminal fields for each projection were constrained based on anatomical studies of the respective axonal distributions. The initial perforant path projection was shown to organize random Poisson activity into finite regions of spatially and temporally dense spiking activity that we called clusters. These clusters persisted throughout the trisynaptic pathway in various forms as they were propagated through the network. Topography was ultimately found to determine the spatial extent of the clusters, predominantly along the longitudinal axis of the hippocampus. This was further quantified as the longitudinal distance at which neuronal spiking could be correlated.
The results of this work demonstrate a clear role for topography in determining the spatio-temporal dynamics of a neural system. The work also establishes a neuronal network model that can be used to investigate the trisynaptic pathway at a scale that encompasses the entire rat hippocampus.

Disclosures: **G.J. Yu:** None. **J.C. Bouteiller:** None. **D. Song:** None. **T.W. Berger:** None.

Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.15/DD20

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

Support: NIH Grant 5R01MH110932
NSF NeuroNex MINT Grant 1707316

Title: NTracer2: An ImageJ/Fiji plug-in for multispectral neuronal tracing and annotation in densely labeled brains

Authors: ***L. A. WALKER**[1], H. CHENG[2], A. DIZAJI[2], F. Y. SHEN[3], Y. LI[2], M. CHEN[2], D. CAI[2,1,3];

[1]Biophysics, [2]Dept. of Cell and Developmental Biology, Med. Sch., [3]Neurosci. Grad. Program, The Univ. of Michigan, Ann Arbor, MI

Abstract: Techniques for neural tracing and reconstruction plays an important role in our understanding of nervous system development. Multispectral labeling techniques, such as Brainbow [Livet, et. al, Nature 2007; Cai, et. al, Nat. Methods 2013], allow the tracing of densely-labeled neurons by expressing different, random combinations of fluorescent proteins in each cell. These sample can then be imaged with, for example, a confocal or lightsheet fluorescence microscope. While many automated techniques exist for the reconstruction of sparsely labeled neurons from optical microscopy data, no automated approach is currently capable of properly handling the multichannel nature of the Brainbow datasets. To address this, we created nTracer [Roossien, et. al, Bioinformatics 2019], a semi-manual tracing and annotation tool for the analysis of Brainbow datasets. nTracer runs as a plugin to the ubiquitous ImageJ/Fiji software, performing path-optimized tracing based on user-supplied starting and ending points along individual cell bodies or processes. Uniquely, our model includes color, in addition to position, as optimization dimensions, allowing the color of the cell to guide the tracing. We have updated the software (nTracer2) with algorithmic performance upgrades, support for terapixel-scale images via the HDF5 file format, multi-tracer collaboration, and an expanded toolset for the registration and normalization of tiled images. Additionally, user interface components have been optimized according to user feedback, leading to a more efficient workflow overall. Together, these changes allow nTracer to be applicable to much larger experiments than previously reported, such as the manual proofreading of whole-brain imaging experiments. In this interactive presentation, we will discuss these new developments to nTracer and provide a live demonstration of the utility it provides to scientists studying neuronal structure.

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Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.16/DD21

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

Support: NIH NS107148
NIH NS079507

Title: Toward high-throughput, non-invasive seizure detection using piezoelectric motion sensors

Authors: *D. HUFFMAN[1], F. DUQUE QUICENO[1], S. CARRIZOSA[1], A. AJWAD[1], J. WANG[1], E. JOHNSON[2], K. HARGIS-STAGGS[2], K. DONOHUE[3,6], B. F. O'HARA[4,6], B. BAUER[5], E. BLALOCK[2], S. SUNDERAM[1];
[1]Biomed. Engin., [2]Pharmacol. and Nutritional Sci., [3]Electrical and Computer Engin., [4]Biol., [5]Pharmaceut. Sci., Univ. of Kentucky, Lexington, KY; [6]Signal Solutions, LLC, Lexington, KY

Abstract: Outcomes in preclinical models of epilepsy-i.e., the likelihood and timing of epileptogenesis, seizure yield and frequency—can be quite variable. It is often desirable for experimental studies to commence only after spontaneous seizures have been verified and their frequency has stabilized. This determination is complicated by the diversity of seizure-related behaviors and the unpredictability of seizure recurrence. While invasive electroencephalography (EEG) is the standard for accurate seizure detection, it is resource-intensive and impractical in large cohorts. Visual observation or video analysis, while non-invasive, are labor-intensive and tedious. Thus, convenient non-invasive automated methods for seizure screening in animal models are desirable. Here, we investigate the utility of a piezoelectric ("piezo") motion sensor for noninvasive seizure screening in two rodent epilepsy models: mice and rats. Mice and rats of both sexes were treated with pilocarpine i.p. to induce acute status epilepticus (SE). In this model, seizures typically subside within 1-2 hours post-injection and spontaneously recurring seizures—the hallmark of chronic epilepsy—may emerge after a latent period of several weeks. Animals that survived SE (12 mice; 7 rats) were monitored continuously for 12 weeks in individual cages, where signals from the piezo sensors and continuous video were collected. Piezo signals were screened weekly for seizures using an algorithm that responded to significant deviations in piezo signal features from a moving baseline. Video review of the detections enabled timely identification of animals that developed chronic epilepsy. This demonstrates the feasibility of noninvasive epilepsy screening without exhaustive review of video. Animals with good seizure yield were then instrumented with EEG headmounts and, after recovery from surgery, monitored for four more weeks with simultaneous EEG, piezo, and video recording. Seizures detected from the EEG were used as the truth set to validate the performance of the piezo detection algorithm. In mice, the piezo detector gave a maximum sensitivity of 80% to EEG-verified seizures but at the cost of low precision (11%). Nevertheless, this greatly reduced the amount of data to be reviewed to about 20 minutes per 24-hour period. Using a supervised logistic classifier for the rats, sensitivity and precision reached peaks of 68 and 62% respectively. Efforts to improve these preliminary results, such as feature selection and algorithm development, are ongoing.

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Poster

431. Computational Tools for Neuronal Mapping, Activity, and Networks

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 431.17/DD22

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

Support: NSF OAC-1730655
NSF ENG-1801666

Title: Software automation for research and training in neural engineering

Authors: *S. S. NAIR[1], T. BANKS[2], B. LATIMER[2], Z. CHEN[1], Z. LYU[2], Z. CHEN[2], D. DOPP[3], A. FOTOOHIGHIAM[6], P. CALYAM[4], T. JOSHI[5], D. XU[4];
[1]Electrical Engin. and Computer Sci., Univ. of Missouri Columbia, Columbia, MO; [2]Univ. of Missouri, Columbia, MO; [3]Univ. of Missouri, columbia, MO; [4]Electrical Engin. and Computer Sci., [5]Hlth. Mgmt. and Informatics, Univ. of Missouri, Columbia, MO; [6]Rock Bridge High Sch., Columbia, MO

Abstract: Research and training in neural science and engineering necessitates the use of heterogeneous software and data analytic tools, multiple data archives and computing resources. Effective use of such resources and technologies is essential for bold advances in neuroscience, including bridging across the various ‘levels’ in neuroscience. However, to accomplish this, neuroscientists (often with limited software skills) are required to take valuable time away from their focus on knowledge discovery, to learn how to use such technology. Development of appropriate software automation and cyberinfrastructure (CI) technologies hold significant promise to aid the neuroscientist in this regard on both research and training fronts. We report the results from an on-going effort to develop automated modules of multiple types with application across the K-20 spectrum. The automation modules for research and training include the following: transcriptomics (RNAseq) analytic tool, and a job submission tool named SimAgent. The transcriptomics tool automates usage of genomic and molecular databases in neuroscience, HPC resource brokering options, and visualization tools, to simplify the diverse tasks for the neuroscientist. The SimAgent tool has two core functions of automated job submission and parameter sweep, with a point and click interface that accepts any project directory, submits the project files from a laptop and configures them to run remotely and watches it until completion with live updates to the user. The parameter sweep feature allows the same functionality with the added ability to specify sections of code to automatically change with each run. SimAgent presently supports NEURON and MATLAB scripts and python code. A series of Jupyter notebook modules for teaching and self-learning have been developed for cellular neuroscience topics such as Nernst and rest potential, spike generation, bursting, synaptic transmission, and the development of small networks. Another set of modules cover the

fundamentals of neural signals and digital signal processing concepts such as Fast Fourier Transforms (FFTs), band pass filters, power spectral densities, and local field potentials. A JupyterHub server was established on the JetStream cloud computing platform to host several of the teaching notebooks. Some of the K-12 neural engineering modules for teacher training will also be presented. One of these focuses on the basics of brain waves using python notebooks after a hands-on introduction to the topic using scalp-recording kits from BackyardBrains.com. This project was supported in part is supported by the grant NSF grants OAC-1730655 and ENG-1801666.

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Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.01/DD23

Topic: I.07. Data Analysis and Statistics

Support: NSF 1840218

Title: Enabling data reproducibility with open science chain

Authors: *S. SIVAGNANAM, V. NANDIGAM, K. LIN, S. SAKAI;
San Diego Supercomputer Ctr., UCSD, San Diego, CA

Abstract: In neuroscience large amounts of experimental and imaging data are produced in a wide variety of formats and are utilized to develop data-driven computational models for elucidating neuronal and network functions. Significant advances in brain research have been made, however investigators working with these massive data require techniques to ensure its integrity, especially when trying to build upon prior research work done by other scientists. Reproducibility is fundamental to scientific inquiry and independent verification of results is an essential component. Several journals and funding agencies mandate that data and experimental details be made accessible and archived to allow for replication and consequent studies. However several independent studies show that the data withholding rates remain constant for various reasons including protecting scientific lead, and fear of misinterpretation of data. There are no cyberinfrastructure resources available for researchers to independently validate the authenticity of datasets, track the provenance and lineage of the data, and verify ownership information. Open Science Chain (OSC) provides a cyberinfrastructure platform, built using distributed ledger technologies, where verification information about scientific dataset is stored and managed in a consortium blockchain. OSC provides a unique verification identifier for the information stored

on blockchain. Other researchers have the ability to independently verify authenticity of scientific data using the information stored in the blockchain and provide feedback when the data cannot be validated.
In the initial implementation of OSC, the location of the dataset, information to verify the dataset (SHA256 hash) and the contributor information are required as the mandatory parts of the metadata but will gradually expand to other metadata elements based on feedback from the user community. In order to lower the complexity barrier for use of this technology and to promote adoption, a web-based portal is developed with seamless user interfaces and multi-platform client for interacting with the underlying blockchain. Neuroscience researchers, especially those involved in collaborative research will benefit from using the OSC to track the dataset that maybe generated and maintained at various locations. Members of research labs will benefit from tracking the provenance and lineage of data produced and referenced during different stages of research by various members. OSC aims to enhance data sharing and reproducibility in the neuroscience community by increasing the confidence of the scientific results.

Disclosures: **S. Sivagnanam:** None. **V. Nandigam:** None. **K. Lin:** None. **S. Sakai:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.02/DD24

Topic: I.07. Data Analysis and Statistics

Support: NIH NS074540
NIH EB023398

Title: NIH funded nitrc's triad of services: Software, data, compute

Authors: *D. N. KENNEDY[1], C. HASELGROVE[2], N. PREUSS[3];
[1]U. Massachusetts Med., Worcester, MA; [2]Univ. of Massachusetts Med. Sch., Worcester, MA; [3]Preuss Enterprises, Miami, FL

Abstract: Aim: NeuroImaging Tools and Resources Collaboratory (NITRC) is a neuroinformatics knowledge environment for MR, PET/SPECT, CT, EEG/MEG, optical imaging, clinical neuroinformatics, computational neuroscience, and imaging genomics tools and resources.
Methods: Initiated in 2006 through the NIH Blueprint for Neuroscience Research, NITRC's mission is to foster a user-friendly knowledge environment for the neuroinformatics community. By continuing to identify existing software tools and resources valuable to this community, NITRC's goal is to support its researchers dedicated to enhancing, adopting, distributing, and contributing to the evolution of neuroinformatics analysis software, data, and compute resources.

Results: Located on the web at www.nitrc.org, the Resources Registry (NITRC-R) promotes software tools and resources, vocabularies, test data, and databases, thereby extending the impact of previously funded, neuroimaging informatics contributions to a broader community. NITRC-R gives researchers greater and more efficient access to the tools and resources they need, better categorizing and organizing existing tools and resources, facilitating interactions between researchers and developers, and promoting better use through enhanced documentation and tutorials—all while directing the most recent upgrades, forums, and updates. All services freely downloadable, NITRC-R offers over 1,090 public resources; NITRC-Image Repository (NITRC-IR) offers 16 data projects, 10,860 subjects, and 12,440 imaging sessions, and NITRC Computational Environment (NITRC-CE) provides cloud-based computation services downloadable to local machines or via commercial cloud providers such as Amazon Web Services.
Conclusions: In summary, NITRC is now an established knowledge environment for the neuroimaging community where tools and resources are presented in a coherent and synergistic environment. NITRC is a trusted source for the identification of resources in this global community. With over 6,230 citations on Google Scholar, NITRC has supported over 26,000 registered users, served up 10.8 million total, and of that, 9.7 million data downloads, to over 1.1 million users generating 2.5 million sessions. In addition to untold downloaded Virtual Machines, NITRC-CE currently supports over 180 subscriptions on AWS Marketplace running over 216,400 compute hours. We encourage the neuroinformatics community to continue providing valuable resources, design and content feedback and to utilize these resources in support of data sharing requirements, software dissemination and cost-effective computational performance.

Disclosures: D.N. Kennedy: None. **C. Haselgrove:** None. **N. Preuss:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.03/DD25

Topic: I.07. Data Analysis and Statistics

Title: Schol-AR an augmented reality platform for scientific communication and data visualization

Authors: *T. ARD, A. W. TOGA;
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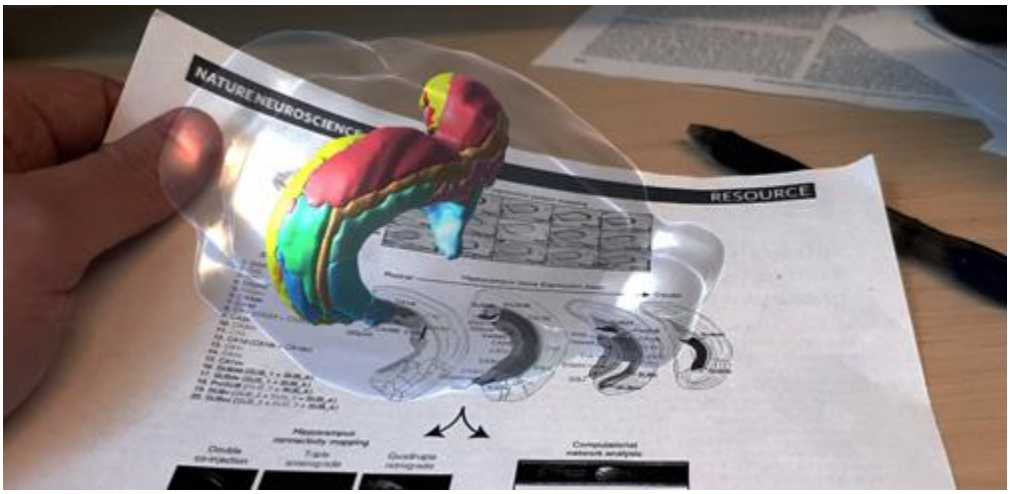
Abstract: Scientific communication is dominated by the printable media of journals and papers, which have not kept pace with the rapid technological evolution of the ways we view information and communicate. While several attempts have been made to amend this, such as

providing links to data and expanded supplementary materials, we have largely failed to adopt digital media in standard scientific communication. This dramatically impedes the exchange of concepts and information, particularly as modern scientific fields are producing more complex data and results which remain constrained and simplified by what can be represented by the standard publishing of 2D static images.

Here, we present 'Schol-AR,' an augmented reality (AR) mobile application designed to provide printable scientific communications with the ability to seamlessly integrate digital media. Schol-AR enables you to point a smartphone at a scientific figure and have any accompanying digital media associated with that figure appear on the phone, 'augmented' over the printed figure. This approach combines the advantages and establishment of printable media with interactive 3D objects, video, original data, and more. The fluidity of this approach remedies critical issues of previous attempts such as needing specific software to view data, cumbersomely tracking down supplementary links and materials, and overall ease of access.

While the possible uses of this approach are numerous, one example is augmenting a single-slice printed image from a functional MRI into a full neuroimaging volume, showing the entire brain volume over time. Additionally, complex anatomy such as arterial networks which are simplified onto a projected 2D printed image can be augmented to a 3D interactive object, presenting the data in all of its structure and detail. Overall, our approach aims to support the modernization of scientific communication, offering seamless integration of complex data and digital media with standard printable communication.



Disclosures: **T. Ard:** None. **A.W. Toga:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.04/DD26

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 1R24MH116922-01

Title: Neurodata without borders: Neurophysiology as a cross-species standard for electrocorticography

Authors: *B. K. DICHTER[1], M. DOUGHERTY[2], V. BARATHAM[3], K. NASIOTIS[4], A. TRITT[5], O. RUEBEL[5], E. F. CHANG[6], K. E. BOUCHARD[7];
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Abstract: Electrocorticography (ECoG) provides a rare opportunity to perform comparative analyses between humans and other species; however, sharing these datasets between the research communities is nascent, and there is a need for a neural data tools that facilitate cross-species data sharing, analyses and collaboration. Here, we demonstrate the use of Neurodata Without Borders: Neurophysiology (NWB:N) 2.0 format (Ruebel et al. 2019) for storage and analysis of ECoG data from humans and rats.
In human ECoG data, the precise position of each electrode with respect to the gyral patterns of the cortex is essential information, and since gyral patterns differ from subject to subject, the shape of the cortex must be stored for each. We present an extension to NWB:N 2.0 that allows for storage of cortical and subcortical surfaces as triangular meshes, as well as the position of the ECoG electrodes. We demonstrate high-quality rendering of 3D cortical surfaces in MATLAB using Brainstorm (Nasiotis et al. 2019), and in python using img_pipe (Hamilton et al. 2017).
Spectral decomposition is a standard analytical technique applied to ECoG data, allowing neuroscientists to study fluctuations of neural activity across both broad and fine spatial scales. We leverage recent advances in NWB:N 2.0 that facilitate storage of time-frequency decomposition with the necessary meta-data for analysis, visualization, and sharing with collaborators. We demonstrate the utility of NWB:N 2.0 in cross-species comparisons by performing a Hilbert transform spectral decomposition to data recorded from micro-ECoG arrays in rats and high-density ECoG arrays in humans. By analyzing these datasets side-by-side, we can precisely identify differences between species, motivating further modeling work to explain why such differences occur.

Disclosures: **B.K. Dichter:** None. **M. Dougherty:** None. **V. Baratham:** None. **K. Nasiotis:** None. **A. Tritt:** None. **O. Ruebel:** None. **E.F. Chang:** None. **K.E. Bouchard:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.05/DD27

Topic: I.07. Data Analysis and Statistics

Title: Canonical pipelines for neurophysiology in DataJoint

Authors: S. SHEN[1], **T. T. NGUYEN**[1], *D. YATSENKO[2,1], E. Y. WALKER[2,1], J. REIMER[2], A. S. TOLIAS[2], Z. V. GUO[3], H. INAGAKI[3], M. N. ECONOMO[3], S. A. HIRES[3], J. YU[3], N. LI[3,2], K. SVOBODA[3];
[1]DataJoint Neuro, Vathes LLC, Houston, TX; [2]Neurosci., Baylor Col. of Med., Houston, TX; [3]HHMI / Janelia Farm Res. Campus, Ashburn, VA

Abstract: Many neuroscience research teams have designed specialized and efficient data pipelines for the collection and processing of experiment data with the use of DataJoint — a free, open-source framework for scientific databases and computational pipelines. Even in similar studies, their data pipelines vary in their design to accommodate lab-specific configurations and workflows. To provide starting points for new projects and to promote greater interoperability across groups, we introduce a collection of Canonical Pipelines for common neurophysiology data modalities. The Canonical Pipelines borrow best design elements from leading projects and include animal management, trial-based sensory stimulation, anatomical imaging, trial-based behavior, trial-based perturbation experiments (including optogenetics), extracellular and intracellular electrophysiology, and calcium imaging. Through modularity and parameterization, the Canonical Pipelines strike a careful balance between flexibility and standardization. Modules may be combined and extended to suit the specific evolving needs of active projects. Alternative preprocessing and analysis computations may be used. Principled data organization during experiments simplifies data sharing with collaborators for uniform analysis, publishing, and archiving. The Canonical Pipelines include examples of exporting data into the NeuroData Without Borders format NWB 2.0. We envision that the Canonical Data Pipelines will serve as starting points for accelerated development in collaborative research projects, evolving to reflect new experimental paradigms.

Disclosures: S. Shen: None. **T.T. Nguyen:** None. **D. Yatsenko:** None. **E.Y. Walker:** None. **J. Reimer:** None. **A.S. Tolias:** None. **Z.V. Guo:** None. **H. Inagaki:** None. **M.N. Economo:** None. **S.A. Hires:** None. **J. Yu:** None. **N. Li:** None. **K. Svoboda:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.06/DD28

Topic: I.07. Data Analysis and Statistics

Title: How to collect genuine TEPs: A graphical user interface to control data quality in real-time

Authors: *S. CASAROTTO, M. FECCHIO, S. PARMIGIANI, S. SARASSO, C. C. DERCHI, A. MAZZA, A. VIGANÒ, T. NIEUS, M. MASSIMINI, M. ROSANOVA;
Univ. of Milan, Milan, Italy

Abstract: Electroencephalography (EEG) combined with transcranial magnetic stimulation (TMS) represents a unique tool to investigate brain potentials (TMS-evoked potentials - TEPs) evoked by a direct and non-invasive perturbation of cortical neurons. Obtaining genuine TEPs involves minimizing artifacts (magnetic pulse, muscle activation) and confounding factors (the effects of concurrent acoustic and somatosensory stimulation) while maximizing the direct impact of TMS on cortical circuits. The latter, depends on several factors that a priori are hard to control for, including coil design, E-field orientation with respect to axons, 3D morphology and cytoarchitectonics of the underlying cortical tissue in individual brains. We present a Matlab-based Graphical User Interface (GUI) that provides an optimal online data visualization tool allowing the experimenter to simultaneously minimize the contribution of artifacts and confounding factors while maximizing the signal-to-noise ratio of brain responses during data collection. Specifically, this GUI allows i) to remove the pulse artifact otherwise masking the early electrophysiological responses; ii) to visually inspect single-trial responses in order to rule out the presence of unwanted muscle activation or capacitive discharging artifacts; iii) to dynamically reject artifact-contaminated channels during the recordings; iv) to compute the average reference from artifact-free channels only; v) to display and measure the amplitude of the initial EEG response (first 50 ms) from the electrodes under the coil (the initial impact of TMS) as well as the time course of average TEPs according to their topographical distribution on the scalp, thus enabling the user for a real-time assessment of the presence/absence of confounding factors. We show that maximizing cortical impact using the GUI results in TEPs that are specific for the angle and site of stimulation and that are very different, at both early and late latency, from those obtained from the peripheral evoked potentials evoked by realistic sham (Conde et al., Neuroimage 2019). This GUI represents a fundamental software complement to any TMS-compatible EEG system providing appropriate visualization features for assessing data quality in real-time. In its present version, the GUI is compatible with different EEG amplifiers from BrainProducts, ANT and GTEC companies and will be released under the CC BY-NC-SA license. This GUI allows employing EEG as an immediate readout for an optimal real-time

titration of the stimulation parameters to collect genuine TEPs and to foster standardization within the TMS/EEG community.

Disclosures: **S. Casarotto:** None. **M. Fecchio:** None. **S. Parmigiani:** None. **S. Sarasso:** None. **C.C. Derchi:** None. **A. Mazza:** None. **A. Viganò:** None. **T. Nieus:** None. **M. Massimini:** None. **M. Rosanova:** None.

Poster

432. Software Tools: Analysis II

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Program #/Poster #: 432.07/DD29

Topic: I.07. Data Analysis and Statistics

Support: NSF/EPSCoRgrant #1632849
nVIDIA GPU grant

Title: Delineate: A deep learning toolbox for neuroscientists

Authors: ***K. M. KUNTZELMAN**[1], J. M. WILLIAMS[2], A. SAMAL[2], P. K. RAO[2], M. R. JOHNSON[3];
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Abstract: Contemporary approaches to the analysis of neuroscience data often make use of machine learning (ML) classifiers in order to determine whether there is information present in a complex multivariate signal that can reliably distinguish between cognitive/behavioral states or experimental conditions in a manner unconstrained by an experimenter's preconceptions. In neuroimaging such techniques are most frequently referred to as multivariate pattern analysis (MVPA), although similar approaches are used in other fields, sometimes going by other names. The relatively simple linear algorithms that underlie most such analyses, however, are computationally inadequate for large datasets, and in at least some domains (e.g., image recognition) easily outperformed by deep neural networks (DNNs). Consequently there has been a recent surge of interest in approaches to data analysis that capitalize on these "deep learning" techniques. Still, adoption of DNNs within the neurosciences has been slowed by the greater complexity of DNN architectures, the programming expertise required to use current DNN tools, and the lack of built-in support for specific methodological requirements of the neuroimaging and systems neuroscience communities. To address these issues, we have created an open-source Python toolbox called Deep Learning In Neuroimaging: Exploration, Analysis, Tools, and Education (DeLINEATE). Notable features include support for several cross-validation and rescaling schemes; a text-based job description file format that requires no Python coding knowledge from users; and backend support for PyMVPA, enabling direct comparisons to

traditional techniques. Across multiple datasets analyzed with DNNs, we demonstrate advantages in computational speed and/or classification performance relative to traditional ML/MVPA techniques. DNNs can also implement novel forms of classifier and tackle research questions that were unavailable to older methods. The current release is available on the project website (http://delineate.it) and all project code is hosted on Bitbucket (https://bitbucket.org/delineate/delineate/src/master/). Development is ongoing and we invite feature requests from the community.

Disclosures: **K.M. Kuntzelman:** None. **J.M. Williams:** None. **A. Samal:** None. **P.K. Rao:** None. **M.R. Johnson:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.08/DD30

Topic: I.07. Data Analysis and Statistics

Support: The Scientific and Technological Research Council of Turkey (TUBITAK), Grant No: EEEAG-117E286

Title: A low-cost fast scan cyclic voltammetry system for measurement of striatal dopamine concentration in behaving rats

Authors: R. RIAZ[1], M. HAZIQ[1], A. AYYAZ[1], S. ZAIDI[1], ***M. KOCATURK**[1,2];
[1]Deparment of Biomed. Engin., [2]Regenerative and Restorative Med. Res. Ctr. (REMER), Istanbul Medipol Univ., Istanbul, Turkey

Abstract: Phasic changes in the activity of dopamine neurons and the ensuing alterations in the dopamine concentration in the target structures are thought to have critical roles in reinforcement learning, goal-directed behavior and locomotion. Fast scan cyclic voltammetry (FSCV) enables detection of dopamine release in the structures innervated by dopaminergic axons with high, subsecond temporal resolution. In this work, we present a low-cost FSCV system for the longitudinal measurement of dopamine in the rat striatum chronically implanted with carbon fiber microsensors. The hardware has been implemented using a microcontroller and a combination of analog integrated circuits (ICs) in order to generate triangular voltammetric waveforms. The timing of the voltammetric waveforms are controlled by a phase-locked loop so that the noise caused by the frequency drifts in the power line can be eliminated. The voltammetric current through the microsensor is converted to voltage, amplified and filtered using the present hardware and then fed to a data acquisition card on a PC. Applications developed using LabVIEW and MATLAB are used to control the data acquisition hardware and the behavioral experiments. The electrically controlled components in the behavioral

environment are optically isolated so that the voltammetric signal drifts caused by these components are eliminated. In order to validate the practicality of the system, we chronically implanted one rat with a carbon fiber microsensor in the ventromedial striatum (VMS) and recorded the voltammetric signals in response to presentation of unexpected primary rewards. Based on the recordings performed 176 days after the implantation surgery, the increase in the dopamine concentration in response to presentation of 0.16 ml 30% sucrose solution rewards was measured as 11.85±8.75 nM (n = 20 trials). Our results based on the longitudinal recordings from one rat indicate that phasic increases in dopamine concentration in the VMS during behavior can be detected using the present system.

Disclosures: R. Riaz: None. **M. Haziq:** None. **M. Kocaturk:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

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Program #/Poster #: 432.09/DD31

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant 1515587

Title: V-NeuroStack: 3-D time stacks for finding patterns in spontaneous activity of neurons in mouse brain slice

Authors: *A. G. NAIK[1], R. V. KENYON[2], T. BERGER-WOLF[2], B. IBRAHIM[3], D. A. LLANO[3];
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Abstract: V-NeuroStack is a web application which uses 3-D time stack approach to identify patterns in the neuronal activity and to understand functional relationships between neurons. Our research group aims to understand the auditory corticothalamic activity in the mouse. We used GCaMP6s calcium imaging to capture spontaneous activity of cortical neurons. V-NeuroStack uses a point cloud to visualize the behavior of neurons during spontaneous activity while also having the capability to drill down on a neuron at a single timestep. In V-NeuroStack we assemble the 2-D movie frame data captured during spontaneous activity to visualize it as a 3-D time stack that the user can manipulate using given controls. Additionally, for every cross-sectional frame of the 3-D time stack, we generate a 2-D projection of the data at any time step which may then be explored for detailed analysis of the activity. A line graph depicting the intensities over the entire set of timesteps can be generated by clicking on a neuron in the 2-D projection. Button and slider controls can be used to change the portion of data used for

visualization and a user interactive pointing device can be used to manipulate the 3-D time stack. Multiple data files are fed to our application: a timeseries file contains calcium intensities for each neuron at each timestep, an XYcoordinates file contains the location of each neuron on the image. Finally a file for each timestep identifies clusters of like-responding neurons; a Python script is used to extract the color associated with each cluster for the visualization. To generate the point cloud, V-NeuroStack uses the same x and y value for each neuron and populates a new z value to stack the neuronal activity in time. The result is a tube-like structure generated for each neuron for some or all available timesteps in the data. V-NeuroStack is written in JavaScript. In conclusion, we have implemented a tool to view patterns during spontaneous activity in mouse brain tissue that cannot be captured using a single 2-D view of the data. A visible pattern may either show like-colored neurons that belong to a particular cluster during a certain period of the activity or help in identifying strong correlation between the neurons for a given set of timesteps. An accompanying 2D image for any timestep can be viewed to gain greater detail of the neuronal activity. This flexibility in exploring patterns in firing of neurons has the potential to open up further research questions on understanding spontaneous as well as other forms of neuronal activity in any brain region.

Disclosures: A.G. Naik: None. **R.V. Kenyon:** None. **T. Berger-Wolf:** None. **B. Ibrahim:** None. **D.A. Llano:** None.

Poster

432. Software Tools: Analysis II

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Program #/Poster #: 432.10/DD32

Topic: I.07. Data Analysis and Statistics

Support: NSF Grant EF1822476
Huck Institutes of the Life Sciences at Penn State University

Title: Birdsong syllable recognition with sparse spike sequence representations

Authors: *L. E. S. TAVARES, D. Z. JIN;
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Abstract: Birdsongs consist of sequences of stereotypical syllables. For species such as the Bengalese finch and the canary, song sequences are variable and can follow elaborate probabilistic rules. To study the structure and neural basis of birdsongs, it is critical to label large numbers of syllables from continuous recordings of individual birds. Since manual labeling is tedious and time consuming, a number of standard machine learning techniques have been applied with varying degrees of success to automate syllable recognition. Artificial deep neural networks (DNNs) in particular, the state of the art in automatic speech recognition, can be

trained to achieve very low error rates [1,2]. But the high performance comes at the expense of some limiting factors. DNNs require large numbers of pre-labeled training syllables, which directly translates into intensive manual labor. They also often need specialized hardware such as GPUs for training. In practice, most DNN implementations actually rely on sequence information to achieve low error rates. This is undesirable if the goal is to analyze the sequence itself.

Here we present an alternative approach for labeling birdsong syllables from continuous recordings. Most importantly, we have designed our method to minimize the number of training exemplars required. We make no use of sequence information and rely completely on pattern recognition. No special hardware is required.

In our method, we represent relevant sounds by sparse spike sequences of feature-detecting neurons. This approach has been previously shown to perform well in noise-robust speech recognition [3]. Two distinct ensembles of feature detectors are trained by support vector machines (SVMs) to respond to specific acoustic features. Given a continuous recording, one set of non-linear SVMs performs the discovery, segmentation, and initial classification of syllables. Meanwhile, a second set of linear SVMs produces sparse sequences of spikes from the discovered segments, which are then compared to pre-trained templates of spike patterns to check and correct the initial labels. Using only 10 training exemplars per syllable type, we achieve a 5% error rate on Bengalese finch songs. Our system can be efficiently trained and run on basic computers, and requires little technical expertise from the user. As more syllables are classified, our system can be re-trained online to further reduce the error rate.

We have encoded our method into an open-source software in Python for the community to use.

[1] Koumura et. al. (2016). [2] Nicholson and Cohen. Tweetynet. [3] Schafer and Jin. (2014)

Disclosures: L.E.S. Tavares: None. **D.Z. Jin:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.11/DD33

Topic: I.07. Data Analysis and Statistics

Support: DOD Grant W81XWH- 17-C-0032

Title: WAAVES+: An environment- and animal-agnostic automated ultrasonic vocalization scoring tool

Authors: *C. L. DUVAUCHELLE[1], R. SNYDER[3], K. ABER[3], N. MITTAL[4], C. CERVANTES[1], J. BAILEY[2], E. J. PARK[1], W. T. MADDOX[5];
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Abstract: Ultrasonic vocalizations (USVs) are known to reflect emotional processing, brain neurochemistry, and brain function. USVs in the 22-28 kHz and 50-55 kHz frequency range are widely recognized forms of social and emotional expression in rodents. 22-28 kHz USVs reflect negative affect status and are initiated by regional activation along the ascending mesolimbic cholinergic (ACh) pathways. 50-55 kHz USVs reflect positive affect status and are initiated by regional activation along the mesolimbic dopaminergic (DA) pathway. Collecting and processing USV data is manual, time-intensive, and costly creating a significant bottleneck by limiting researchers' ability to employ fully effective, and nuanced experimental designs, and serving as a barrier to entry for other researchers. Duvauchelle et al (2013, 2014) addressed this bottleneck by developing WAAVES, an automated USV assessment program that utilizes a decision tree architecture. Unfortunately, WAAVES requires manual threshold calibration for each unique environmental context reducing its benefit to the wider research community. To expand WAAVES' capabilities and to generalize the tool for widespread use, UT collaborated with Cornerstone Research Group to develop "WAAVES+" (Snyder et al, 2018). WAAVES+ aims to 1) develop an environment-agnostic, automated USV scoring tool that works "out-of-the-box", 2) to automate call classification, and 3) to expand the parameterization of acoustic properties to provide researchers with additional data for investigating significant and behaviorally-relevant outcomes. WAAVES+ uses supervised and unsupervised machine learning techniques to detect, isolate, parameterize, group, and classify USVs. Rapid screening is used to limit complex calculations providing fast analysis while limiting required computing resources. Unlike template matching or neural networking approaches, the WAAVES+ parametric approach provides accurate detection and classification without the need for large training sets or lengthy trial and error environment-specific tuning. WAAVES+ has demonstrated fast (i.e., 20x faster than time-intensive hand scoring), accurate detection (88%) of USVs across three disparate rodent recording environments, as well as over 90% classification accuracy on a subset of calls.

Disclosures: C.L. Duvauchelle: None. **R. Snyder:** None. **K. Aber:** None. **N. Mittal:** None. **C. Cervantes:** None. **J. Bailey:** None. **E.J. Park:** None. **W.T. Maddox:** None.

Poster

432. Software Tools: Analysis II

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Program #/Poster #: 432.12/DD34

Topic: I.07. Data Analysis and Statistics

Support: NSF GRFP
NSF GRFP
DOE/LBNL LDRD, Deep Learning for Science (PI, Prabhat)

Title: Open-source classification of rodent sleep states with a convolutional neural network

Authors: *Z. K. BARGER[1], C. G. FRYE[1,2], D. LIU[3], Y. DAN[3], K. E. BOUCHARD[1,2];
[1]Helen Wills Neurosci. Inst., [2]Redwood Ctr. for Theoretical Neurosci., [3]Mol. and Cell Biol., UC Berkeley, Berkeley, CA

Abstract: Sleep is a fundamental animal behavior and has long been the subject of intensive basic and clinical research. Studying sleep involves determining a subject's sleep state at each point in time. When performed manually, this step is time-intensive, scales poorly with both number of subjects and samples per subject, and could raise issues for reproducibility. Manual sleep state scoring remains prevalent in studies of rodent sleep despite advances in machine learning-based approaches. To make the benefits of machine learning more accessible, we present free, open-source MATLAB and Python implementations of a convolutional neural network architecture that achieves state-of-the-art accuracy for classification of rodent sleep states. Furthermore, we provide tools for interactive data visualization and model retraining. The inputs to the network are composite 'images' of electroencephalogram (EEG) spectrograms and electromyogram (EMG) data. We chose a convolutional neural network design because this class of models excels at image classification and has previously been used to classify human sleep stages from EEG spectrograms. To train the network and validate its performance, we recorded 440 hours of EEG and EMG data from 10 adult C57BL/6 mice during both light and dark cycles. Two expert scorers independently labeled the recordings at 2.5-second resolution. The network's classification accuracy approaches inter- and intra-rater reliability for all three brain states—wakefulness, non-REM sleep, and REM sleep—and comparable accuracy can be achieved with only a few hours of training data. In summary, we built a full-featured, flexible, and scalable sleep state classifier that accelerates data analysis and opens the door to increased data sharing and reproducibility of results.

Disclosures: Z.K. Barger: None. **C.G. Frye:** None. **D. Liu:** None. **Y. Dan:** None. **K.E. Bouchard:** None.

Poster

432. Software Tools: Analysis II

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Program #/Poster #: 432.13/DD35

Topic: I.07. Data Analysis and Statistics

Support: the Basic Science Research (2017R1A2B1009234)
the Brain Research Program (2016M3C7A1947307, 2017M3A9F1030063) of the National Research Foundation (NRF) of Korea
funded by the Ministry of Education, and Hallym University Research Fund (HRF-201707-011 to U.L.)

Title: Automatic and qualitative scoring of interlocking pentagon drawing test based on U-Net and mobile sensor data for a cognitive screening of older adults with Parkinson's disease

Authors: *I. PARK, S. U. MARWAT, I. A. WAHLA, S. KAMAL, U. LEE;
Dept. of Electronic Engin., Hallym Univ., Chuncheon, Korea, Republic of

Abstract: Pentagon Drawing test (PDT) can be used for the prediction of a global cognitive dysfunction as well as the evaluation of praxis function. It has long been used in the assessment of the cognitive function of older adults since it may be possible to suggest the presence of neurodegenera¬tive disease, such as Alzheimer's disease, vascu¬lar dementia, or Parkinson's disease, among others, from apraxia. The interpretation of the PDT is usually binary. Bourke et al. described an easy scoring scale for the PDT with a total of 6 points. Micaela Mitolo et al. developed a qualitative scoring of the PDT (QSPT), which encompasses the assessment of different parameters of the drawing, such as number of angles, distance/intersection, closure/opening, rotation, and closing-in. The QSPT scores were used to compare between groups, in which it suggested that the QSPT might be a sensitive measure of visuo-constructive abilities. However, the scoring needs to be done manually and is prone to human errors. The necessities of automatic scoring have been increased. Therefore, we implemented the PDT as of a mobile phone application and develop a noble automatic scoring method of the mobile PDT based on U-Net, a convolutional networks for biomedical image segmentation, and the mobile sensor data obtained from the mobile PDT. The U-Net was trained with 480 512*512 PDT images to get a trained model for segmenting a right or left pentagon. The U-Net was also trained with 480 512*512 PDT images to get a trained model for segmenting an interlocking figure. Here, epochs were iterated until the accuracy is greater than 98% and it is saturated. The mobile senor data consisted of the coordinates of all the samples with 20ms sampling period extracted from the mobile touch sensor. The velocities and accelerates were then calculated using the coordinates and then smoothed. Number of angles, distance/intersection, closure/opening, rotation, and closing-in were estimated by using the trained model and sensor data, resulting in scaling with total 10 points. The Institutional Review Board of the Hallym University Sacred Heart Hospital approved this study. A total of 57 PD patients and 87 controls recruited. Two mobile PDTs were performed for each subject and the resulted scorings of number of angles, distance/intersection, closure/opening, rotation, and closing-in were compared the grounded truths to those obtained manually and cross-checked by 10 volunteers. The performance showed specificity of 94% and sensitivity of 89%, suggesting our method may be useful in clinical practice or field studies.

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Poster

432. Software Tools: Analysis II

Location: Hall A

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Program #/Poster #: 432.14/DD36

Topic: I.07. Data Analysis and Statistics

Title: A standardized framework for sorting, analysis, and evaluation of extracellular recordings

Authors: *C. L. HURWITZ[1], A. P. BUCCINO[2], J. MAGLAND[3], K. Z. GERLEI[1], M. F. NOLAN[4], M. H. HENNIG[5];
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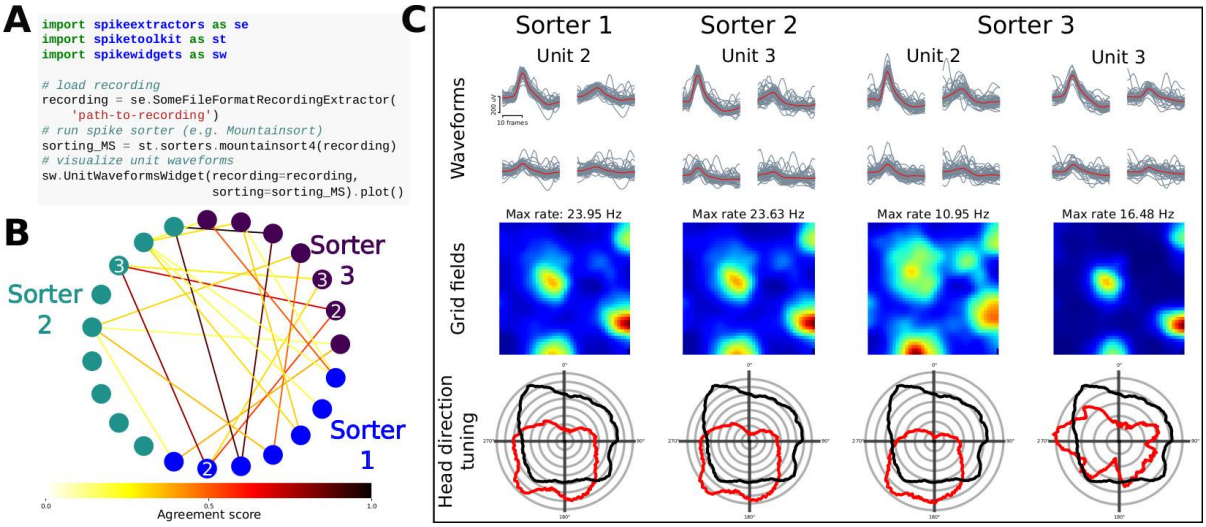
Abstract: Recent breakthroughs in microelectronics have enabled high precision extracellular recording of thousands of neurons both *in vitro* and *in vivo*. While the increased data volume and complexity offers unprecedented opportunities for understanding brain function, it also heightens the need for standardized, reproducible analysis techniques. In particular, inferring the activity of single neurons from raw recorded signal, a blind source separation problem called spike sorting, is an essential preprocessing step in many electrophysiological studies, yet remains largely non-standardized across labs. Since sorting errors can affect subsequent analysis, it is important to establish community-accepted standards by validating and comparing preexisting spike sorting algorithms. Traditionally, running and comparing multiple spike sorting software packages is a laborious process, complicated by file format and algorithm differences. To enable straightforward validation and comparison, we developed SpikeInterface (https://github.com/SpikeInterface), a simple framework for extracting and analyzing relevant information from both raw and spike-sorted extracellular datasets of any established file format. SpikeInterface was designed to standardize how data is retrieved from files, rather than how it is stored, allowing users to access, sort, and analyze extracellular datasets with the same tools, regardless of the underlying file format. This design paradigm also allows for wrapping preexisting spike sorting algorithms within SpikeInterface, making the process of running and comparing multiple spike sorters accessible. We demonstrate SpikeInterface by analysing tetrode recordings from the entorhinal cortex of awake behaving mice with three popular spike sorters. A comparison of the sorted units and their functional characteristics shows how this tool can facilitate more reliable interpretation of extracellular recordings.



Disclosures: **C.L. Hurwitz:** None. **A.P. Buccino:** None. **J. Magland:** None. **K.Z. Gerlei:** None. **M.F. Nolan:** None. **M.H. Hennig:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.15/DD37

Topic: I.07. Data Analysis and Statistics

Support: NIMH R01MH064537
NIMH R37MH087027

Title: Multivariate phase coupling across brain regions

Authors: ***J. ORELLANA**[1], N. KLEIN[1], S. L. BRINCAT[2], E. K. MILLER[3], R. E. KASS[1]; [1]Carnegie Mellon Univ., Pittsburgh, PA; [2]MIT, Arlington, MA; [3]Picower Inst. Learning Memory, Massachusetts Inst. Technol., Cambridge, MA

Abstract: The standard method for analyzing phase coupling in a pair of oscillating local field potentials (LFPs) is Phase Locking Value (PLV), which, under certain conditions, is a natural analogue to correlation for angles. When LFPs are recorded from multiple electrodes across several brain regions, it is possible to examine pairwise interactions by applying PLV to all pairs, but this does not take advantage of the multivariate nature of the recordings and can produce misleading results. We have developed a multivariate method that is much more powerful, producing a complete description of interacting phase relationships in a graphical model, i.e., a set of edges that connect nodes, where each node corresponds to the phase of a particular LFP (at a particular location) and the presence of an edge between two nodes indicates a direct functional

interaction of the corresponding LFP phases. In data from 24 electrodes in prefrontal cortex (PFC) and three subregions of hippocampus, CA3, Dentate Gyrus (DG), and subiculum (Sub), during an associative memory task, we find that PFC is functionally connected to CA3 and Sub but not DG. That is, phase locking between PFC and DG is driven by indirect rather than direct functional connectivity.
Because each phase is an angle on a circle and, mathematically, a product of circles is a torus, we call our graphical models Torus Graphs. We have shown that Torus Graphs are the natural analogue to standard multivariate Gaussian models known as Gaussian Graphical Models (GGM): as in GGM, the absence of an edge between two nodes indicates that the corresponding signals (here, phases) are conditionally independent given all the other nodes. We have also shown that all previous approaches to determining multivariate phase relationships arise as special cases of Torus Graphs under restrictive assumptions. In our numerical simulations, designed to be similar to the real data, Torus Graphs perform extremely well in the sense of correctly identifying presence or absence of direct functional connections.

Disclosures: **J. Orellana:** None. **N. Klein:** None. **S.L. Brincat:** None. **E.K. Miller:** None. **R.E. Kass:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.16/DD38

Topic: I.07. Data Analysis and Statistics

Support: NIH 1R01EB026955-01

Title: Software for extracting interpretable components in ensemble neural activity from multi-channel brain recordings

Authors: *S. MACKESEY[1], F. T. SOMMER[2];
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Abstract: With the size of experimental recording data sets exponentially growing, the exploration of data sets with respect to specific coding hypotheses becomes increasingly laborious. To address this problem, we present the prototype of a cross-platform desktop application for extracting and visualizing functional components of multi-channel electrophysiology data. The application is demonstrated on local field potentials from publicly available dataset “hc3” (Buzsaki lab) from CRCNS.org, consisting of 442 multielectrode hippocampal recording sessions on rats navigating a variety of environments. Our software enables visualization of the activity of LFP components extracted by a variety of methods (e.g.

ICA on a phase-demodulated signal), facilitating visual comparison of components extracted using different algorithms and/or hyperparameter settings from different sessions. Our software also allows the user to fit arbitrary tuning models to components and search, sort, and filter by variance explained, allowing users to test a range of modeling hypotheses over large datasets. We present a variety of spatially-tuned (e.g. place, border) components matching the activity patterns seen in hippocampal neurons. Our goal is to promote exploratory analysis of large electrophysiology datasets by providing users with rapid visual feedback on the results of different analysis techniques. Currently, the scope of our prototype is limited to datasets formatted in the style of CRCNS hc3. However, we plan to provide a common public interface to promote use of the tool for arbitrary data formats.

Disclosures: S. Mackesey: None. **F.T. Sommer:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.17/DD39

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R24 MH114811-02

Title: JRCLUST: Toward comparing spike-sorting approaches for high-density extracellular electrophysiology

Authors: *A. C. LIDDELL, Jr, N. G. CLACK, B. KIMMEL;
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Abstract: JRCLUST is an open-source software application for visualization and semi-automated spike sorting with an emphasis on high-density extracellular electrode arrays, in particular, the Neuropixels probe. Our goal is to enable long-term support for JRCLUST as a community resource by maintaining a professional-quality code base and documentation while providing a platform for both official and peer support. As an open source project, we also want to encourage user contribution to the code. In order to achieve these goals, we have restructured the code base with an emphasis on interpretability and extensibility. This restructuring has had the additional benefit of enabling the incorporation and comparison of results from other sorting algorithms, such as Kilosort 2 and MountainSort. We have rearchitected the user interface so that visualizations pertaining to specific algorithms can be easily incorporated. Additionally, we have written and published comprehensive user documentation, including a tutorial, and have set up a real-time chat room. Both the chat room and the Github-based issue tracker are actively monitored. Algorithm development for spike sorting is an active field with several existing approaches optimized for various probe configurations. Our ultimate aim is to accelerate the

development of high-quality spike sorting approaches by identifying and incorporating algorithms and validation data sets into JRCLUST to facilitate comparison of these approaches, thus empowering users to select the best approach for their data.

Disclosures: **A.C. Liddell:** A. Employment/Salary (full or part-time):; Vidrio Technologies. **N.G. Clack:** A. Employment/Salary (full or part-time):; Vidrio Technologies. **B. Kimmel:** A. Employment/Salary (full or part-time):; Vidrio Technologies.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.18/DD40

Topic: I.07. Data Analysis and Statistics

Support: NIH R01DK120759

Title: Machine-learning driven approach for the analysis of calcium spatio-temporal maps

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Abstract: The widespread use of high-resolution Ca^{2+} imaging to study cellular Ca^{2+} dynamics, kinetics, and behavior has led to the creation of large datasets with a profound need for standardization and accurate analysis. In order to study these Ca^{2+} dynamics in a variety of cell types, researchers often make use of spatio-temporal maps (STMs). STMs allow for 2D visualization of Ca^{2+} signals as a function of time such that Ca^{2+} events can be detected. Previous methods of STM analysis rely on user defined segmentation of ROIs and involve a highly arduous process of event based data retrieval. This process is both time consuming and lacks accuracy. To ameliorate these limitations, we propose a machine-learning based pipeline for the analysis of Ca^{2+} STMs. This approach includes optimized tools for preprocessing, segmentation, and automated extraction of key STM Ca^{2+} event information such as: frequency, propagation velocity, intensity, area, and spatial spread. Our analysis relies on the use of multiple elements that work in concert to achieve high throughput analysis. To illustrate our analysis, we have generated STMs from a diverse group of cells, Interstitial Cells of Cajal (ICC), in situ. These cells are pacemakers with robust Ca^{2+} dynamics that mediate neurotransmission, slow wave potential, and muscle contractions in the gut.
Generation of ICC STMs, using Volumetry software (version G8d), enables detection of both stochastic and concerted Ca^{2+} events. Once an STM is generated, our method is fully implemented in ImageJ software and optimized to detect and analyze Ca^{2+} events from STMs. STMs are first preprocessed through our sequence of filtration steps that reduces background streaking and blur. Thereafter, STM Ca^{2+} events are accurately identified using a modified

machine-learning based Trainable Weka Segmentation algorithm. This algorithm implements a trainable automated process enhanced for STM event segmentation. Subsequent thresholding facilitates our analysis of STM event features and structured export of data. Our machine-learning based method dramatically reduces opportunities for user error and provides a consistent method to allow for high throughput analysis of STM datasets.

Disclosures: S.A. Baker: None. **W. Leigh:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.19/DD41

Topic: I.07. Data Analysis and Statistics

Support: NSF DMS-1547394

Title: A feature extraction method for noisy electrophysiology data

Authors: *E. M. JOHNSON[1], W. L. KATH[2];
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Abstract: Extraction of features such as action potential peaks or widths, and determining how they change under different conditions, is an essential component of analyses of electrophysiology data. Current methods range from using simple voltage thresholds to more complicated wavelet decompositions, but all perform relatively poorly when applied to noisy or low-resolution data. Furthermore, many of these methods require user input or refinement of algorithm parameters, making them ill-suited for large data sets. A suite of tools for robustly extracting features automatically from electrophysiology data has been developed using the Python programming language. The tools in this package make use of the statistics of the data themselves to estimate algorithm parameters, minimizing user intervention while remaining robust to noise. To demonstrate the use of these methods, we have applied them to a set of electrophysiology recordings of DN1 "clock" neurons in *Drosophila* (from Flourakis [Cell, 162 (2015)]) to determine electrophysiological features that are coupled to the circadian rhythm.

Disclosures: E.M. Johnson: None. **W.L. Kath:** None.

Poster

432. Software Tools: Analysis II

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.20/DD42

Topic: I.07. Data Analysis and Statistics

Support: European Commission: Marie Curie European Joint Doctorate 'Complex oscillatory systems: Modelling and Analysis (COSMOS)', project 642563

Title: Neuronal population coding: A new algorithm to discriminate stimuli according to the labeled line hypothesis

Authors: E. A. SATUVUORI[1,2,4], ***T. KREUZ**[3];
[1]Univ. of Florence, Florence, Italy; [2]Cnr, [3]Inst. for Complex Systems, Florence, Italy; [4]Vrije Univ. Amsterdam, MOVE Res. Institute, Dept. of Human Movement Sci., Amsterdam, Netherlands

Abstract: The nervous system is believed to employ large populations of neurons to code and broadcast information. Population coding can be considered a more reliable and robust manner than coding via single neurons **[1]**. In neuronal recordings population coding can appear in two ways. Either responses to external stimuli are generated by the neuronal population as a whole (*Summed Population, SP*) or each individual neuron may have a unique and distinguishable role (*Labeled Line, LL*). Accordingly spike train distances such as the SPIKE-distance **[2]** can evaluate either the pooled activity over the whole population or each neuron separately. The aim is to identify the neuronal subpopulation that discriminates a given set of stimuli best.
Following our proposals of algorithms to deal with the SP case **[3,4]**, we here introduce a new LL optimisation algorithm **[5]**, which finds the most discriminative subpopulation by analysing the discrimination performance of every neuron separately. First, for every individual stimulus pair the algorithm identifies the discriminative neurons and selects the best one. These best neurons are then combined to form the optimal LL-population. The algorithm can handle quite involved coding scenarios, even though its computational complexity is much lower than in the SP case. Moreover, we are guaranteed to find the best subpopulation since this time no search in a high-dimensional subpopulation space is needed.
The three measures ISI-distance **[6]**, SPIKE-distance **[2]** and SPIKE-Synchronization **[7]** and their adaptive versions **[8]** as well as the new directional measure SPIKE-Order **[9]** are implemented in the Matlab-based graphical user interface SPIKY **[7]**, the Matlab command line library cSPIKE, and the Python library PySpike **[10] [11]**.
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Mulansky M, Kreuz T. Software X **5**, 183 (2016)
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http://www.fi.isc.cnr.it/users/thomas.kreuz/Source-Code/cSPIKE.html and
https://github.com/mariomulansky/PySpike

Disclosures: E.A. Satuvuori: None. **T. Kreuz:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.21/DP14/DD43

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: I.07. Data Analysis and Statistics

Support: Duke Institute for Brain Sciences Research Incubator Award
BD2K Career Development Award (K01-ES-025442)

Title: Adaptive platform for online characterization of neural data

Authors: *A. DRAELOS[1], M. NIKITCHENKO[2], E. E. THOMSON[2], E. PNEVMATIKAKIS[3], A. GIOVANNUCCI[4], E. A. NAUMANN[2], J. M. PEARSON[1,2];
[1]Biostatistics & Bioinformatics, [2]Neurobio., Duke Univ., Durham, NC; [3]Ctr. for Computat. Biol., Flatiron Institute, Simons Fndn., New York, NY; [4]UNC/NCSU Joint Dept. of Biomed. Engin., Univ. of North Carolina, Chapel Hill, NC

Abstract: Recently developed imaging and microscopy techniques have enabled the recording of whole-brain activity at cellular resolution, generating terabytes of data from the activity of thousands of individual neurons in a single experiment. While new algorithms have made true real-time preprocessing of such data feasible, these advances have not yet been matched by an equally powerful set of tools for analyzing and modifying neural activity during live experiments. Here, we report on the design and implementation of an online data pipelining and analysis system capable of both characterizing neural activity and adaptively selecting targets for intervention in real time.

This online analysis and adaptive experimental platform can ingest data from common experimental sources (calcium imaging, electrophysiology), apply standard preprocessing methods, and integrate custom visualization and streaming statistical analysis, offering experimenters real-time insights into their data. Our system is designed to meet the stability requirements of experimental use while ensuring zero data loss, offering speed (via parallelization) and data integrity (through intensive logging). Moreover, it facilitates the rapid prototyping of new analysis and visualization methods through a streamlined Python application programming interface with minimal configuration syntax. The result is a flexible preprocessing and analysis pipeline that can be customized for each experimenter's needs in a few lines of code, one that is agnostic to the experimental setup or model organism.
As proof of concept, we implement a streaming algorithm that computes tuning curves online and identifies candidate ensembles of functionally related neurons. Such curves represent a functional characterization of cells into distinct types, each of which contributes differently to behavior. Groups of functional neural subtypes can then be selected out for specific desired features (e.g., all neurons strongly tuned to one given stimulus). By facilitating the online assembly of such functional groups, this work offers both real-time insights and a highly configurable platform on which to build experiments that modify themselves adaptively in response to data.

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Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.22/DP15/DD44

ControlExtraData.DynamicPosterDisplay:
Dynamic Poster
Topic: I.07. Data Analysis and Statistics

Support: Wellcome Trust grant 209558
Simons Foundation

Title: The IBL data architecture: Tools for storing and accessing diverse datasets collected at multiple sites

Authors: N. BONACCHI[1], G. A. CHAPUIS[2], A. K. CHURCHLAND[3], ***K. D. HARRIS**[2], C. ROSSANT[2], O. WINTER[4], M. J. WELLS[2], T. IBL COLLABORATION[2];
[1]Neurosci., Champalimaud Ctr. for the Unknown, Lisbon, Portugal; [2]Univ. Col. London, London, United Kingdom; [3]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [4]Champalimaud Inst. for the Unknown, Lisbon, Portugal

Abstract: The International Brain laboratory (IBL) is a collaboration aiming to understand the neural basis of decision-making. Ten experimental labs will use multiple neural recording modalities in diverse brain structures of mice making perceptual decisions. A primary requirement of IBL is to establish a data architecture that integrates data from all labs and modalities together. We have developed a system that allow users across 5 countries to automatically contribute data and metadata, search for relevant data, and share code for exploratory analysis.

To accurately record metadata about subjects and experiments in a searchable and accessible way, we have developed a user-friendly, web-based electronic lab notebook system for colony and experiment management (Alyx - https://github.com/cortex-lab/alyx). Alyx is a small relational metadatabase that records relevant information about each subject (age, strain, procedures), alongside information about experiments and their resulting data files. Once an experiment is completed, data registered in Alyx is automatically uploaded to a central server via Globus transfer.

Users can search and access the data using a lightweight interface called the Open Neurophysiology Environment (ONE - https://bit.ly/2V1v1ZW), implemented in Python and MATLAB. ONE defines a list of DatasetTypes, as arrays of predetermined shapes and units. For example, “spikes.times” defines a column vector of spike times in seconds, while “spikes.clusters” defines a column vector of integers of the same shapes, specifying each spike’s cluster assignment. Users search the database by running a command one.search that returns an ID identifying experiments matching their criteria. A command one.load returns the requested DatasetTypes as a numerical array, downloading it from the central server and caching on the user’s machine to avoid repeated downloads. Additional commands provide further ways to list experiments on the server, and the dataset types they each contain.

This system allows users to search, load and process data that was collected in laboratories spanning multiple geographical locations. To visualize these data, we have developed a pipeline for automated analysis, based on the DataJoint framework, described in a companion poster.

Disclosures: **N. Bonacchi:** None. **G.A. Chapuis:** None. **A.K. Churchland:** None. **K.D. Harris:** None. **C. Rossant:** None. **O. Winter:** None. **M.J. Wells:** None. **T. IBL Collaboration:** None.

Poster

432. Software Tools: Analysis II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 432.23/DD45

Topic: I.07. Data Analysis and Statistics

Support: Wellcome Trust grant 209558
Simons Foundation

Title: The IBL data architecture: DataJoint pipeline

Authors: *S. SHEN[1,2], M. SASAKI[1], A. E. URAI[3], E. Y. WALKER[1,2], C. A. TURNER[1,2], O. WINTER[4], N. BONACCHI[4], C. ROSSANT[5], G. A. CHAPUIS[5], M. J. WELLS[5], D. YATSENKO[1,2], A. K. CHURCHLAND[3], K. D. HARRIS[5], .. IBL COLLABORATION[5];
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Abstract: Large-scale international collaborative projects require standardized and well-organized data architecture that supports timely data ingestion, robust data maintenance, and flexible data access and processing. Part of the International Brain Laboratory (IBL) data architecture, developed in collaboration with Vathes LLC, is a standardized data pipeline based on DataJoint (datajoint.io), a general purpose framework for building scientific data pipelines with a database backend. DataJoint allows users to quickly develop processing pipelines using Python or MATLAB, that automatically run as new data is collected. For example, a current application of this system automatically produces a daily graphical web snapshot of all animals' behavioral performance history, which serves as a convenient tool for users to visualize the training status of a subject. These snapshots are updated automatically as new data is collected, with no need for the user to run an update function. We are currently developing a website that provides interactive visualization of physiological data and includes multiple methods to search and navigate experiments performed across IBL labs.
Behind the scenes, the IBL DataJoint pipeline is composed of three parts. The first part is metadata, ingested from the Alyx colony management database (see companion poster). The second part contains behavioral and electrophysiological data with basic pre-processing, ingested using the Open Neurophysiology Environment protocol (ONE; see companion poster). The third part contains analyses results created within the DataJoint pipeline, for example, behavioral results such as psychometric functions and reaction times for specific stimulus conditions. These results are computed automatically as new data is recorded, using DataJoint's auto-populate features. The IBL DataJoint pipeline supports flexible data searching, fetching, and processing, allowing users to analyze the data in a sophisticated manner and keep track of their analyses. All code is available as open source, and we envision that this system can be used as a general framework for the neuroscience community.

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Poster

433. Novel Approaches in Neuromodulation II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.01/DD46

Topic: I.08. Methods to Modulate Neural Activity

Title: Behavioral and physiological measures of stress in the head-fixed method combined with the air-lifted platform using a mouse model

Authors: *K. JUCZEWSKI, J. KOUSSA, A. KESNER, D. M. LOVINGER;
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Abstract: Head-fixing of an awake rodent is a great method to overcome common limitations related to physiological measurements and manipulations of cellular networks in the brain of anesthetized or awake and behaving rodents. We used the Mobile HomeCage system (Neurotar, Finland) for its “stress-free” conditions and multitude of potential behaviors that may be used with this system. It is a research device designed to enable recordings from awake mice that are head-fixed to an aluminum frame, but otherwise are freely moving in an ultralight carbon container floating above an air-dispensing base. To better understand this experimental environment before planned physiological and behavioral studies, we analyzed locomotion and head-fixation-induced stress during the extended habituation protocol. In our study, 1 week after the head-plate surgery and 2 days of handling and flannel-wrapping, we began 25-days habituation experiments. Each day the mouse was wrapped in the flannel, transferred to the head-fixation frame and left in the head-fixed position on the air-lifted container for 2 hours. Throughout 25 days, blood samples were taken periodically from the tail vein to measure corticosterone concentration that is known to correspond to the stress level. Additionally, locomotion data from the head-fixed mice were obtained from video recordings of the floating platform and/or the tracker sensor board compatible with the Mobile HomeCage system. Finally, at the end of the 25-days, animals were subjected to several behavioral tests commonly used as stress indicators, including the open-field, forced-swim, elevated plus maze, nesting behavior and sucrose preference tests. Our results show that habituation procedures reduce the stress level across the habituation days visible as a gradual decrease in the corticosterone levels. Further, they reveal that the head-fixation stress is not entirely relieved by giving the mouse ability to move its body in the air-lifted container, even with extended habituation time. In fact, in our initial analysis we observed changes in the stress-evaluating behavioral paradigms mentioned above after 25-days of habituation, but this data is still under evaluation. Therefore, even extended habituation does not make the head-fixation entirely “stress-free” which should be taken into consideration when interpreting physiological data obtained from the head-fixed animals.

Disclosures: **K. Juczewski:** None. **J. Koussa:** None. **A. Kesner:** None. **D.M. Lovinger:** None.

Poster

433. Novel Approaches in Neuromodulation II

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.02/DD47

Topic: I.08. Methods to Modulate Neural Activity

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NIH R01DA037152
NIDA R01DA033396-S1
NIDA F32DA043999
Mallinckrodt Professorship

Title: Smartphone controlled optofluidic device with replaceable Lego drug cartridges for chronic wireless *in vivo* pharmacology and optogenetics

Authors: R. QAZI[1,2], A. M. GOMEZ[3], D. C. CASTRO[3,4], Z. ZOU[2], J. SIM[5], Y. XIONG[2], J. ABDO[2], C. KIM[1], A. ANDERSON[2], F. LOHNER[2], S.-H. BYUN[1], B. LEE[6], K.-I. JANG[7], J. XIAO[2], M. R. BRUCHAS[3,4], *J.-W. JEONG[1,2];
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Abstract: Optogenetics and pharmacology are powerful techniques for dissection of complex neural circuitry with high spatiotemporal specificity. Conventional methods used optical fibers and metal cannulas for deliveries of light and drugs, respectively. However, these approaches significantly limit *in vivo* studies due to their tethered operation and mechanical mismatch between rigid devices and soft neural tissue, which cause increased tissue damage and inflammation response. Recent advances in materials, microfabrication, and wireless technology have enabled minimally invasive polymeric optofluidic probes, which allow wireless drug delivery and micro-LED based photostimulation in freely behaving animals. Although this wireless device overcame the limitations associated with the traditional tools, they still lack capabilities to repeatedly deliver drugs over long periods of time and to offer reliable wireless control without line-of-sight (LoS) handicap, control orientation dependency, and need for a special, expensive remote controller. To address these issues, here we present a smartphone-controlled, programmable optofluidic neural device that can wirelessly deliver drugs and light indefinitely for “chronic” in vivo pharmacology and optogenetics. This novel neural implant can provide photostimulation at two distinct wavelengths (470 nm and 589 nm) and deliver multiple different drugs for desired periods of time through replaceable ‘plug-n-play’ Lego drug

cartridges. Furthermore, its integration with Bluetooth low energy (BLE) facilitates wireless control via an easy-to-use, yet powerful smartphone app. This BLE wireless approach offers a long range of operation (up to ~100 m), omnidirectional control access, no LoS handicap, highly accurate target selectivity, wirelessly reprogrammable configurations, and facile setup for scalable closed loop controls, therefore substantially leveraging behavior neuroscience research involving complex 3D environment and multiple animals. Through various *in vivo* studies, we highlighted its unique capabilities for chronic drug delivery, selective animal control within a group, high temporal control for multidrug deliveries and closed loop control with optogenetic and pharmacological effects. These experiments signify its powerful applications for chronic wireless *in vivo* pharmacology and optogenetics as well as its potential for drug development and optopharmacology.

Disclosures: **R. Qazi:** None. **A.M. Gomez:** None. **D.C. Castro:** None. **Z. Zou:** None. **J. Sim:** None. **Y. Xiong:** None. **J. Abdo:** None. **C. Kim:** None. **A. Anderson:** None. **F. Lohner:** None. **S. Byun:** None. **B. Lee:** None. **K. Jang:** None. **J. Xiao:** None. **M.R. Bruchas:** None. **J. Jeong:** None.

Poster

433. Novel Approaches in Neuromodulation II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.03/DD48

Topic: I.08. Methods to Modulate Neural Activity

Support: Canadian Institutes of Health Research (FDN 154292)

Title: Effects of low intensity focused ultrasound on motor cortex excitability in human

Authors: ***K.-H. S. CHEN**[1,2], A. FOMENKO[3], J. SARAVANAMUTTU[2], M. EL-BABA[2], J.-F. NANKOO[2], A. M. LOZANO[4], R. CHEN[5];
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Abstract: Background Focused ultrasound (FUS) is as an approved non-invasive intracranial ablation therapy for essential tremor. Low intensity FUS can also be used to reversibly modulate the human cortex non-invasively. We investigated how different fundamental sonication parameters influence the effects of low intensity FUS on the motor cortex.
Method We studied 11 healthy subjects (six women). A dual-element, annular phase arrayed FUS transducer was attached to a 70 mm transcranial magnetic stimulation (TMS) coil. The center of FUS transducer was placed on the motor hot spot of right first dorsal interosseous muscle. TMS was delivered at 10 ms before the end of FUS. Different fundamental FUS

parameters, including output power, pulse repetition frequency, duty cycle and sonication duration, were tested together with the sham (near-zero output) condition. Motor evoked potential (MEP) elicited by FUS plus TMS were measured. TMS conducted with flipping the FUS transducer with the active surface pointing away from the scalp was used as a second sham condition.
Results: No subjects reported any adverse effects. FUS showed significant motor cortical suppression with longer sonication durations of 0.4 and 0.5 second (p=0.0013; Kruskal-Wallis test). FUS with 10% duty cycle also showed significantly reduced MEP amplitude (p=0.037). Other FUS settings did not elicit significant changes. FUS sonication showed significant MEP suppression compared to sham FUS with the
transducer flipped over (p=0.011).
Conclusion Low intensity FUS is a safe method for non-invasive neuromodulation in human. It suppressed motor cortex excitability with certain parameter settings.

Disclosures: K.S. Chen: None. **A. Fomenko:** None. **J. Saravanamuttu:** None. **M. El-Baba:** None. **J. Nankoo:** None. **A.M. Lozano:** None. **R. Chen:** None.

Poster

433. Novel Approaches in Neuromodulation II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.04/DD49

Topic: I.08. Methods to Modulate Neural Activity

Title: Non-invasive, receptor-specific, millimetre-precision manipulation of brain circuits for neuropsychiatric disorders

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Abstract: Central nervous system (CNS) disorders arise from dysfunctions in brain networks, involving different brain areas as well as different cell-types and molecular targets. Non-invasive, receptor-specific, focal modulation of different brain circuits in a controlled, reliable manner can lead to breakthroughs in future treatments of brain disorders. To achieve this, we systemically deliver engineered ultrasound-controllable therapeutic drug carriers. We then apply a unique focused ultrasound pulse sequence at a region of interest inside the brain which concentrates the drug carriers by orders of magnitude and uncages the cargo locally with millimeter precision. Upon release from the carriers, the drug locally crosses the intact blood-brain barrier (BBB), achieving high target specificity and low off-target effects. We show a proof-of-concept circuit-specific manipulation of sensory signaling in motor cortex in rats by

locally concentrating and releasing a $GABA_A$ receptor agonist from ultrasound-sensitive carriers. This approach not only uses orders of magnitude lower amount of drug than is otherwise required by systemic injection, but also requires very low ultrasound powers. Additionally, we also show that BBB remains intact using sensitive measures. Furthermore, we apply our technique to alleviate a neuropsychiatric disorder in the SAPAP3 KO mouse model by focused drug delivery. The technology we developed enables the most precise type of non-invasive circuit manipulation by combining molecular/receptor specificity of existing small molecules with a spatially-targeted delivery technique.

Disclosures: **A. Shah:** None. **M.S. Ozdas:** None. **P.M. Johnson:** None. **N. Patel:** None. **M. Marks:** None. **T. Yasar:** None. **W. von der Behrens:** None. **S.R. Sirsi:** None. **M.F. Yanik:** None.

Poster

433. Novel Approaches in Neuromodulation II

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Program #/Poster #: 433.05/DD50

Topic: I.08. Methods to Modulate Neural Activity

Support: Predoctoral translational fellowship Indiana CTSI #UL1TR002529
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Title: Magnetoelectric nanoparticles cobalt ferrite-barium titanate ($CoFe_2O_4$-$BaTiO_3$) for non invasive brain stimulation

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Abstract: Brain stimulation is widely used to diagnose and treat various neurological diseases. Current clinical stimulation techniques, however, are either invasive (e.g. deep brain stimulation) or have very low spatial and temporal resolutions as well as penetration depth (e.g. transcranial magnetic stimulation). Therefore, developing a non-invasive, efficient, precise, and translational brain stimulation approach will have significant value for brain study and disease treatments. In this study, we have developed a non-invasive brain stimulation technique using magneto-electric nanoparticles (MENs). The MENs have core-shell structures consisting of cobalt ferrite-barium titanate ($CoFe_2O_4$-$BaTiO_3$). They can be delivered to a focal brain region using a strong permanent magnetic field (~5000 Gauss), and produce an electric field only in the presence of a

weaker magnetic field (~450 Gauss) at specific frequency ranges. Their magnetic-electric property arises from its $CoFe_2O_4$ core vibration, in response to the weak magnetic field, which induces redistribution of the outer $BaTiO_3$ shell surface's charges to generate an electric field. After injecting fluorescently labeled MENs through a vein and attracting them to a specific cortical region using a strong magnetic field, we determined their distribution in the target cortical region. To assess the effect of MENs on activating neurons, we imaged calcium signals in organotypic cortical slices and *in vivo* in GCaMP6-expressing transgenic mice. On MENs-loaded slices or cortex *in vivo*, turning on the weak magnetic wave stimulation caused a dramatic increase in calcium transients of cortical neurons. Whole brain mesoscopic calcium imaging through a glass window of GCaMP6 mice showed a focal increase in calcium signal at the area where MENs localized and the signal attenuated at areas further away. To evaluate whether MENs resulted in brain toxicity and inflammation, we used immunostaining to IBA1 and GFAp for microglia and astrocyte activations and found no significance increases up to one week after MENs delivery. Taken together, the results indicate that MENs can be focally delivered to a specific cortical region and wirelessly activate cortical activity using magnetic wave with minimal toxicity. This technique shows promising potential to be a new approach for treating neurological diseases.

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Poster

433. Novel Approaches in Neuromodulation II

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Program #/Poster #: 433.06/DD51

Topic: I.08. Methods to Modulate Neural Activity

NIH K12HD043494
NIH T32HD043728

Title: Control of neuronal excitability with radiofrequency magnetic fields

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Abstract: The ability to control neuronal excitability with high spatial and temporal resolution is essential to understanding the complex circuitry of the brain. We have devised a technique termed “FeRIC” (Ferritin-iron Redistribution to Ion Channels) to remotely control neuronal excitability with non-invasive radio-frequency (RF) magnetic fields. FeRIC technology couples modified membrane ion channels with ferritin. Specifically, the intracellular domain of the

transient receptor potential vanilloid 1 and 4 (TRPV1 and TRPV4), and the Ca^{2+}-activated Cl^- channel Anoctamin 1 (Ano1 or TMEM16A) were fused with the ferritin-binding region (domain 5) of Kininogen. The resulting FeRIC channels redistribute endogenous ferritin to their proximity and could be activated with RF. Here we show that RF activates cultured hippocampal and cortical neurons expressing $TRPV1^{FeRIC}$ and $TRPV4^{FeRIC}$ channels. In those neurons, RF depolarizes the membrane potential, triggers Ca^{2+} transients, and increases the firing rate. Conversely, RF inhibits neurons expressing $TMEM16A^{FeRIC}$ via membrane hyperpolarization. To conclude, present work shows the feasibility of FeRIC technology to remotely control the neuronal excitability with spatiotemporal resolution.

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Poster

433. Novel Approaches in Neuromodulation II

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Program #/Poster #: 433.07/DD52

Topic: I.08. Methods to Modulate Neural Activity

Support: NIBIB U18EB021793
NINDS F31NS103472
NIDDK F32DK115122
NIDA T32DA007261

Title: A fully implantable optofluidic cuff system for wireless optogenetic and pharmacological neuromodulation of peripheral nerves

Authors: *A. D. MICKLE[1], Y. ZHANG[3], P. GUTRUF[4], L. MCILVRIED[1], J. GOLDEN[1], J. G. GRAJALES-REYES[1], Y. HUANG[5], J. A. ROGERS[5], R. W. GEREAU, IV[2];
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Abstract: Traditional neuromodulator technologies utilize fiber optic cables, catheters, injection needles and/or tethered systems that cannot be fully implanted. Furthermore, these are mostly constructed of hard, high modulus materials that can damage fragile tissues, create persistent inflammation at the biotic/abiotic interface and alter natural behaviors. Recent advances in implantable technologies that utilize thin, lower modulus materials have mitigated some of these limitations. Here we present a suite of technologies that advances on previous fully wireless implantable systems providing discrete optical and pharmacological stimulation, directly to peripheral nerves in a soft, bio-compliant cuff interface. This system provides the opportunity to selectively manipulate the activity of distinct neuron populations in awake, freely behaving

animals while removing experimental artifacts and confounds related to anesthesia and off-target pharmacological effects. This device utilizes near-field wireless communication to power independent time-locked delivery of up to four different pharmacologic agents as well as local light delivery at the nerve-cuff interface. Importantly, the cuff is designed with soft materials that mimic the physical properties of the peripheral nerve and as a result it imparts minimal effects on nerve health or function after chronic implantation. This opto-fluidic cuff will allow for future work discriminating the role of afferent populations in different aspects of nociception and sensory perception, with the potential to pair both optogenetics and local pharmacological approaches to increase neuromodulatory specificity and complexity of experiments. This work is supported by NIBIB U18EB021793 to JR/RG, NINDS F31NS103472 to JGGR, NIDDK F32DK115122 to AM. and NIDA T32DA007261 to LM.

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Poster

433. Novel Approaches in Neuromodulation II

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Topic: I.08. Methods to Modulate Neural Activity

Support: Grants-in-Aid for Scientific Research (15H05879, 16H02454, 17H05565) from MEXT
Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from AMED

Title: Retrograde gene transfer efficiency and inflammatory response of two types of lentiviral vectors in the motor cortex input system of nonhuman primates and rodents

Authors: Y. OTSUKA[1], H. TSUGE[1], S. UEZONO[1], S. TANABE[1], M. FUJIWARA[1], M. MIWA[1], S. KATO[2], K. NAKAMURA[1], K. KOBAYASHI[2], K.-I. INOUE[1], *M. TAKADA[1]; [1]Primate Res. Institute, Kyoto Univ., Inuyama, Japan; [2]Fukushima Med. Univ., Fukushima-City, Japan

Abstract: Pseudotyped lentiviral vectors that allow retrograde gene transfer are powerful tools to achieve pathway-selective gene manipulation in the brain. We have so far developed two types of vectors, HiRet vector and NeuRet vector. The former vector is pseudotyped with the B2 type

of fusion envelope protein, which is composed of a combination of vesicular stomatitis virus glycoprotein (VSV-G) and rabies virus glycoprotein (RV-G), while the latter is pseudotyped with E or E2 type. We have observed that these HiRet (B2) and NeuRet (E and E2) vectors show a high efficiency of retrograde gene delivery in the striatal input system of nonhuman primates and rodents. However, since the efficacy of retrograde gene transfer varies depending on the species and input systems, the properties of these vectors in corticocortical projections need to be clarified to analyze the roles of complex circuits involving widespread cerebral cortical areas. Here, we investigated the pattern and efficiency of transgene expression of the HiRet and NeuRet vectors in the cortical input system of nonhuman primates (rhesus monkeys, common marmosets) and rodents (rats) by injecting the GFP-expressing vectors into the supplementary motor area, medal area 6, and the secondary motor cortex, respectively. In common marmosets, many GFP-positive neurons were located in regions projecting to the injection site, such as the ipsilateral motor thalamus and the cortical motor-related areas of both hemispheres. We found that retrograde gene transfer of the NeuRet vectors was equivalent to or greater than that of the HiRet vector. In macaque monkeys, injections of the NeuRet vector with higher titer resulted in sufficient efficiency. Moreover, the NeuRet vectors displayed a higher transduction efficiency in the rat. We also found that the HiRet vector yielded gene delivery into many glial cells around the injection site, whereas the NeuRet vectors preferentially transduced neuronal cells in both primates and rodents. In addition, neuroinflammation characterized by microglial and CD8+ lymphocytic infiltration appeared after the injection of the HiRet vector, but not of the NeuRet vectors in nonhuman primates. The present results indicate that the NeuRet vectors are more suitable than the HiRet vector for retrograde gene delivery into neural networks involving the cerebral cortex in both nonhuman primates and rodents.

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Poster

433. Novel Approaches in Neuromodulation II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.09/DD54

Topic: I.08. Methods to Modulate Neural Activity

Support: KAKENHI(19H03335)
KAKENHI(19H04984)
PREST(JPMJPR1683)
AMED(dm0307021)

Title: The modified adeno associated virus vectors enable neuron specific efficient gene transduction in the primate brain

Authors: *K. KIMURA[1], Y. NAGAI[2], *S. TANABE[1], A. ZHENG[1], M. FUJIWARA[1], M. NAKANO[1], T. MINAMIMOTO[2], K.-I. INOUE[1,3], M. TAKADA[1];
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Abstract: Recombinant adeno-associated virus (AAV) vectors are promising tools to deliver functional molecules into the brains of nonhuman primates (NHP). Although the use of the AAV vectors for optogenetic and chemogenetic manipulation in the NHP demonstrates the validity of this strategy to elucidate neural network functions, the development of the AAV vectors that are more effective for foreign gene transfer is still needed. High-level expression of functional molecules is required for inducing reliable changes of neuronal activity and behavioral actions. However, viral infection to glial cells and/or transgene expression therein may cause inflammation. Therefore, it is critical that the development of a novel AAV vector with both high-level transgene expression and neuron-specific infection is desirable for controlling neural circuits in NHP. In the present study, we have developed modified AAV vectors (AAV2.1-A and AAV2.1-B), composed of AAV1 and AAV2 capsids. We injected these vectors into the cerebral cortex of macaque monkeys and examined the pattern and efficiency of their transgene expression by comparing with those via the AAV1 and AAV2 vector itself. We found that the AAV2 and AAV2.1-A vectors showed higher neuronal specificity, whereas the AAV1 and AAV2.1-B vectors had higher glial infectivity. On the other hand, it was revealed that both the AAV2.1-A and the AAV2.1-B vectors exhibited high-level transgene expression which was equivalent to that of the AAV1 vector and 3-4 times higher than that of the AAV2 vector. These results indicate that the newly developed AAV2.1-A vector possesses both high-level transgene expression capacity and neuron-specific infectivity. To examine the utility of the AAV2.1-A vector as a tool for neural manipulation, we injected the vector expressing the excitatory DREADD receptor into the striatum of macaque monkeys. We found that high level DREADD receptor expression and significant changes in regional brain glucose metabolism, an index of neuronal/synaptic activation by FDG PET imaging. Thus, the AAV2.1-A vector might be an ideal candidate for delivering functional molecules into neurons of the NHP brain.

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Poster

433. Novel Approaches in Neuromodulation II

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Program #/Poster #: 433.10/DD55

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant T32GM008555
New Innovator (1DP2-NS111505)
Beckman Young Investigator
Vallee Young Investigator
Sloan Fellowship

Title: A high-speed, bright, red fluorescent voltage sensor to detect neural activity

Authors: *C. BECK, Y. GONG;
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Abstract: Genetically encoded voltage indicators (GEVIs) form a powerful class of tools that can effectively probe neural circuitry and function. Genetically encoded indicators enable recordings of neural activity with a combination of relative non-invasiveness, temporal precision, and cell-type specificity. GEVIs have demonstrated ultra-fast kinetics and high spike detection fidelity *in vivo*, but the spectral diversity of this class of sensors limits their potential applications. Expanding the spectral diversity of GEVIs would carry three benefits for the neuroscience community. First, a red-fluorescent GEVI would enable simultaneous investigations of the connections and interactions between distinct neural populations when used in parallel with existing green-fluorescent GEVIs. Second, a red-fluorescent GEVI would enable simultaneous, spectrally separable optogenetic control and voltage imaging with minimal crosstalk between imaging and excitation wavelengths, allowing researchers to probe the effects of manipulations of neural circuits without inadvertently influencing endogenous spiking activity. Third, a red-shifted fluorescent GEVI with a high quantum yield capable of resolving single action potentials would emit light that is less prone to scattering than green-fluorescent proteins, allowing for deeper imaging in tissue. We have developed a red-fluorescent GEVI that achieves these myriad benefits by fusing Ace, a voltage-sensitive inhibitory rhodopsin, with a bright red-fluorescent protein. Our sensor takes advantage of fluorescence resonance energy transfer to produce a fast, nearly linear change in fluorescence in response to changes in membrane potential. Our sensor detects action potentials *in vitro* with high signal-to-noise and with comparable kinetics to state-of-the-art redfluorescent GEVIs. When paired with appropriate optical design and existing protein sensors or actuators, this new sensor enables complete spectral separation in simultaneous two-channel experiments such as multispectral imaging applications and optogenetic “read-write” applications.

Disclosures: **C. Beck:** None. **Y. Gong:** None.

Poster

433. Novel Approaches in Neuromodulation II

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Program #/Poster #: 433.11/DD56

Topic: I.08. Methods to Modulate Neural Activity

Support: U18EB021716-01
T32GM008471
T32DA007234

Title: AAV-mediated gene transfer targeting colon-innervating sensory neurons

Authors: *R. GORE[1], T. EMAIL[5], E. MARRON[2], K. KITTO[1], M. RIEDL[1], K. PFLEPSEN[3], A. KARLEN[4], S. MCIVOR[4], C. HONDA[1], C. FAIRBANKS[1], L. VULCHANOVA[1]; [1]Neurosci., [2]Phamacology, [3]Pharmaceutics, [4]Genetics, Cell Biology and Develop., Univ. of Minnesota, Minneapolis, MN; [5]Psychology, Macalester Col., Minneapolis, MN

Abstract: Adeno-associated viruses (AAV) have emerged as a preferred tool for gene transfer to neurons in basic research applications and as promising candidates for therapeutic gene delivery vectors. While AAV vectors have been widely used for gene transfer in the central nervous system, peripheral nervous system applications have been more limited. The colon has a dense intrinsic neural network and is also innervated by extrinsic afferent and efferent fibers. We previously demonstrated that intracolonic injection of AAV9 vectors leads to transgene expression in both dorsal root ganglion (DRG) neurons and enteric neurons. In this study, we test the hypothesis that a dual vector strategy that employs vectors injected intrathecally (i.t.) and intracolonically (i.c.) can achieve selective targeting of transgenes to colon innervating sensory neurons. First, we compared the capacity of AAV9.hSyn.GFPcre (i.t.) and AA2retro.hSyn.GFPcre (i.t.) to transduce DRG neurons and the percentage of neurons labelled with AAV9.CAG.tdTomato (i.c.), which was delivered a week after the intrathecal injections. AAV9 resulted in higher percentage of GFP+ neurons in the DRG (mean=30.6%, SD=19) as compared to AAV2retro (mean=2.4%, SD=2.1). In contrast, percent of tdTomato+ DRG neurons in AAV9 (i.t.) (mean=0.42%, SD=0.5) was lower compared to AAV2retro (i.t.) (mean=2.7%, SD=1.5) injected animals. We then injected AAV9.hSyn.GFPcre (i.t.) followed by AAV9.CAG.tdTomato (i.c.) on the same day or a week later to determine optimal injection paradigm to increase probability of expression of both vectors in colon-innervating sensory neurons. We found that injecting AAV9.CAG.tdT one week after AAV9.hSyn.GFPcre resulted in fewer tdT+ neurons (mean=0.1, SD=0.2) in lumbosacral DRG compared to the DRG of animals that received the two vectors on the same day (mean=70.8, SD=36.4). The same-day injections of two vectors resulted in overlap of the two fluorescent proteins in 6-20% of DRG

neurons (mean=11%, SD=5.1). Next, we used same-day treatment with AAV9 carrying Cre-dependent inhibitory DREADD (DREADDi) and AAV9.hSyn.GFPcre to express DREADDi in DRG neurons, and confirmed DREADDi expression using qPCR and immunohistochemistry. Work in progress is evaluating whether this expression is sufficient to modulate capsaicin induced nociceptive behaviors in mice. This study provides proof-of-concept for the application of combinatorial AAV vector strategies in the peripheral nervous system.

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Poster

433. Novel Approaches in Neuromodulation II

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Topic: I.08. Methods to Modulate Neural Activity

Support: NSF NeuroNex N3 1707562
DARPA N3 Award
Welch Foundation

Title: Fast magnetogenetics in *Drosophila*

Authors: ***C. SEBESTA**[1], G. DURET[2], D. TORRES[1], C. TZOUANAS[1], S. TONG[1], G. BAO[1], H. DIERICK[3], J. T. ROBINSON[2];
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Abstract: Magnetic activation of genetically targeted neurons enables circuit-level manipulations of deep-brain regions in freely moving animals without using implanted devices that could restrict animal movement or disrupt the natural neural architecture. Indeed, recent demonstrations of “magnetogenetic” technology have shown that coupling synthetic magnetic nanoparticles to temperature-gated TRPV1 channels enables remote, magnetic stimulation of select neurons in mice. While these landmark studies have demonstrated the tissue penetration capabilities of magnetogenetics, the latency of the magnetogenetic response limits some potential applications that require precise timing of neural activation. In fact, prior work has shown that for cells in a dish, activation occurs several seconds after the start of the magnetic stimulus. Response latencies are even longer *in vivo* with behavioral responses appearing tens of seconds after the magnet is activated. Here we demonstrate a magnetogenetic technology for *Drosophila* that has a ten-times-faster behavioral response than what has been reported in rodents. This fast magnetogenetic technology uses the temperature rate sensitive *Drosophila* channel TRPA1 in

place of the previous temperature threshold gated channels. Our results suggest that optimizing these channels that respond to the rate of temperature change provides a path toward fast magnetogenetic technologies that could rival the millisecond temporal resolution of optogenetics while maintaining the minimal invasiveness of magnetic activation. Additionally, magnetogenetics in *Drosophila* provides a neural activation technology that is orthogonal to the animal's natural senses allowing minimal off target effects compared to optogenetic stimulation.

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Poster

433. Novel Approaches in Neuromodulation II

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Program #/Poster #: 433.13/DD58

Topic: I.08. Methods to Modulate Neural Activity

Title: Nanotechnology-driven non-viral modulation of gene expression in the central nervous system enables fundamental and translational studies with minimum alterations to the native tissue and/or cell culture niche

Authors: *D. ALZATE-CORREA, W. LAWRENCE, L. ORTEGA, N. HIGUITA-CASTRO, *D. GALLEGO-PEREZ;
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Abstract: A major challenge for the development of effective gene therapies for disorders related to the Central Nervous System (CNS) is the lack of safe and controlled gene transfer approaches to neurons, which hampers the study of basic neuronal physiology and the identification of novel therapeutic targets. Currently gene transfer to neurons is achieved via transfection with synthetic nanoparticles, or viral transduction, either locally or systemically with adeno-associated viral vectors. These approaches, however, are fraught with caveats, including low transfection efficiencies and high toxicity, especially when using synthetic nanoparticles, or calcium phosphate precipitation. Alternative methods such as standard electroporation require significant disruption of the native tissue or cell culture niche. Viral vectors, on the other hand, are prone to insertional mutagenesis and to induce adverse immune responses. To address these complications, we developed nanotechnology-driven platforms to efficiently and safely deliver genes to nerve tissue, both *in vitro* and *in vivo*, without the need for viral vectors, and without having to disrupt the cell culture or tissue niche, respectively. Such nanoscale systems rely on the use of nanochannels or engineered exosomes as delivery vehicles for the gene of interest. We tested both of these systems with *in vitro* cultures of mouse primary hippocampal neurons, and with hippocampal neurons *in vivo.* When nanochannels or exosomes were used to deliver a reporter protein gene, *GFP*, minimal perturbations to cell physiology and low to no toxicity were

observed, with cell viabilities close to 100%. In order to demonstrate the capacity of nanochannel- and exosome-mediated transduction to deliver therapeutic cargo to the CNS, we successfully transfected and controlled the expression level of the circadian clock gene Period 1 (*Per1*) in hippocampal neurons *in vitro* and *in vivo*. Changes in electrophysiological activity were monitored in response to nanochannel- or exosome-based delivery of *Per1*. A number of neurodegenerative disorders are accompanied by disruptions of circadian rhythms together with a decline of long-term memory. Circadian modulation of neuronal excitability likely underlies the pathophysiological changes observed in numerous neurodegenerative disorders. Here we demonstrate for the first time that nanochannels or exosomes could be used as powerful tools to control gene expression in neurons *in vitro* and *in vivo* without causing adverse responses. Such systems could potentially be implemented for fundamental studies, and in the development of highly promising and benign gene therapies for the CNS.

Disclosures: D. Alzate-Correa: None. **W. Lawrence:** None. **L. Ortega:** None. **N. Higuita-Castro:** None. **D. Gallego-Perez:** None.

Poster

433. Novel Approaches in Neuromodulation II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.14/DD59

Topic: I.08. Methods to Modulate Neural Activity

Support: MH113780
AA025128
GM095480

Title: A strategy for cell type-specific CRISPR/Cas9 activation and visualization in mice

Authors: *A. CRUZ, J. CAVARETTA, M. PAUKERT;
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Abstract: CRISPR/Cas9 is used to generate knockout animals in a fraction of the time of conventional knockouts, however for acute gene editing in living animals there are still weaknesses in the approach. When delivering single guide RNAs (sgRNAs) *in vivo* using viruses, the extent of knockdown of the gene of interest is unknown, due to both the efficiency of the sgRNA and delivery of the virus, which influences the interpretation of data collected. Therefore, having a method to accurately determine which cells in a population have been exposed to CRISPR/Cas9 is essential to determine the effects of the targeted gene of interest. We have developed a construct linking expression of an sgRNA and tdTomato. After viral injection into a mouse ubiquitously expressing Cas9, the construct can undergo permanent Cre recombination, allowing for the expression of tdTomato and an sgRNA. Cells that have

tdTomato expression therefore have the sgRNA expressed and have CRISPR activity, while cells that do not have tdTomato expression are wildtype. With the dependence on Cre recombinase, this construct can be used with a cell type-specific Cre-driver mouse line for select recombination, and an sgRNA targeting a sequence of interest can be quickly added using standard cloning protocols. The expression of tdTomato after recombination was found to be successful in transfection tests in HEK cells, and preliminary *in vitro* tests of sgRNAs targeting eGFP knockdown have shown successful CRISPR/Cas9 activity. *In vivo* tests are ongoing.
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Poster

433. Novel Approaches in Neuromodulation II

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.15/DD60

Topic: I.08. Methods to Modulate Neural Activity

Support: CNT - EEC-1028725
NIH - 5R01NS086804

Title: Multifunctional polymer-based fibers enable *in vivo* photopharmacology

Authors: J. FRANK[1], ***M.-J. ANTONINI**[2], P.-H. CHIANG[2], A. CANALES[2], I. C. RICE[4], P. ANIKEEVA[3];
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Abstract: Chemical neurotransmission is a tightly regulated process that underlies animal behavior, perception and disease pathology. To reversibly manipulate these signalling events with increased spatial and temporal precision, photoswitchable drugs can add an optical switch to a target receptor or cell. Although azobenzene-based probes are widely used to understand cell physiology, their application to behavioral experiments has been hindered by the lack of available hardware for exploiting these probes to deep brain regions in freely moving rodents. Here, we will outline the development of a photoswitchable capsaicin derivative that can reversibly activate the TRPV1 cation channel with green/violet light. To expand our probes' use *in vivo*, we developed a flexible fiber-optic implant with microfluidic and optical capabilities that can deliver and stimulate photochemical tools within deep brain regions of freely moving mice. Combined, these technologies can be leveraged to manipulate reward behaviors via the mesolimbic reward system, and future efforts targeting endogenous receptors in the central

nervous system will allow us to explore the cellular mechanisms underlying animal behavior with increased precision.

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Poster

433. Novel Approaches in Neuromodulation II

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Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.16/DD61

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 1R43NS102088-01A1

Title: A chemically defined hydrogel substrate promotes accelerated maturation and neurite extension of cortical glutamatergic neurons for high-throughput screening

Authors: T. L. SAMPSELL[1], ***G. KAUSHIK**[1], W. D. RICHARDS[1], K. XU[2], Z.-W. DU[2], M. HENDRICKSON[2], C. S. LEBAKKEN[1];
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Abstract: Human neural cells manufactured from patient-derived induced pluripotent stem cells (iPSCs) hold great promise for modeling neurodevelopmental disorders, discovering precision therapies, and screening for potential risks from environmental toxins. However, many neurological phenotypes arise in mature neurons, and human iPSC-derived neurons can require extensive time in culture (1-3 months) to reach full maturity. These lengthy cultures slow the discovery process and are costly due to labor and reagent requirements. We hypothesized that optimizing culture substrate properties through the use of tunable synthetic matrices would improve maturation. Currently, neurons are cultured on a variety of substrates including charged polymers (poly-lysines or poly-ornithines) or animal-derived matrices. Using JMP™ software, we employed Design of Experiment (DOE) methodology utilizing Box-Behnken response surface modeling to screen for synthetic polyethylene glycol-based (PEG) hydrogel formulations that promoted viability, cell adhesion, desired morphology, and accelerated maturation of cortical glutamatergic neurons. In the experimental design, we varied PEG concentrations, crosslinkers and cell adhesion peptide composition and concentrations. To facilitate the quantitative DOE analysis, we utilized neurons derived from a human iPSC reporter line with a fusion protein comprising nanoluciferase (Nluc, Promega) and synaptophysin (SYP), a synaptic vesicle glycoprotein that is expressed in virtually all mature neurons and acts as a marker for quantification of synapses. We identified hydrogel formulations that 1) support cortical glutamatergic neuron adhesion as scored morphologically and assessed quantitatively via cellular ATP (Cell Titer Glo 2.0, Promega) and 2) accelerate maturation, as demonstrated through a time-

course of synaptophysin expression. Finally, these hydrogel formulations supported over two-fold increases in neurite length over a poly-D-lysine substrate. When incorporated into neuronal high-throughput screening efforts, the identified hydrogel substrates will improve overall outcomes and decrease the culture time required to reach the necessary maturation state.

Disclosures: T.L. Sampsell: A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. **G. Kaushik:** A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stem Pharm, Incorporated. **W.D. Richards:** A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stem Pharm, Incorporated. **K. Xu:** A. Employment/Salary (full or part-time):; BrainXell, Incorporated. **Z. Du:** A. Employment/Salary (full or part-time):; BrainXell, Incorporated. **M. Hendrickson:** A. Employment/Salary (full or part-time):; BrainXell, Incorporated. F. Consulting Fees (e.g., advisory boards); Stem Pharm, Incorporated. **C.S. Lebakken:** A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH sponsored SBIR grants. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stem Pharm, Incorporated.

Poster

433. Novel Approaches in Neuromodulation II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.17/DD62

Topic: I.08. Methods to Modulate Neural Activity

Title: Scaled expansion of pluripotent stem cells in suspension culture followed by direct neural differentiation from 3D cell aggregates

Authors: M. DERR, M. AKENHEAD, J. SAGAL, R. NEWMAN, D. KUNINGER; Thermo Fisher Scientific, Frederick, MD

Abstract: Culture systems for pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While two-dimensional (2D) feeder-free expansion of PSCs is well established, the scale at which PSCs and subsequent PSC-derived cell types can be efficiently manufactured using traditional methods is limited without a significant increase in hands-on time, as well as a potential risk of contamination. Therefore, to fully realize the potential of PSCs in downstream applications where large numbers of cells are required, such as cell therapy and

high-throughput screening applications, alternative expansion methodologies may be beneficial. Here we describe a new system for highly scalable expansion of PSCs as three-dimensional (3D) spheroids in suspension as self-assembled aggregates followed by neural induction and differentiation starting from the expanded PSC aggregates or spheroids. While expansion potential is an important parameter for assessing a fit for purpose medium system (i.e., 2D vs. 3D), another important consideration is compatibility with downstream differentiation protocols. In recent years, 3D aggregate cell culture has been gaining traction as an enhanced culture technique which provides more physiologically relevant cell-cell interactions over the traditional 2D cell culture protocols. When determining whether to move from 2D culture environments to 3D culture environments, a number of considerations need to be made. These considerations include the quantity of desired cell type(s) required for downstream applications, compatibility of reagents and experimental endpoints designed for 2D, and importantly how neurons derived using 2D and 3D compare and contrast to each other. Here, we demonstrate the feasibility of directing expanded PSC 3D aggregates to neurons using neural induction and differentiation reagents designed originally for monolayer applications. Key parameters and consideration for both PSC expansion and neural differentiation are presented and discussed, which include scalability, expansion rates, and differentiation efficiency. Notably, 3D neural differentiation resulted in significantly higher expansion rates of neural stem and progenitor cells and similar marker expression compared to standard 2D methods. Finally, the impact of 2D vs 3D neural differentiation and expansion on neuronal maturation will be presented.

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Poster

433. Novel Approaches in Neuromodulation II

Location: Hall A

Time: Monday, October 21, 2019, 1:00 PM - 5:00 PM

Program #/Poster #: 433.18/DD63

Topic: I.08. Methods to Modulate Neural Activity

Support: DFG CRC 870
DFG 487/3-1
German Federal Ministry of Education and Research under the Grant Code 01 EO 0901

Title: Oxygen producing algae permit functional recovery of neuronal activity in *Xenopus laevis* under hypoxia

Authors: ***S. OEZUGUR**[1], M. N. CHÁVEZ[2], J. NICKELSEN[2], L. KUNZ[1], H. STRAKA[1]; [1]Dept. Biol. II, Ludwig-Maximilians-University Munich, Germany, Munich, Germany; [2]Dept. Biol. I, Ludwig-Maximilians-University Munich, Germany, Munich, Germany

Abstract: Neuronal activity in the brain depends to a large extent on ATP generation and thus on the availability of oxygen. This makes the concentration of the latter molecule a highly relevant parameter for studying the interrelation between neuronal metabolism and computation. In order to evaluate the dependency of neuronal activity from oxygen availability, we employed semi-intact preparations of *Xenopus laevis* tadpoles with functional central and peripheral nervous systems. Trochlear motor nerve spike discharge served as physiological correlate for neuronal activity, while oxygen concentrations in the bath chamber and the brain, were concurrently monitored using a Clark-type oxygen microsensor during superfusion of Ringer solution with various levels of oxygen. The oxygen concentration was accurately set to a desired value by aeration with carbogen (95% O_2, 5% CO_2) or nitrogen. In air-saturated Ringer solutions (280 µM O_2), the IVth ventricle was devoid of oxygen likely due to consumption in the adjacent brain tissue. At oxygen bath concentrations > 300 µM, spontaneous burst discharge of the trochlear nerve caused a transient drop of the oxygen level within the IVth ventricle, indicating a neuronal activity-related increase in the local demand for oxygen. In contrast, decreasing the bath concentration of oxygen below ~40 µM completely ceased the trochlear motor nerve activity after ~30 min. Aiming at a spatially more accurate and faster means for the modulation of the oxygen level in the brain, we exploited the natural capability of algae to produce oxygen upon illumination. Injection of the green algae *Chlamydomonas reinhardtii* or the cyanobacteria *Synechocystis sp.* into the vascular system of *Xenopus* tadpoles immediately prior to the generation of the semi-intact preparation distributed these single celled eukaryotic and prokaryotic organisms throughout the blood vessels of the entire brain. While the hypoxic condition within the IVth ventricle persisted in such preparations in darkness, illumination with visible light increased the oxygen level up to ~80 µM. In addition, the abolished trochlear motor nerve activity in oxygen-depleted bath solutions restarted upon illumination, suggesting that algal oxygen production is sufficient to restore the energy equivalents required for maintained neuronal activity. Accordingly, introduction of algae and illumination represents a promising method to augment the oxygen level in any diffusion-limited *in vitro* neuronal preparation devoid of a functional circulation and potentially also under *in vivo* conditions.

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Poster

433. Novel Approaches in Neuromodulation II

Location: Hall A

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Program #/Poster #: 433.19/DD64

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant DA 040965

Title: Efficacy of aged chondroitinase-ABC in degrading perineuronal nets

Authors: *A. DEAN[1], J. H. HARKNESS[2], B. A. SORG[3];
[1]Integrative Physiol. and Neurosci., Washington State Univ. - Vancouver, Vancouver, WA; [2]Neurosci., Washington State University, Vancouver, Portland, OR; [3]Integrative Physiol. and Neurosci., Washington State Univ., Vancouver, WA

Abstract: Perineuronal nets (PNNs) are dense extracellular matrix structures surrounding fast-spiking, parvalbumin-containing GABAergic interneurons throughout the brain. Within the medial prefrontal cortex (mPFC), PNNs are important for the stabilization of memories following learning. Much of our research focuses on fluctuations in the staining intensity of these PNNs, which may reflect their thickness, and in turn, their ability to help form and maintain memories for drugs of abuse. The most common method to degrade PNNs is by giving an intracerebral injection of chondroitinase-ABC (Ch-ABC), an enzyme that digests chondroitin sulfate proteoglycans, a key structure in PNNs. However, it is not known how long this enzyme remains active once it is dissolved in buffer and stored frozen. Therefore, we determined the period over which dissolved Ch-ABC that was maintained at -20 C would be enzymatically viable. To test the lifespan of enzymatic activity, we identified aliquots of Ch-ABC that were stored at -20 C for 0, 0.5, 1, 4, 5, 8, 13, 18, or 20 months. A 0.4 µL volume/hemisphere of Ch-ABC or vehicle was microinjected into the mPFC of adult male rats (n=9). Rats were perfused three days following injections, brains were then frozen, sliced into 40 µm coronal sections, and stained with *Wisteria floribunda* (WFA) to identify PNNs. The intensity and number of PNNs were analyzed for each time period using the PIPSQUEAK program and grouped into three time bins (0-4 mo., 5-8 mo., 13-20 mo.). While every age of Ch-ABC significantly reduced WFA intensity, Ch-ABC from the 0-4 mo. and 4-8 mo. time points were more effective at reducing the intensity of PNNs compared with Ch-ABC from the 13-18 mo. time point. Additionally, Ch-ABC from the 5-8 mo. time point significantly reduced the intensity of PNNs compared to all other time points. These data indicate that Ch-ABC was the most effective between five to eight months post reconstitution; however, significant enzymatic activity may still occur even after being maintained at -20 C for up to 20 months.

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